Université de Montréal

Le mercure en Arctique, de l'environnement à la santé humaine : photodéméthylation aquatique, bioaccessibilité alimentaire et interactions avec le microbiome intestinal humain

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Résumé

Le mercure (Hg) est un contaminant d'envergure en Arctique, tant pour la santé écosystémique que la santé humaine. Émis par des activités anthropiques aux latitudes industrialisées, le Hg est transporté vers le Nord. Il peut ensuite être déposé dans les milieux aquatiques arctiques, où il peut être transformé en méthylmercure (MeHg), la forme organique et la plus toxique de ce contaminant. Le MeHg étant bioamplifiable dans les réseaux trophiques, il peut contaminer les animaux chassés par les Inuits, exposant cette population à des concentrations élevées de cette neurotoxine. L'objectif de cette thèse est d'améliorer notre compréhension des transformations que subissent le Hg et le MeHg dans le Nord, afin de mieux évaluer le risque posé par ce contaminant pour les milieux aquatiques et pour les Inuits.

Nous nous penchons d'abord sur la photodéméthylation du MeHg, un processus abiotique survenant dans l'environnement aquatique qui déméthyle le MeHg en sa forme moins toxique, le Hg(II). Nos expériences démontrent que si la photodéméthylation est un phénomène observable dans des mares de fonte de pergélisol, elle est quasi-absente dans les lacs oligotrophes. Ceci contredit l'hypothèse couramment acceptée qu'il s'agit d'un phénomène ubiquitaire, et nous proposons que la matière organique expliquerait cette différence par des mécanismes indirects. Enfin, nous démontrons que la photodéméthylation n'a pas d'impact sur le bilan net de MeHg d'une mare de fonte. Nous proposons que l'importance de la photodéméthylation dans le Nord pourrait changer avec les apports en matière organique et en Hg vers les milieux d'eau douce, dus aux changements climatiques (Girard et al. 2016).

Le MeHg qui n'est pas photodéméthylé dans l'environnement peut entrer dans les réseaux trophiques, et être absorbé par les humains via leur alimentation. Les lignes directrices visant à protéger les consommateurs du Hg assument que 100% du MeHg dans le poisson est absorbé par le corps humain. Toutefois, des études récentes suggèrent qu'il pourrait s'agir d'une surestimation, puisque ces recommandations ne tiennent pas compte de l'effet des pratiques alimentaires qui pourraient altérer la spéciation et la complexation du MeHg, affectant sa capacité à être absorbé. Nous avons utilisé un modèle de digestion *in vitro* pour étudier l'effet de pratiques alimentaires sur la bioaccessibilité (la solubilisation dans le tractus digestif) du MeHg. Nos résultats démontrent que la cuisson et la co-ingestion de composés riches en

polyphénols (comme le thé) diminuent significativement la bioaccessibilité, et peuvent avoir un effet cumulatif. Cette étude *in vitro* devra être validée dans un modèle *in vivo*, mais ouvre de nouvelles avenues de recherche pour mieux comprendre le sort du Hg dans le corps et pour améliorer les lignes directrices actuelles (Girard et al. 2017a).

Une autre variable pouvant affecter le sort du MeHg dans le corps est le microbiome intestinal, cet écosystème diversifié de microorganismes colonisant le tractus digestif. Les microorganismes étant les moteurs biogéochimiques du cycle du Hg dans l'environnement, ils pourraient également interagir avec le Hg et MeHg ingérés avec la nourriture, particulièrement chez les Inuits qui sont exposés à des concentrations importantes via leur diète traditionnelle. Nous présentons la première description du microbiome inuit, réalisé par séquençage de nouvelle génération d'un gène marqueur. Nous démontrons que le microbiome inuit est généralement similaire au microbiome d'occidentaux en termes de composition et de diversité, sans doute dû à la transition alimentaire actuellement en cours dans les communautés nordiques. Par contre, grâce à des analyses de souches bactériennes, nous avons identifié plusieurs marqueurs subtiles associés à la diète traditionnelle inuite (Girard et al. 2017b). Grâce au séquençage métagénomique, nous présentons le premier profile fonctionnel du microbiome inuit, et nous montrons la signature unique des gènes mobiles (comme les plasmides) chez les Inuits. Puis, nous avons exploré comment le microbiome de cette population répond au Hg. D'abord, nous analysons la présence de l'opéron de résistance au mercure (*mer*) dans le microbiome intestinal humain, qui pourrait permettre la volatilisation du Hg dans le tractus digestif en une forme moins biodisponible. Enfin, nous démontrons l'absence de potentiel de méthylation du Hg par le gène *hgcAB* dans le microbiome (Girard et al. *in prep*).

Cette thèse contribue de nouvelles observations sur le cycle du Hg et du MeHg en Arctique. Dans le contexte où les changements climatiques risquent d'augmenter l'apport en Hg vers les écosystèmes aquatiques nordiques et de faciliter sa méthylation en MeHg, ces résultats nous permettent de mieux comprendre ce que devient ce contaminant dans l'environnement et chez l'humain, afin de mieux cerner les risques de santé auxquels les Inuits font face tout en permettant la valorisation de la nourriture traditionnelle. **Mots-clés** : mercure, méthylmercure, Arctique, photodéméthylation, bioaccessibilité, polyphénols, microbiome, diète traditionnelle inuite, résistance au mercure (opéron *mer*), méthylation du mercure (*hgcAB*)

Abstract

Mercury (Hg) is a contaminant of major interest in the Arctic, both for human and ecosystem health. Produced at industrialized latitudes by human activity, Hg is easily carried to the Arctic. It can then be deposited in the North and accumulate in aquatic ecosystems, where it can be transformed into its organic and toxic form of methylmercury (MeHg). As MeHg can biomagnify through foodwebs, it can have important health consequences on the Inuit, whose traditional diet relies on fauna that can be contaminated. The goal of this thesis is to improve our understanding of the cycle of Hg and MeHg in the North, to better assess the risk it represents to aquatic ecosystem health and to the Inuit.

First, we focus on the photodemethylation of MeHg, an abiotic process occurring in aquatic ecosystems that degrades MeHg into the less toxic Hg. Our results show that while photodemethylation occurs in permafrost thaw ponds, it is barely detectable in Arctic oligotrophic lakes. This contradicts the commonly accepted idea that photodemethylation is ubiquitous in aquatic ecosystems, and we hypothesized that the lack of organic matter in oligotrophic lakes may explain this difference. Finally, by manipulating an entire pond, we show that photodemethylation did not impact this small ecosystem's net MeHg budget. However, we suggest that the importance of photodemethylation may change with increased input of organic matter and Hg into thaw ponds due to climate warming (Girard et al. 2016).

MeHg that is not degraded by photodemethylation can enter food webs and bioamplify in aquatic animals, and thus be absorbed by humans through their diet. Guidelines aiming to protect consumers from Hg assume that 100% of MeHg in fish is absorbed by the human body, but recent studies suggest this may be an overestimate. Indeed, these recommendations do not take into account dietary practices, which may alter speciation and complexation of MeHg, changing the way it is absorbed. We used an *in vitro* digestion model to study the impact of dietary practices on bioaccessibility (the fraction of a contaminant solubilized in the gastrointestinal tract) of MeHg. Our results show that cooking and co-ingesting polyphenol-rich foods (such as tea) can significantly reduce bioaccessibility, and both treatments can have a cumulative effect. While this *in vitro* study must be validated in an *in vivo* model, it improves our understanding of the mechanistic fate of Hg in the body, and offers novel ways of improving current guidelines (Girard et al. 2017a).

Another variable that could potentially affect how MeHg behaves in the human body is the gut microbiome, the complex microbial ecosystem that colonizes the human intestine. Since microorganisms are the drivers of biogeochemical cycling of Hg in the environment, it is possible bacterial members of the microbiome can interact with Hg and MeHg ingested with food, notably in the Inuit who are exposed to MeHg through their traditional diet. Here, we provide the first description of the Inuit microbiome using next generation sequencing of a marker gene. We show that the taxonomic composition and diversity of the Inuit microbiome is broadly similar to that of Westerners, likely due to the dietary transition currently underway in Northern communities. However, using fine-scale strain-level analyses, we identified many markers associated with the traditional Inuit diet (Girard et al. 2017b). Using shotgun metagenomic sequencing, we also present the first functional profile of the Inuit microbiome, and show that mobile genes such as plasmids have a unique signature in the Inuit. We then explored how the Inuit microbiome can interact with dietary Hg: first, we show evidence for the presence of the Hg resistance operon (*mer*) in the human gut microbiome, which shows that Hg could be volatilized in the human gut into a less bioavailable species. Finally, we present our survey of the methylator gene *hgcAB*, and suggest that it is unlikely that Hg is methylated into toxic MeHg in the human microbiome (Girard et al. *in prep*).

This thesis contributes novel observations on Hg and MeHg in the Arctic, especially in its cycling in the human body. Climate change is likely to increase Hg input into Arctic aquatic ecosystems and facilitate its methylation into MeHg, as well as to contribute to altering the Inuit diet. It is thus critical to better understand how this contaminant behaves in the environment and in the human body. This knowledge on the transformations of MeHg in the Arctic will allow us to improve our assessments of potential health risks Inuit populations face, while encouraging traditional food consumption. **Keywords**: mercury, methylmercury, Arctic, photodemethylation, bioaccessibility, polyphenols, microbiome, traditional Inuit diet, mercury resistance (*mer* operon), mercury methylation (*hgcAB*)

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Liste des abréviations

(Les caractères italiques indiquent les termes en anglais)

AMAP : *Arctic Monitoring Assessment Programme*

AMDE : événement de déplétion de mercure atmosphérique | *atmospheric mercury depletion event*

ANCOVA : *analysis of covariance*

ANOVA : *analysis of variance*

BA : bioaccessibilité | *bioaccessibility*

BLAST : *Basic Local Alignment Search Tool*

BMI : *body-mass index*

Br : brome | *bromine*

C : carbone | *carbon*

CALA : *Canadian Association for Laboratory Accreditation*

CCA : *canonical correspondence analysis*

CD-HIT-EST : *Cluster Database at High Identity with Tolerance*

CERFAS : Comité d'éthique de la recherche en arts et en sciences

Cl : chlore | *chloride*

Cond. : *conductivity*

CVAFS : spectroscopie de fluorescence atomique à vapeur froide | *cold vapor atomic fluorescence spectroscopy*

DMSO : *dimethyl sulfoxide*

DNA : *deoxyribonucleic acid*

DO : *dissolved oxygen*

DOC : *dissolved organic carbon*

DOM : *dissolved organic matter*

EGCG : *epigallocatechin gallate*

FDR : *false discovery rate*

GC-FID : *gas chromatography with flame ionization detector*

GSH : glutathion | *glutathione*

Hg : mercure | *mercury*

HMP : *Human Microbiome Project*

HPLC : *high-performance liquid chromatography*

I : *light intensity*

ICP-MS : *inductively coupled plasma mass spectrometry*

IDBA-UD : *Interative De Bruijn graph* de novo *Assembler for short reads sequencing data with hingly Uneven sequencing Depth*

JSD : *Jensen-Shannon divergence*

k : *light attenuation coefficient*

KEGG : *Kyoto Encyclopedia of Genes and Genomes*

k_{PD} : taux de photodéméthylation | *photodemethylation rate*

MeHg : méthylmercure | *methylmercury*

Lat. : *latitude*

LDA : *linear discriminant analysis*

LefSe : *LDA Effect size*

LMW : *low molecular weight*

Long. : *longitude*

MED : *Minimum Entropy Decomposition*

MG-RAST : *Metagenomics RAST server*

OTU : *operational taxonomic unit*

PAR : *photosynthetically-active radiation*

PBET : *Physiologically-based extraction test*

PCA : *principal component analysis*

PCR : *polymerase chain reaction*

PCoA : *principal coordinates analysis*

PD : photodéméthylation | *photodemethylation*

PDI : *probable daily intake*

PDI_{BA} : *bioaccessibility-corrected PDI*

PTDI : *provisional tolerable daily intake*

QIIME : *Quantitative Insights Into Microbial Ecology*

ROS : *reactive oxygen species*

rRNA : *ribosomal ribonucleic acid*

S : soufre | *sulfur*

SBD-F : *7-Fluorobenzofurazan-4-sulfonic acid*

SCFA : *short chain fatty acids*

SD : *standard deviation*

SHIME : *Simulator of Human Intestinal Microbial Ecosystem*

STAMP : *Statistical analysis of taxonomic and functional profiles*

$SUVA_{254}$: *specific ultraviolet absorbance at 254 nm*

TA : *thioglycolic acid*

Temp. : *temperature*

UPLC-MSMS : *ultra performance liquid chromatography tandem mass spectrometer*

UV : rayonnement ultraviolet | *ultraviolet radiation*

WHAM : *Windermere Humic Aqueous Model*

z_{max} : *maximum depth*

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Avant-propos

Historiquement, l'Arctique a été principalement défini par les puissances du Sud. Cette perspective colore notre imaginaire du Nord : on l'imagine souvent comme un grand espace vide bleu et blanc, inhabité. Pourtant, le Nord est occupé depuis des milliers d'années par les Inuits, qui sont témoins des changements importants qui s'opèrent en Arctique depuis le début de l'Anthropocène. Bien que la recherche en milieu arctique soit de plus en plus populaire, souvent elle n'implique pas les gens qui y vivent. Dans le cadre de cette thèse, des efforts ont été mis de l'avant pour générer de la recherche novatrice, mais également bilatérale et utile, tant pour la communauté scientifique et les populations nordiques.

Introduction

Le mercure, un contaminant d'envergure

Le mercure (Hg) est un contaminant d'intérêt pour les populations humaines depuis des milliers d'années. Il est d'abord connu des Grecs et des Romains comme *quicksilver*, dû à ses propriétés liquides uniques à température pièce (Clarkson 2008). Depuis, le mercure a été utilisé à de nombreuses fins, du traitement des fourrures à l'extraction minière, en passant par la vaccination et la médecine dentaire. Malgré ses sources naturelles comme les éruptions volcaniques et l'érosion de la roche-mère, les sources modernes de Hg sont principalement anthropiques : la combustion du charbon, la fusion des métaux ainsi que les usines chloro-alkali sont actuellement les contributeurs principaux aux émissions (Pacyna et al. 2006).

La forme organique du Hg, le méthylmercure (MeHg) est particulièrement d'intérêt. Bioaccumulable et bioamplifiable, le MeHg peut se retrouver en quantités appréciables dans les poissons et les mammifères marins, faisant de l'alimentation le vecteur d'exposition principal à ce contaminant (Clarkson and Magos 2008). En tant que neurotoxine capable de traverser la barrière hémato-encéphalique, le MeHg cause des troubles neurologiques et de développement et est donc d'intérêt pour la santé humaine (Clarkson 2008).

La signature de la Convention de Minamata en 2013 par 128 pays vise à diminuer ces émissions, et témoigne de l'importance continue de ce contaminant (United Nations Environment Programme 2013).

L'Arctique : un puits pour le Hg

Transport du Hg vers l'Arctique

Si le Hg contemporain est principalement émis par des sources anthropiques, pourquoi s'y intéresser dans des régions isolées et peu développées comme l'Arctique ? Lorsqu'il est produit par des activités industrielles, le Hg est émis sous sa forme volatile gazeuse de Hg(0), qui possède une durée de vie atmosphérique de 1-2 ans (Dastoor and Larocque 2004; Schroeder and Munthe 1998; Seigneur et al. 2004). Ceci lui permet d'être transporté sur de grandes

distances et de contaminer des écosystèmes éloignés de sources d'émissions, comme les régions nordiques (Durnford et al. 2010; Steffen et al. 2014). Les courants atmosphériques dirigent le Hg produit à des latitudes plus basses vers le Nord (Figure 1), et l'Arctique est maintenant reconnu comme étant un puits pour le Hg (Ariya et al. 2004).

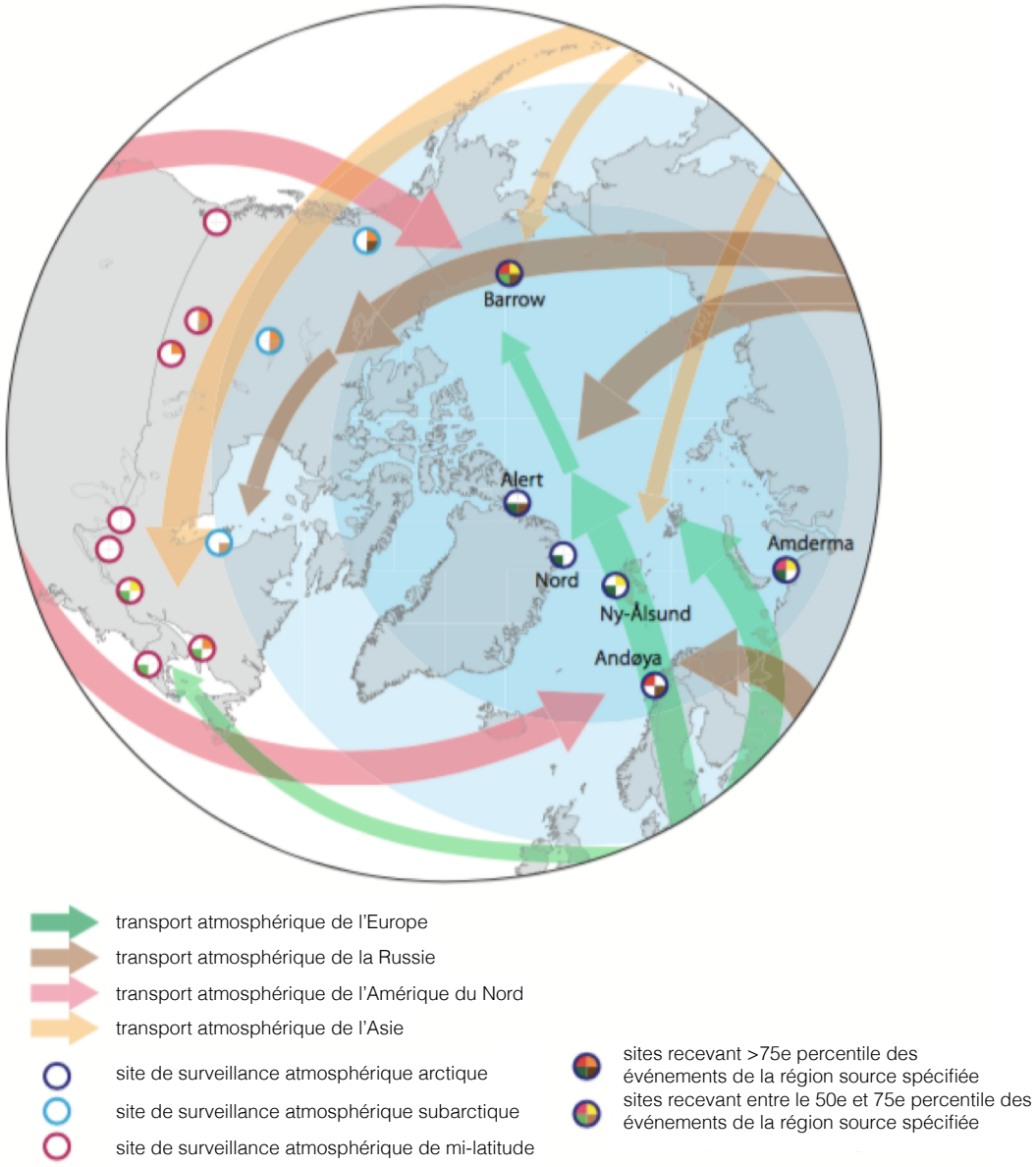

FIGURE 1. Carte des patrons des transports atmosphériques principaux du Hg vers l'Arctique depuis des latitudes moyennes. Adapté de AMAP (2011).

Le Hg(0) dans l'atmosphère peut être oxydé en Hg mercurique (Hg(II)) par des halogènes, principalement bromés (Ariya et al. 2002; Steffen et al. 2014). Le brome (Br), produit par les embruns marins et le regel de chenaux de saumure, devient réactif dans l'atmosphère en se liant à l'ozone (BrO), et peut ensuite catalyser l'oxydation du Hg(0) en Hg(I) ou Hg(II) (AMAP 2011; Ariya et al. 2002), qui se déposent facilement sur les environnements terrestres et aquatiques du Nord. Ces phénomènes d'oxydation et de déposition du Hg ont lieu partout sur le globe, mais en Arctique, le cycle lumineux et la composition atmosphérique favoriseraient la réalisation de ces réactions sur des intervalles plus courts, menant à des événements de déposition atmosphérique de masse (*atmospheric mercury depletion events*, AMDE) (Steffen et al. 2008). Le retour de la radiation solaire au printemps polaire après plusieurs mois d'obscurité totale permettrait une succession rapide des réactions photochimiques permettant la production de BrO, qui cause l'oxydation massive du Hg. L'importance relative des AMDE est contestée depuis quelques années, dû à des observations de ré-émission rapide du Hg vers l'atmosphère (Kirk et al. 2006; Poulain et al. 2004), ainsi qu'aux tendances d'augmentation du Hg bioaccumulé chez dans les animaux alors que les concentrations atmosphériques sont stables ou en déclin (Outridge et al. 2008). Le Nord est toutefois toujours considéré comme un puits pour le Hg atmosphérique, comme le soulignent des observations récentes sur la captation du Hg(0) par la végétation, qui en font une source de Hg non-négligeable pour la toundra (Obrist et al. 2017). Avec son transport atmosphériques, ses échanges et ses transformations complexes dans l'environnement Arctique, le Hg demeure donc un contaminant d'importance dans le Nord (AMAP 2011).

Méthylation et déméthylation en milieu aquatique

Le cycle du Hg en Arctique est complexe, avec de nombreuses transformations possibles depuis le milieu terrestre (dans le pergélisol (Rydberg et al. 2010) ou la neige (Poulain et al. 2007)) et dans l'océan (Braune et al. 2014), catalysées par des voies photochimiques ou microbiogéochimiques (Barkay and Poulain 2010). Toutefois, nous nous concentrerons ici sur les milieux lacustres arctiques, qui représentent 18% des ressources d'eaux douces canadiennes, et recouvrent une superficie de plus de 135 000 km^2 des territoires du Nord du pays (Prowse

1990). Les milieux d'eaux douces arctiques incluent une grande gamme de types d'écosystèmes, des lacs oligotrophes d'origine glaciaire aux mares thermokarstiques, issues de la fonte dynamique du pergélisol. Ces écosystèmes sont assujettis à de hauts niveaux de radiation solaire en été, des apports en eau ponctuels importants à la fonte des neiges (Pienitz et al. 2008), et potentiellement à des apports de Hg stocké dans le pergélisol en fonte (Klaminder et al. 2008). L'étude des lacs arctiques est critique pour mieux comprendre le cycle du Hg, et peut être d'importance pour la santé humaine si la pêche y est pratiquée. Dans le cas des mares thermokarstiques, si ces petits écosystèmes ne contiennent pas de poissons, ils demeurent néanmoins importants car ils peuvent représenter une source de MeHg pour les milieux environnants (Annexe I, MacMillan et al. 2015). Il est donc capital de comprendre le sort du Hg déposé dans ces environnements.

Une fois déposé dans les milieux aquatiques arctiques, le Hg(II) issu de l'oxydation atmosphérique a une très grande affinité pour la matière organique, particulièrement pour les groupes réduits de soufre. La matière organique aura donc des conséquences importantes sur la spéciation, la mobilité et la toxicité du Hg (Ravichandran 2004). La matière organique peut par ailleurs stimuler l'activité microbienne et faciliter la méthylation du Hg(II) en sa forme organique, le MeHg (Paranjape and Hall 2017). Si la méthylation peut se produire à de faibles taux par des mécanismes abiotiques (Eckley and Hintelmann 2006), la majorité de la production de MeHg est réalisée par des processus biotiques. Les bactéries sulfato-réductrices ont longtemps été considérées comme les méthylateurs principaux des milieux lacustres (Compeau and Bartha 1985; Paranjape and Hall 2017), mais d'autres groupes ont depuis été identifiés comme des contributeurs, notamment les méthanogènes (Hamelin et al. 2011), les ferro-réductrices (Si et al. 2015) et potentiellement les phototrophes (Grégoire and Poulain, 2014). La méthylation peut avoir lieu dans les zones humides et les sédiments, mais également dans le périphython et la colonne d'eau (Paranjape and Hall 2017). La transformation du Hg en MeHg est catalysée par le groupe de gènes bactériens *hgcAB*, identifiés en 2013 (Gilmour et al. 2013; Parks et al. 2013). Ces gènes encodent une protéine corrinoïde (HgcA) et une ferredoxine (HgcB) qui ensemble, permettent l'ajout d'un groupement méthyl au Hg (Parks et al. 2013). Tout comme le Hg, le MeHg ainsi produit a également une haute affinité pour la matière organique, et sa complexation peut contribuer à en limiter le transfert vers les réseaux

trophiques. La matière organique est donc un ligand important pour les différentes espèces de Hg, ainsi que pour permettre la méthylation bactérienne.

À l'inverse, la matière organique peut également participer à la dégradation du MeHg en Hg(II) via la photodéméthylation. La photodéméthylation du MeHg est un processus abiotique, initialement identifié dans la région des lacs expérimentaux de l'Ontario (*Experimental Lakes Area*) suite à des observations de déclin des concentrations de MeHg après exposition à la lumière du soleil (Sellers et al. 1996). Depuis, le phénomène a été rapporté dans plusieurs autres écosystèmes (Black et al. 2012; Fernández-Gómez et al. 2013; Hammerschmidt and Fitzgerald 2006; Suda et al. 1993; Sun et al. 2015), et semble dépendant de la radiation ultraviolette (UV) (Lehnherr and St Louis 2009). La matière organique aurait un rôle majeur à jouer dans le processus, tant via la production de molécules impliquées dans la réaction (Fernandez-Gomez et al. 2015; Tai et al. 2014) que par l'atténuation lumineuse dans la colonne d'eau.

La déméthylation du MeHg peut aussi être médiée par des processus biotiques, par l'opéron *mer* trouvé dans une large gamme de bactéries Gram-négatives et Gram-positives (Schaefer et al. 2004). Cet opéron, qui permet la réduction du Hg(II) en Hg(0) via une réductase (MerA), peut également opérer la séparation du groupement méthyl du MeHg via une lyase organomercuriale (MerB) (Barkay et al. 2003).

Malgré les processus complexes de méthylation et de déméthylation du MeHg dans les milieux aquatiques, il n'en demeure pas moins qu'une quantité suffisante de MeHg demeure pour entrer dans la faune et la flore aquatique, permettant un transfert vers les maillons supérieurs des réseaux trophiques de la forme la plus biodisponible du Hg.

Transfert vers les réseaux trophiques

Si plusieurs métaux traces peuvent être bioaccumulés dans les organismes aquatiques, le MeHg a la particularité d'être bioamplifiable (Morel et al. 1998) : on observe donc une augmentation des concentrations de MeHg depuis l'environnement et en remontant les réseaux trophiques (Figure 2). Dans les poissons, vecteur principal du MeHg vers l'humain, le MeHg

est principalement lié aux cystéines des protéines, et se trouve majoritairement dans le muscle (Harris 2003).

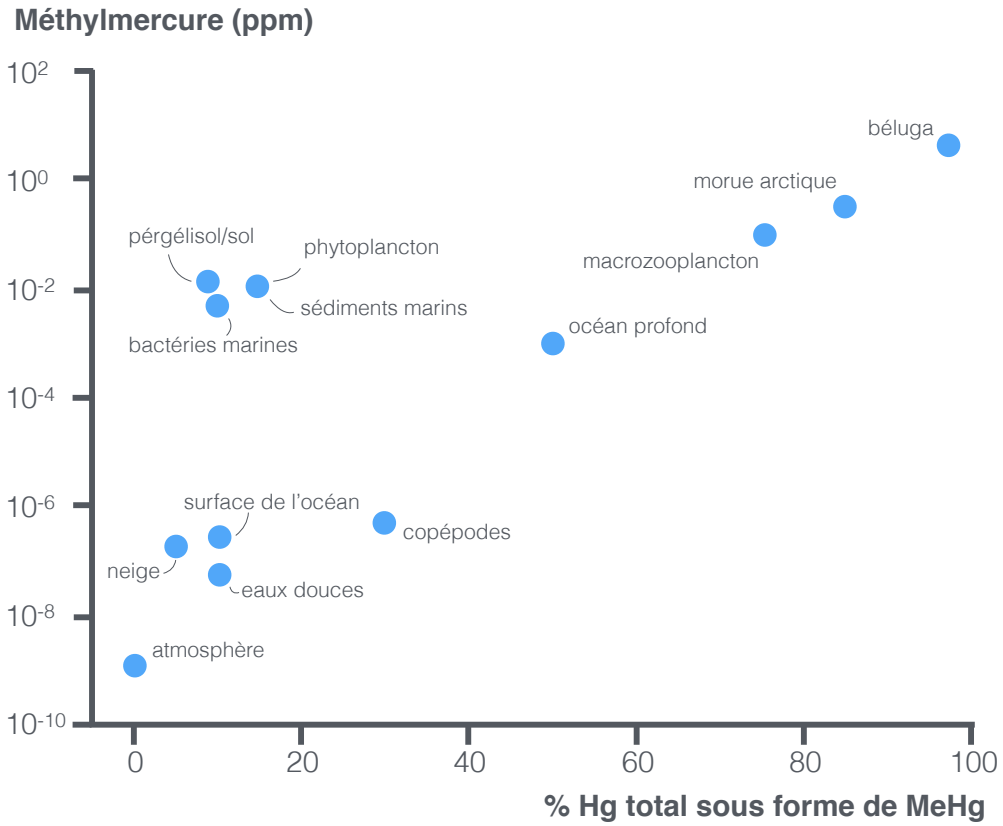

FIGURE 2. Bioaccumulation et bioamplification du MeHg dans l'environnement arctique, montrant que la forme organique du Hg devient l'espèce chimique la plus importante dans les niveaux trophiques supérieurs. Adapté de AMAP 2011.

En Arctique, la bioamplification du Hg fait en sorte que des organismes de haut niveau trophique contiennent parfois des concentrations excédant les seuils d'effets nocifs obtenus pour les animaux en laboratoire, et il semblerait que la bioamplification du MeHg soit accélérée dans les conditions froides et peu productives de l'Arctique (Lavoie et al. 2013). Le Hg peut donc avoir des conséquences importantes sur la santé des populations animales (Scheuhammer et al. 2014). Par extension, la présence de concentrations élevées de Hg dans la faune arctique pose également problème pour les populations humaines qui en dépendent pour leur alimentation (Figure 3).

Santé humaine

Nourriture traditionnelle dans le Nord

Les pratiques de chasse et de pêche sont profondément ancrées dans la culture des Inuits du Nord du Canada (Sharma 2010). Les proies consommées incluent le poisson (comme l'omble chevalier), les animaux terrestres (caribou, bœuf musqué, lagopède) et les mammifères marins (phoque, morse, baleine), et la viande peut être consommée crue, cuite, congelée ou fermentée. Des baies, plantes et fruits de mer peuvent aussi faire partie de l'alimentation, selon leur disponibilité dans la région. Cette diète est associée à un apport plus important en protéines et en gras d'origine animale que dans la diète de populations occidentales plus méridionales, et comparativement plus pauvre en fibres (Sharma 2010; Sharma et al. 2013).

La diète inuite a un statut nutritionnel très élevé, et est une source importante de vitamines, de minéraux et d'acides gras essentiels (Kuhnlein et al. 2006; 2002). De plus, sa récolte est associée à un niveau d'activité physique plus important (Donaldson et al. 2010) et à un risque de maladie cardio-vasculaire plus faible (Munch-Andersen et al. 2012). La nourriture traditionnelle présente également plusieurs avantages socioculturels : elle s'inscrit dans une culture de partage communautaire, favorise la cohésion sociale, et représente un ancre culturel important à l'identité inuite (Donaldson et al. 2010). Enfin, étant donné le coût élevé associé à l'importation de nourriture et de denrées périssables vers l'Arctique (Donaldson et al. 2010), la chasse et la pêche sont associées à une meilleure sécurité alimentaire (Chan et al. 2016), un enjeu d'envergure dans les communautés autochtones. Toutefois, le gibier faisant partie de cette diète peut également être un vecteur important d'exposition aux contaminants, incluant les polluants organiques et les métaux comme le Hg (Figure 3) (Van Oostdam et al. 2005). Il y a donc un besoin de réconcilier les bienfaits de la nourriture traditionnelle et le risque potentiel d'exposition, afin de protéger la santé publique dans les communautés nordiques (Laird et al. 2013; Lemire et al. 2014).

Malgré le fait que les bénéfices associés à la consommation de nourriture traditionnelle excèdent les risques du Hg (Chan 2012), la présence de ce contaminant dans la diète des peuples nordiques demeure une préoccupation importante (Binnington et al. 2016; Donaldson et al. 2010). En effet, les Inuits présentent des concentrations de Hg sanguin ou dans les cheveux qui

sont plus élevées que chez les Canadiens non-autochtones (Donaldson et al. 2010), et plus élevées que dans d'autres peuples nordiques (Gibson et al. 2016). Ces concentrations sont généralement corrélées à la consommation de nourriture traditionnelle (Muckle et al. 2001). Des études épidémiologiques sur d'autres populations côtières consommant de grandes quantités de poisson ou de mammifères marins ont montré que l'exposition au Hg est associée à des effets toxiques neuro-comportementaux (Grandjean et al. 1998) et cardiovasculaires (Grandjean et al. 2004; Weihe et al. 2016). Chez les Inuits, l'exposition accrue au Hg a été associée à plusieurs problèmes de santé, notamment au diabète de type II (Jeppesen et al. 2015), à des perturbations de la vision (Saint-Amour et al. 2006) ou du temps de réaction (Boucher et al. 2010).

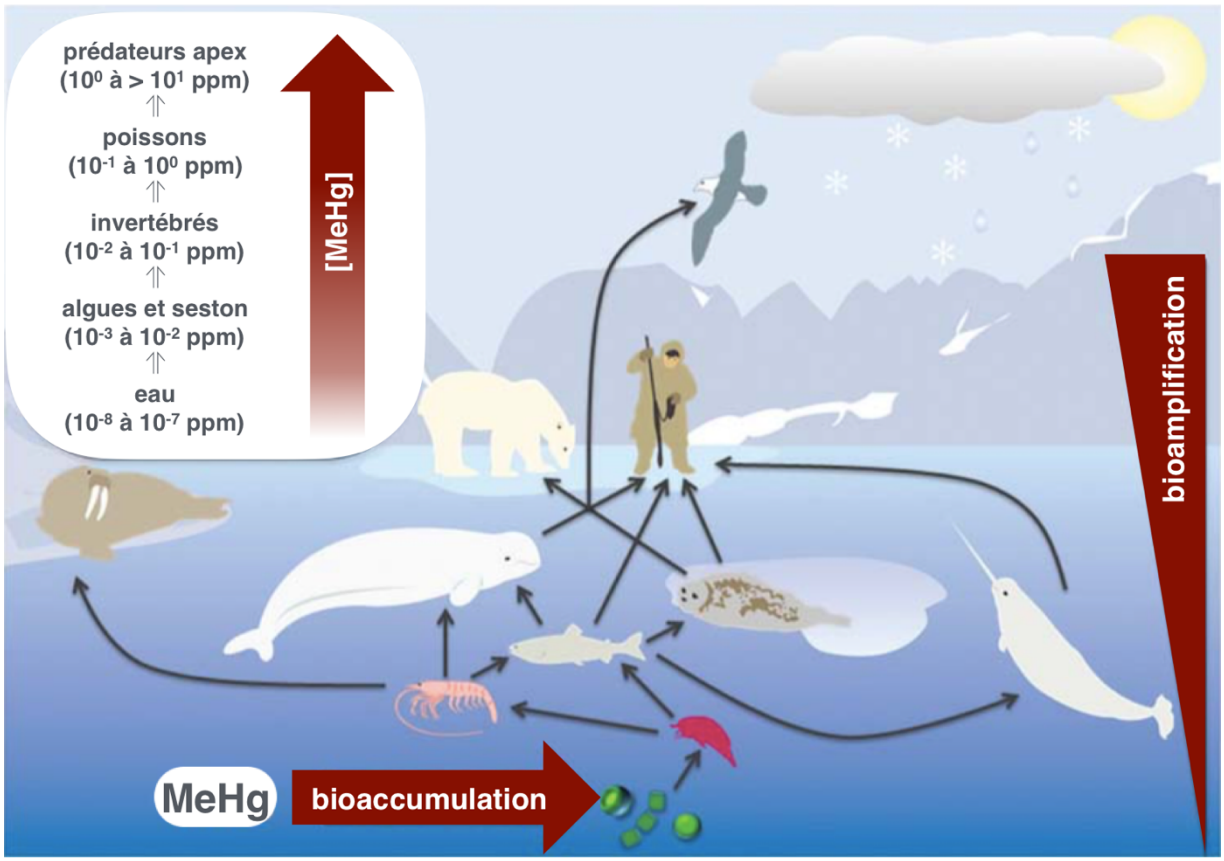

FIGURE 3. Transfert trophique du MeHg dans les réseaux trophiques arctiques, démontrant la bioaccumulation depuis l'eau et la bioamplification vers la nourriture traditionnelle. Adapté de Lehnherr 2014.

La problématique du Hg (et de d'autres contaminants) dans la nourriture traditionnelle est un enjeu de santé publique critique dans le Nord. Dans le contexte où les Inuits sont la population canadienne souffrant le plus d'insécurité alimentaire (Egeland et al. 2011), la gestion ainsi que la perception du risque posé par les contaminants dans la nourriture traditionnelle prend une grande importance (Bordeleau et al. 2016).

Absorption du Hg par le corps humain

Si des études épidémiologiques nous informent sur les effets toxicologiques du Hg, le sort du contaminant dans le corps après son ingestion est moins clair. La plupart des lignes directrices visant à limiter l'exposition au Hg à partir du poisson (vecteur principal du Hg alimentaire dans le monde) (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States 2010) assument que 100% du Hg trouvé dans le poisson est sous forme de MeHg, et que 100% de ce MeHg est absorbé par le corps (et donc biodisponible). Ces chiffres viennent d'études datant des années 70, qui démontraient une absorption presque totale du contaminant chez l'homme (Aberg et al. 1969) et le rat (Miettinen et al. 1971). Toutefois, ces études ne sont pas nécessairement représentatives de l'absorption réelle du Hg : en effet, ces expériences avaient été réalisées avec du nitrate de MeHg ($MeHgNO_3$), alors que dans le poisson, le MeHg est plutôt lié aux groupes soufrés et aux thiols dans les protéines (Clarkson and Magos 2008; Harris 2003).

Une autre manière d'étudier le Hg et le MeHg dans le corps est d'utiliser le concept de bioaccessibilité : si la biodisponibilité représente la fraction d'un contaminant qui est absorbée par le corps via l'épithélium intestinal, la bioaccessibilité fait référence à la fraction du contaminant qui est solubilisée depuis la matrice protéique, et qui peut ensuite être absorbée par le corps (Figure 4). La bioaccessibilité est donc une mesure conservatrice de la biodisponibilité, et le fait de pouvoir l'étudier dans des modèles *in vitro* en laboratoire permet d'explorer le sort du MeHg complexé à la chaire de la viande d'un point de vue plus mécanistique. Des études récentes portant sur la bioaccessibilité suggèrent que si le MeHg est sans doute fortement biodisponible (absorbable par le corps), il n'est pas nécessairement bioaccessible (Ha et al. 2016) : la solubilisation du MeHg depuis la matrice alimentaire semble incomplète, limitant la

capacité du contaminant à être absorbé. Ceci a été observé via des simulateurs *in vitro* pour la bioaccessibilité du MeHg dans plusieurs espèces de poisson (Kwasniak et al. 2012; Siedlikowski et al. 2016; Wang et al. 2013), mais également dans des mammifères marins faisant partie de la diète traditionnelle des Inuits (Laird et al. 2009a). Il semblerait donc que l'absorption du MeHg à partir de nourriture contaminée n'est que partielle (Ha et al. 2016).

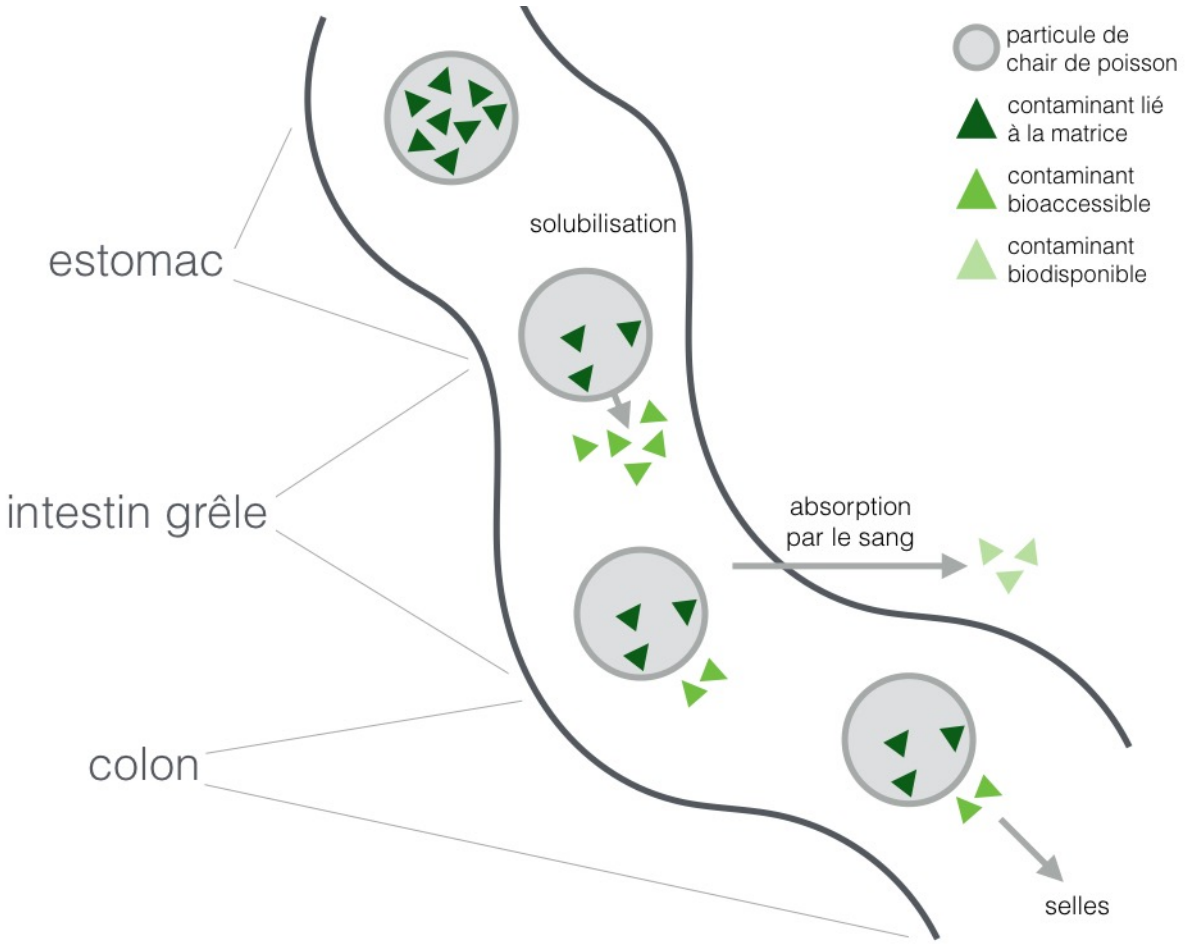

FIGURE 4. Modèle conceptuel de la bioaccessibilité (solubilisation d'un contaminant depuis la matrice alimentaire) et de la biodisponibilité (absorption du contaminant par le corps). Adapté de Denys et al. 2009.

Par ailleurs, les modèles pharmacocinétiques actuels visant à décrire la contamination en Hg et en MeHg chez les humains seraient de mauvais prédicteurs des patrons observés chez les populations autochtones (Canuel et al. 2006a). Il est donc possible que d'autres facteurs, environnementaux, culturels ou génétiques puissent moduler la manière dont le Hg est absorbé par le corps (Canuel et al. 2006a; Chapman and Chan 2000). Certaines études démontrent que la cuisson et la préparation des aliments pourraient altérer l'absorption du Hg et du MeHg (He and Wang 2011; Ouédraogo and Amyot 2011; Torres-Escribano et al. 2011), alors que ce facteur n'est pas pris en compte dans les analyses de risques. D'autres aliments co-ingérés avec de la nourriture contaminée pourraient affecter la spéciation et l'absorption du Hg, comme les fibres (Jadán Piedra et al. 2016; Shim et al. 2009; Yannai and Sachs 1993) ou les polyphénols d'origine végétale (He and Wang 2011; Ouédraogo and Amyot 2011; Shim et al. 2009). Le rôle de ces composés polyphénoliques, qui auraient des propriétés chélatrices envers les métaux (Graham 1992; Hider et al. 2001; Wang et al. 2009) pourrait être d'importance chez les Inuits, étant donné l'abondance de thé et de baies riches en polyphénols dans la diète traditionnelle.

Interactions avec le microbiome

Un autre facteur pouvant expliquer une absorption partielle du MeHg chez les Inuits serait le microbiome gastro-intestinal, qui représente un niveau de variation génétique et métabolique important entre les populations humaines (Mueller et al. 2006; Yatsunenko et al. 2012). Le terme microbiote défini cet écosystème microbien complexe composé de bactéries, d'archées, d'eucaryotes et de virus. L'ensemble de ces microorganismes ainsi que leur matériel génétique forment le microbiome (Whiteside et al. 2015). Ces deux termes sont souvent utilisés de manière interchangeable dans la littérature. Dans cette thèse, par soucis de clarté et d'homogénéité avec les articles publiés présentés ici, nous utiliserons le terme « microbiome » pour englober ces deux concepts.

Si le microbiome est influencé par plusieurs facteurs, la diète semble être la variable principale dirigeant la composition et la structure de cette communauté microbienne (David et al. 2013). Ceci a motivé bon nombre d'études sur le microbiome de différentes populations humaines, afin de mieux comprendre l'impact de l'alimentation sur le microbiome. Une

attention particulière a été accordée aux diètes dites « traditionnelles » qui divergent de la diète occidentale, et ces études ont démontré des différences majeures dans la composition du microbiome de ces individus (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012). Toutefois, la majorité de ces études se sont penchées sur des diètes traditionnelles agraires et riches en fibres de l'Amérique du Sud ou de l'Afrique, et peu d'efforts ont été déployés pour caractériser le microbiome de populations autochtones ayant d'autres types de diètes traditionnelles. Comme le soulignent Sankaranarayanan et al. 2015 dans l'une des rares études sur le microbiome autochtone nord-américain, ceci empêche ces populations d'être représentées dans des études à large échelle, limitant le degré auxquelles elles pourront bénéficier de la recherche biomédicale découlant de percées scientifiques sur le microbiome.

Dans le cas des Inuits, l'absence totale de connaissances sur la composition, la structure et le métabolisme du microbiome est une lacune importante. D'une part, la diète riche en gras et en protéines d'origine animale pourrait avoir une incidence sur la composition du microbiome, comme en témoignent des études comparant des niveaux de consommation de viande contrastés (David et al. 2013; Muegge et al. 2011; Torrey and Montu 1931). De plus, la diète traditionnelle expose le microbiome inuit à des concentrations non-négligeables de Hg et de MeHg. Si les bactéries sont les moteurs du cycle biogéochimique du Hg dans l'environnement, il est important de s'attarder aux interactions potentielles entre bactéries et Hg dans le corps humain. En effet, les transformations bactériennes catalysent les changements de spéciations du Hg au MeHg (et vice versa), ce qui a une incidence majeure sur la toxicité du contaminant. La recherche sur le microbiome intestinal est un milieu foisonnant et très actif, mais peu d'attention a été accordée aux interactions entre le microbiome et les contaminants alimentaires (Snedeker and Hay 2011). Pourtant, ce domaine d'étude semble prometteur, au vu d'études *in vitro* démontrant les changements dans la bioaccessibilité ou dans la spéciation de l'arsenic induits par le microbiome intestinal (Diaz-Bone and Van de Wiele 2010; Laird et al. 2009b; Van de Wiele et al. 2010).

Les microorganismes ont le potentiel de porter dans leurs génomes des gènes impliqués dans le métabolisme du Hg. L'opéron *mer*, qui encode plusieurs protéines, dont une lyase organomercuriale (MerB) pouvant déméthyler le MeHg et une réductase (MerA) permettant la

volatilisation du Hg(II) en Hg(0), a été détecté dans les selles de singes exposés au Hg via des amalgames dentaires (Liebert et al. 1997) : l'exposition au Hg environnemental pourrait donc exercer une sélection positive sur l'opéron (Barkay et al. 2003). Ceci pourrait s'appliquer aux microbiomes d'individus exposés à de hauts niveaux de Hg alimentaire, que ce soit dans le Nord ou dans des populations côtières adeptes de la consommation de poisson. L'opéron *mer* étant souvent porté par des éléments génétiques mobiles, il pourrait être sujet à des transferts horizontaux fréquents, permettant sa transmission au sein de populations bactériennes (Barkay et al. 2003). Dans une étude contrastant des microbiomes d'individus de l'Amérique du Nord, de l'Amérique du Sud et de l'Afrique, la réductase du Hg a été associée aux microbiomes d'Américains, sans informations sur l'exposition des individus au contaminant (Yatsunenko et al. 2012). Elle a également été identifiée dans une analyse du microbiome d'une petite cohorte de femmes enceintes (Rothenberg et al. 2016). Depuis, aucune autre étude ne s'est penchée sur la présence de ce gène (ou du reste de l'opéron *mer*) dans des populations exposées au Hg via leur alimentation.

La méthylation du Hg en MeHg dans le corps humain pourrait également avoir des retombées sur la toxicité du contaminant. Dans l'environnement, cette méthylation est catalysée par le groupe de gènes *hgcAB* (Paranjape and Hall 2017), et peut être opérée en conditions anaérobiques (Lu et al. 2016). Jusqu'à présent, le groupe *hgcAB* n'a pas été observé chez des primates (Gilmour et al. 2013), ni dans une étude sur une cohorte de femmes enceintes (Rothenberg et al. 2016). Par contre, aucune étude jusqu'à présent ne s'est penchée sur la présence d'*hgcAB* dans le microbiome d'une population particulièrement exposée au Hg, comme les Inuits. Enfin, il également est possible que le microbiome interfère avec la spéciation ou l'absorption du Hg dans le corps par des moyens indirects, comme en maintenant une bonne santé de l'épithélium intestinal (Rothenberg et al. 2016), ce mucus pouvant représenter une barrière à l'absorption du MeHg (Vázquez et al. 2013). Bref, le microbiome a le potentiel d'interagir avec le Hg de plusieurs manières différentes, ce qui pourrait affecter son sort dans le corps et expliquer une partie des manquements des modèles pharmacocinétiques actuels pour les populations inuites.

Cadre conceptuel et objectifs généraux de la thèse

Dans le cadre de cette thèse, je me suis intéressée à une partie du parcours du Hg en Arctique, particulièrement sous sa forme organique de MeHg. Les portions du cycle du Hg étudiées dans cette thèse sont présentées dans la Figure 5, et les objectifs et résultats des différents chapitres sont détaillés ci-bas.

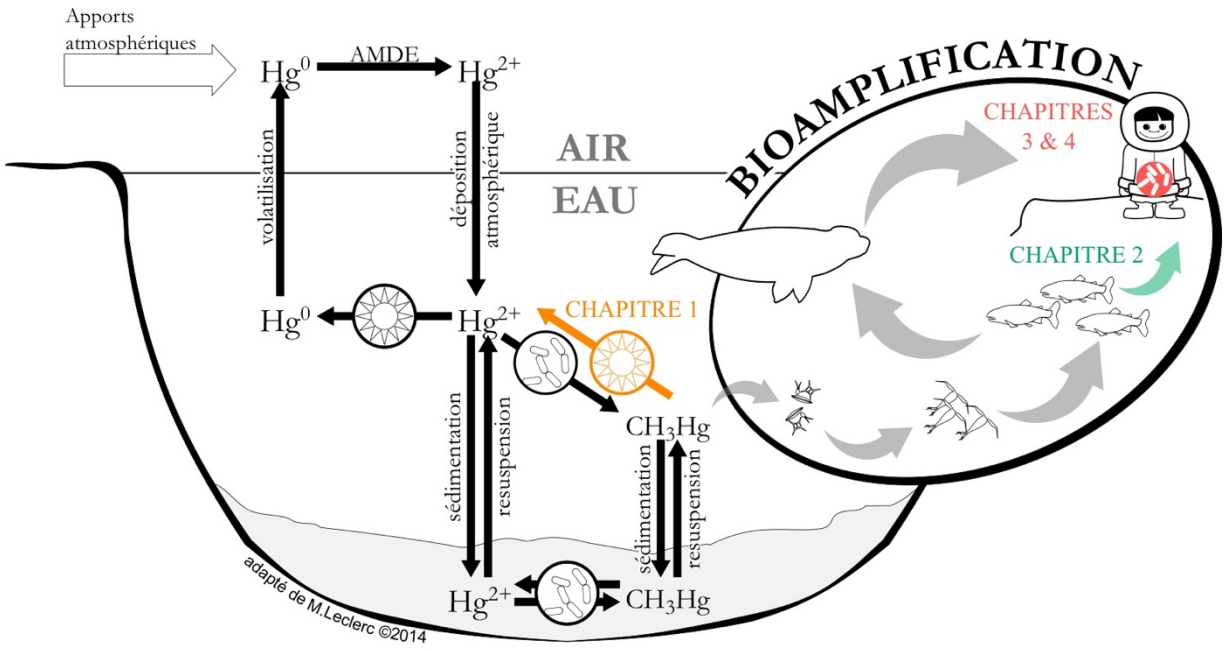

FIGURE 5. Cadre conceptuel de la thèse, présentant les thèmes des chapitres. Adapté de Maxime Leclerc ©2014.

Le MeHg est produit par la méthylation du Hg, et représente un enjeu important pour la santé humaine et des écosystèmes dans le Nord. Qu'arrive-t-il au MeHg suite à sa production dans les écosystèmes d'eaux douces arctiques ? Le **Chapitre 1** de cette thèse se penche sur l'une des voies d'élimination du MeHg, soit la photodéméthylation. Si la photodéméthylation a été identifiée comme un processus majeur dans d'autres écosystèmes, nous avons voulu vérifier son importance relative dans différents types de milieux aquatiques nordiques. Les objectifs de ce chapitre étaient d'abord (1) de déterminer si la photodéméthylation survient dans différents

écosystèmes contrastés en Arctique, et de mesurer des taux *in situ.* (2) Ce chapitre explore ensuite les acteurs chimiques impliqués dans le phénomène de la photodéméthylation. (3) Enfin, nous nous sommes penchés sur les conséquences potentielles de la photodéméthylation sur le bilan total en Hg et MeHg dans une mare de fonte de pergélisol, via une expérience de manipulation d'écosystème.

La photodéméthylation a d'abord été mesurée dans des écosystèmes d'eaux douces à concentration de matière organique contrastées, au moyen d'incubations sur le terrain. La quasi-absence de photodéméthylation dans les sites oligotrophes a démontré qu'il semble s'agir d'un processus intimement lié à la présence de matière organique dans la colonne d'eau. Nous émettons l'hypothèse que la matière organique a un rôle indirect dans la photodéméthylation, par la génération d'espèces réactives d'oxygènes. Enfin, l'impact potentiel de la photodéméthylation sur le bilan total de MeHg d'un écosystème a été mesuré via une expérience *in situ,* par la manipulation de l'exposition au soleil d'une mare thermokarstique. Ceci a montré que si la photodéméthylation survient dans des mares productives, ce processus n'a pas d'impact mesurable au niveau du bilan final de MeHg dans la mare, et que ces écosystèmes aquatiques pourraient demeurer des sources de MeHg pour les milieux environnants (Girard et al. 2016).

Si le MeHg peut être éliminé par photodéméthylation, une portion importante de ce contaminant parvient néanmoins à entrer dans les réseaux trophiques et à s'accumuler dans des organismes consommés par les humains. Le transfert du MeHg depuis la faune aquatique vers l'humain est un enjeu majeur, comme en témoigne la présence de lignes directrices établies par les gouvernements et agences de santé sur la consommation de poisson. Toutefois, ce transfert semble mal compris, au vu d'une littérature croissante suggérant que les lignes directrices estiment mal le risque. Ceci prend une importance particulière dans le Nord, car si les modèles pharmacocinétiques actuels sur le Hg fonctionnent généralement bien, ils sont de mauvais prédicteurs des patrons d'accumulation dans les populations autochtones. Le **Chapitre 2** se penche sur les effets des pratiques alimentaires sur la bioaccessibilité du MeHg, une mesure de la solubilisation du contaminant depuis la chaire du poisson. L'objectif principal de ce chapitre était de (1) déterminer comment les pratiques alimentaires peuvent altérer la solubilisation du MeHg depuis la chair du poisson. (2) Ce chapitre avait également pour but d'émettre des

recommandations pour améliorer les lignes directrices actuelles, pour favoriser la consommation du poisson, une source de protéines d'excellente qualité.

D'abord, il apparaît que la totalité du MeHg n'est pas solubilisée dans le tractus digestif (par simulation *in vitro*), supportant une littérature croissante qui suggère que les lignes directrices actuelles assumant 100% d'absorption du MeHg sont des surestimations. Ensuite, la cuisson a significativement limité la bioaccessibilité du MeHg dans toutes les espèces de poisson testées, potentiellement dû à des changements de conformation protéiques trappant le Hg dans la matrice de protéines de la viande. La co-ingestion d'aliments riches en polyphénols, comme le thé et le café, a également diminué la bioaccessibilité, et le rôle des polyphénols a été confirmé au moyen d'ajouts de molécules purifiées. De plus, la cuisson et les polyphénols agissent cumulativement, diminuant la bioaccessibilité *in vitro* de près de 99%. Enfin, ce chapitre propose des avenues de recherche pour améliorer les lignes directrices actuelles portant sur le MeHg dans le poisson (Girard et al. 2017a).

Une fois ingéré, le Hg et MeHg entrent en contact avec le microbiome intestinal, un écosystème complexe et diversifié. Ce microbiome a le potentiel d'altérer le sort des contaminants dans le corps, et est façonné par la diète et les habitudes de vie. La diète traditionnelle unique des Inuits (qui les expose au Hg) pourrait avoir mené à l'évolution d'un microbiome adapté à leurs conditions de vie. Si beaucoup d'études se sont attardées à décrire les microbiomes de populations partout autour du globe, les communautés autochtones nord-américaines sont très peu représentées. Ainsi, en amont de l'exploration des relations entre le microbiome inuit et le Hg, le **Chapitre 3** avait pour but de (1) présenter la première étude du microbiome inuit, basée sur le séquençage d'un gène marqueur. (2) Ce chapitre avait également comme objectif de déterminer comment la géographie et la diète traditionnelle affectent la composition et la structure de cette communauté microbienne.

Cette section présente d'abord une comparaison des profils taxonomiques de microbiomes d'individus du Nunavut à ceux de Montréalais, pour comprendre les effets de la diète traditionnelle inuit sur la diversité taxonomique dans le microbiome. Ces résultats démontrent que le microbiome inuit présente de grandes similarités avec le microbiome

occidental, potentiellement dû à la transition alimentaire observée dans les communautés nordiques. Par contre, des analyses taxonomiques plus fines (jusqu'aux souches bactériennes) ont identifié des taxons spécifiques aux consommateurs de la nourriture traditionnelle, ainsi qu'une diversité plus faible des bactéries responsables de la dégradation des fibres. Des marqueurs associés à la géographie ont également été identifiés, dont la fonction demeure pour l'instant inconnue, mais qui suggèrent que ce microbiome est unique et bien adapté au mode de vie moderne des Inuits (Girard et al. 2017b).

Étant donné que les bactéries dans l'environnement sont le moteur des transformations biogéochimiques, il est possible que les bactéries du microbiome puissent aussi transformer le Hg dans le corps, ce qui aurait des conséquences sur la santé humaine. Les Inuits étant exposés à davantage de Hg que beaucoup d'autres populations, il devient important de comprendre les transformations potentielles que le Hg pourrait subir au contact du microbiome de cette population. Le **Chapitre 4** avait pour but de d'abord (1) présenter les profiles métaboliques fonctionnels d'un sous-échantillon de 12 participants de l'étude du chapitre précédent, basés sur le séquençage métagénomique, afin d'explorer comment la diète traditionnelle inuite contribue à façonner la composition et les fonctions du microbiome. (2) Ensuite, ce chapitre avait comme objectif d'explorer la diversité des gènes impliqués dans la résistance et les transformations du Hg dans le microbiome intestinal.

Ce chapitre démontre d'abord que si les profils fonctionnels des Inuits ayant une diète traditionnelle sont similaires à des consommateurs de diète occidentale, ils possèdent une collection de gènes mobiles qui leur est propre. Ces gènes mobiles, de fonction inconnue pour l'instant, pourraient caractériser une forme d'adaptation locale à la diète traditionnelle du Nord. De plus, ce chapitre présente une analyse du gène encodant la réductase du Hg *merA*, présent sur un opéron complet de résistance au Hg présent sur un plasmide. Cette section termine avec une analyse de la présence du groupe de gènes *hgcAB* dans les microbiomes des Inuits et de Montréalais, ainsi que dans une cohorte plus large, et conclut qu'il n'y a probablement pas de production de MeHg dans le corps humain via ce complexe de gènes (Girard et al. *in prep*).

Cette thèse comporte également deux articles en annexe, qui ont été réalisés au cours de ce doctorat et qui découlent des chapitres présentés ici. L'**Annexe I** est l'article accompagnateur du Chapitre 1, et présente une évaluation de la contamination en Hg et en MeHg d'écosystèmes d'eaux douces arctiques (incluant les mares thermokarstiques et lacs présentés dans le Chapitre 1), et étudie les variables qui sont associées aux points chauds de contamination. En tant que deuxième auteure, ma contribution à cet article a été d'organiser la moitié des campagnes de terrain et la collecte de ces données, ainsi que de participer aux analyses statistiques et à l'interprétation des résultats (MacMillan et al. 2015). L'**Annexe II** est la suite du projet présenté dans le Chapitre 3. Nous avons émis l'hypothèse que les similarités entre le microbiome Inuit et occidental sont attribuables à la transition alimentaire dans le Nord, vers la nourriture de supermarché. Toutefois, tous les échantillons des Chapitres 3 & 4 ont été recueillis à la fin de l'été, qui n'est pas dans la saison de chasse typique des Inuits. Nous avons supposé que les variations saisonnières dans la diète traditionnelle inuite (due à l'accessibilité aux proies et à leurs patrons migratoires) pourraient induire de la variation dans le microbiome à d'autres moments de l'année. L'**Annexe II** présente un suivi mensuel d'une cohorte de résidents de la même communauté inuite que décrite dans le Chapitre 3, et montre que le microbiome inuit est plus variable dans le temps, sans pour autant exhiber de saisonnalité. Ma contribution à cet article en tant que deuxième auteure a été de développer le projet initial, de participer au recrutement des participants et de contribuer aux analyses de laboratoire et statistiques (Dubois et al. 2017).

CHAPITRE 1. Photodéméthylation du MeHg dans des mares de fonte et lacs de l'Est de l'Arctique canadien

Photodemethylation of methylmercury in eastern Canadian Arctic thaw pond and lake ecosystems

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Abstract

Permafrost thaw ponds of the warming Eastern Canadian Arctic are major landscape constituents and often display high levels of methylmercury (MeHg). We examined photodegradation potentials in high-dissolved organic matter (*DOC*) thaw ponds on Bylot Island (BYL) and a low-*DOC* oligotrophic lake on Cornwallis Island (Char Lake). In BYL, the ambient MeHg photodemethylation (PD) rate over 48 h of solar exposure was 6.1×10^{-3} m^2 E^{-1}, and the rate in MeHg amended samples was 9.3×10^{-3} m^2 E^{-1}. In contrast, in low-*DOC* Char Lake, PD was only observed in the first 12 hours, which suggests that PD may not be an important loss process in polar desert lakes. Thioglycolic acid addition slowed PD, while glutathione and chlorides did not impact northern PD rates. During an ecosystem-wide experiment conducted in a covered BYL pond, there was neither net MeHg increase in the dark nor loss attributable to PD following re-exposure to sunlight. We propose that high-*DOC* Arctic thaw ponds are more prone to MeHg PD than nearby oligotrophic lakes, likely through photoproduction of reactive species rather than via thiol complexation. However, at the ecosystem level, these ponds, which are widespread through the Arctic, remain likely sources of MeHg for neighboring systems.

Introduction

Mercury (Hg) contamination is a major environmental issue in the Canadian Arctic, which is considered to be a sink for volatile anthropogenic Hg produced at lower latitudes (AMAP 2011; Ariya et al. 2004). After deposition in the Arctic environment, Hg can be methylated into methylmercury (MeHg) by certain iron or sulfate-reducing bacteria and methanogens in anoxic environments (Compeau and Bartha 1985; Fleming et al. 2006; Hamelin et al. 2011; St Louis et al. 2005). This dangerous neurotoxin then accumulates in aquatic ecosystems of Arctic Canada (Hammerschmidt and Fitzgerald 2006; Lehnherr et al. 2012). The Arctic's changing climate may also increase the risk posed by MeHg by accelerating permafrost thawing, contributing to a larger release of historically stored inorganic Hg (AMAP 2011; Klaminder et al. 2008; Macdonald et al. 2005; Rydberg et al. 2010) and possibly increasing its microbial methylation rates (Klaminder et al. 2008; Macdonald et al. 2005; Stern et al. 2012). MeHg can represent a potential risk to northern communities by bioaccumulating and biomagnifying in foodwebs, eventually contaminating organisms that are part of the traditional diets of local human populations (Laird et al. 2009a).

Climate warming in the Arctic is currently promoting the formation of thermokarst thaw ponds. These ponds now represent the most abundant type of aquatic systems at Arctic and Subarctic latitudes (Pienitz et al. 2008). In the Eastern Canadian Arctic, they are characterized by very high MeHg levels that are in some instances orders of magnitude higher than neighboring oligotrophic lakes (Annex I, MacMillan et al. 2015). These ponds could constitute a source of MeHg for nearby lakes, streams and coastal waters and therefore contribute to the contamination of traditional food.

Photodemethylation (PD) of MeHg could reduce the risk posed by these high levels of MeHg in thaw ponds and has been documented in other polar ecosystems (Hammerschmidt and Fitzgerald 2006; Li et al. 2010; Sellers et al. 1996; Zhang and Hsu-Kim 2010). Both field and laboratory studies have found PD to be highly dependent on *UV* radiation (Black et al. 2012; Chen et al. 2003; Fernández-Gómez et al. 2013; Lehnherr and St Louis 2009; Rose et al. 2015; Suda et al. 1993; Sun et al. 2013). A study conducted in an Alaskan lake found that PD does not appear to be driven by the direct photolysis of MeHg: an indirect pathway involving naturally

occurring chemical actors seemed to be responsible for the demethylation process (Hammerschmidt and Fitzgerald 2010). In oxygenated freshwater, MeHg is usually bound to organic matter (Hintelmann et al. 1997) which is likely involved in PD (Fernandez-Gomez et al. 2015; Tai et al. 2014). Sulphur-rich functional groups of organic matter (thiols), to which MeHg preferentially binds, are suspected of promoting PD (Chandan et al. 2015; Zhang and Hsu-Kim 2010). Organic matter may also be involved in PD through its photo-mediated production of reactive oxygen species (*ROS*) (Latch 2006; Zhang and Hsu-Kim 2010), or via intramolecular electron transfer (Tai et al. 2014). Source and quality of organic matter have been found to significantly influence PD rates (Black et al. 2012; Fernandez-Gomez et al. 2015; Qian et al. 2014). Organic matter can also reduce light penetration in aquatic ecosystems (Morris et al. 1993). Chloride complexation however has been reported to limit PD, explaining the discrepancy in rates observed between marine and freshwater ecosystems (Black et al. 2012; Zhang and Hsu-Kim 2010).

This study aimed to (1) establish via field-based experiments if PD occurs in permafrost thaw ponds of the Eastern Canadian Arctic and if PD rates are higher than in oligotrophic lakes. We chose sites similar to each other in terms of water chemistry, except for their contrasting levels of organic matter, which could induce variation in PD potential. (2) Using field and laboratory experiments, we also sought to identify the chemical actors involved in PD, namely thiols found either free in the water column or as functional groups of dissolved organic matter, and chlorides. (3) Finally, we conducted an ecosystem-wide experiment to assess the potential impacts of PD on MeHg levels in thaw ponds.

Materials and methods

Field area and sampling sites

Sampling was performed on Bylot and Cornwallis Islands in the Canadian Arctic Archipelago from June to August 2010 and 2011 during the Arctic summer in 24-hour daylight.

Experiments in the polar oasis of Bylot Island (73°9'23.4" N; 79°58'19.38" W) were conducted in permafrost thaw ponds of the Qarlikturvik glacial valley. These ponds (henceforth referred to as BYL sites) are small unstratified aquatic ecosystems formed from degradation of ice wedges and thawing of permafrost (Pienitz et al. 2008) and contain high levels of dissolved organic carbon (Laurion et al. 2010) (*DOC*: 6.20-9.60 mg L^{-1}) and of Hg (Hg: 1.14-30.20 ng L^{-1}; MeHg: 0.06-18.19 ng L^{-1}) (Annex I, MacMillan et al. 2015).

Char Lake was sampled on Cornwallis Island (74°41'11.20" N; 94°54'33.81" W). Due to its low plant productivity, Cornwallis is considered a polar desert, (Chételat et al. 2010) where lakes are highly oligotrophic (*DOC*: 0.94 mg L^{-1}) and typically have low levels of Hg (Hg: 0.22-0.80 ng L^{-1}; MeHg: 0.01-0.07 ng L^{-1}).

Map of sites, surface water chemistry, Hg measurements and light parameters of individual sampled sites are presented in Supporting Information (SI, Figure S1 and Table S1).

Sampling methods

Water was sampled using an acid-washed peristaltic pump and filtered with a high-capacity 0.45 μm filtration cartridge (Pall Life Sciences). Dissolved organic carbon (*DOC*) samples were stored in glass amber bottles that were burnt at 550 °C for 2 hours prior to sampling. High-density polyethylene bottles were used for thiol, cation and anion samples. Thiols and cations were collected in acid-washed bottles, and anion samples in MilliQ-rinsed bottles (ultra-pure 18.2 MΩ cm water, EMD Millipore). PD experiments were conducted with acid-washed Teflon FEP bottles and experimental solutions were kept in amber glass bottles with polytetrafluoroethylene-lined polypropylene caps. Hg and MeHg samples were preserved with OmniTrace Ultra HCl (OmniTrace Ultra, VWR) (final concentration of 0.4%) while following the 'clean hands, dirty hands' protocol (St Louis et al. 1994). Pump and bottle washing procedures are detailed in SI (Supplementary Methods).

Experimental design

PD field experiments were conducted in natural sunlight. Water was collected in clear Teflon bottles with no headspace. At every time-point during incubations, a triplicate of every treatment series (duplicates for some dark controls) was removed from the incubation set-up and preserved with ultrapure HCl to a 0.4% final concentration. Detailed methods are described in SI.

Experiments measuring natural rates in BYL24 and Char Lake included three treatments: dark, exposure to full solar spectrum and exposure to visible light only, using *UV* filters (Lee 226, Lee Filters).

Mechanistic experiments studying the effect of chemical actors on PD over 48 h were conducted in BYL22 and Char Lake, with water amended to 5.0 ng L^{-1} (± 0.4%) of MeHg (Certified standard solution, Alfa Aesar) (Details on certified standards are provided in Supplementary Methods). Treatments included thiols (10.0 nM *GSH*, 10.0 nM thioglycolic acid (*TA*); >98%, MP Biomedical and Sigma-Aldrich) and chlorides (0.6 M NaCl; Certified ACS, Fisher Scientific). Thiol levels were chosen to significantly increase ambient concentrations, while staying in the range of concentrations measured in North American freshwaters (Hu et al. 2006; Labonté-David 2012; Leclerc et al. 2015; Liem-Nguyen et al. 2015; Zhang et al. 2004). Chloride concentration was selected to represent marine conditions, to investigate the wide difference in PD reported for fresh and saltwater (Black et al. 2012; Zhang and Hsu-Kim 2010), and to assess the potential role of marine intrusion into coastal Arctic ponds (Arp et al. 2010). This experiment was repeated in the laboratory with BYL22 and MilliQ water using a solar simulator (Suntest CPS+, Atlas Material Testing Technology). Additional experiments were conducted on waters from a temperate Canadian Shield lake (Perron et al. 2014) (45°59'35" N**;** 74°00'28" W), with amendments of up to 1000 nM *GSH*. To insure comparability between field and laboratory experiments, the solar simulator was programmed to deliver a total dose (E m^{-2}) equal to that received during field incubations (see SI for detailed explanations).

Finally, to assess the potential impact of PD on the MeHg budget of a small aquatic ecosystem, we sampled BYL22 every 6 h (surface and 30 cm depth) over a 14-day period for total Hg and MeHg and water chemistry parameters. During days 4-8, the pond was covered

with a clean opaque plastic tarpaulin anchored into the surrounding permafrost. Pump and sensor tubing were permanently installed at the desired depths to limit disturbances and light penetration associated with sampling. Detailed methods including ambient light and temperature conditions are described in SI.

MeHg photodemethylation rates

PD rates (k_{PD}) were obtained by modeling the evolution of MeHg concentrations against cumulative *PAR* with apparent first-order reaction kinetics:

$$\ln([\mathrm{MeHg}]_t) = \ln([\mathrm{MeHg}]_0) - k_{PD} PAR_t \quad (1)$$

where $(\mathrm{MeHg})_0$ is the initial MeHg concentration (ng L^{-1}), $(\mathrm{MeHg})_t$ is the MeHg concentration (ng L^{-1}) at time *t*, PAR_t is the cumulative *PAR* (E m^{-2}) received at time *t*, and k_{PD} is the PD apparent rate constant (m^2 E^{-1}).

MeHg concentrations also exhibited apparent first-order reaction kinetics as a function of incubation time. However, *PAR* was a better predictor than exposure time, and allowed for comparisons between sites and years. When *PAR* measurements were not available, k_{PD} values were calculated over incubation time.

As Teflon attenuates a small portion of solar radiation, all rates were adjusted to correct the attenuation of light by the bottles' material (Amyot et al. 1997).

Analytical methods

Hg concentrations were quantified using cold-vapor fluorescence spectrometry (CVAFS) (Tekran 2600, Tekran Instruments Corporation), following U.S. EPA method 1631 (detection limit of 0.04 ng L^{-1}). MeHg was measured by gas chromatography and CVAFS (Tekran 2500 and 2700, Tekran Instruments Corporation), according to U.S. EPA method 1630 (detection limits of 0.02 ng L^{-1} and 0.0004 ng L^{-1}, respectively). Detection limits were defined as three times the standard deviation calculated on 10 ultrapure MilliQ blanks. A field detection

limit was also calculated as three times the standard deviation of dark controls. Detailed analytical methods are described in Perron et al. 2014. Hg analyses met the criteria of the Canadian Association for Laboratory Accreditation (CALA) inter-calibration. *DOC*, ion and thiol analyses are described in SI. Speciation calculations were conducted with the Windermere Humic Aqueous Model (WHAM7 - http://www.ceh.ac.uk/services/windermere-humic-aqueous-model-wham) (Tipping et al. 2011).

Statistical analyses

Statistical analyses ($\alpha = 0.05$) were done with R software (R Development Core Team). Linear regressions were used to model changes in MeHg concentrations and to calculate k_{PD}. Only slopes with a significant coefficient were considered and are referred to as significant k_{PDs}. Data points presented are from duplicate (dark controls) or triplicate bottles (treatment series), while error bars show standard deviation. Slopes were compared using analysis of covariance (*ANCOVA*) with Bonferroni's correction where multiple comparisons were made. Analyses of variance (*ANOVA*) paired with Tukey's test and Kruskal-Wallis non-parametric analyses were used to compare conditions before and after manipulations in the covered pond experiment. Means were compared with Student's t-test.

Results and discussion

Photodemethylation in BYL ponds and Char Lake

In all field experiments of Arctic waters, filtered samples incubated in sunlight showed decreasing MeHg concentrations, while MeHg levels in samples kept in the dark remained stable. While phototrophs have been identified as being able to promote MeHg production (Grégoire and Poulain, 2014), our samples were filtered to remove bacterial cells and particulate matter. We therefore conclude that MeHg losses observed in our incubation experiments are attributable to abiotic PD (Figures 1, 2 and 3).

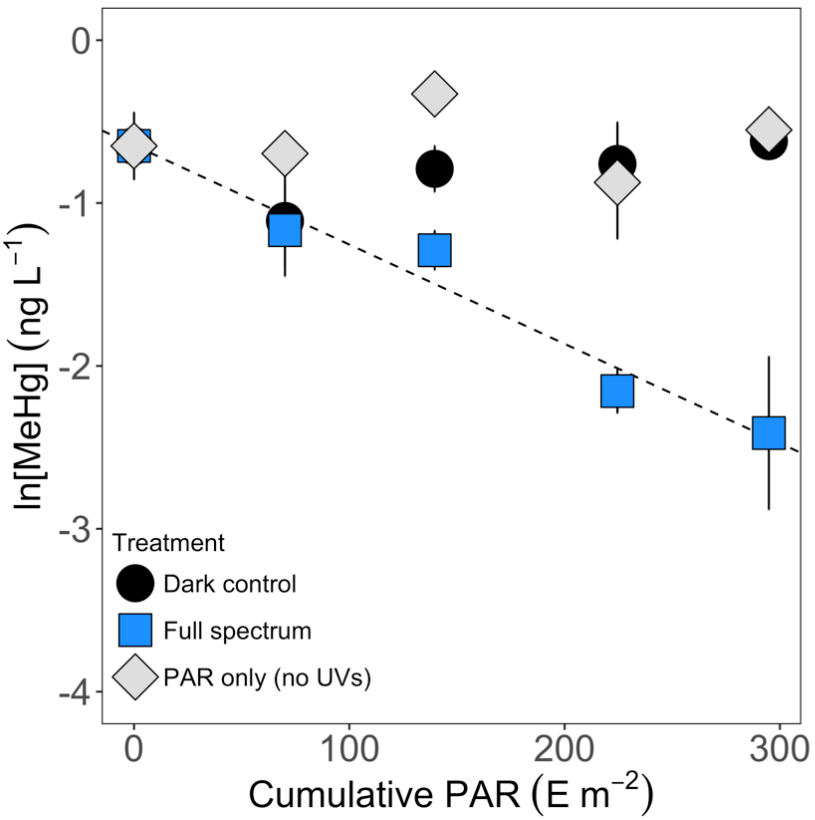

FIGURE 1. Natural logarithm of MeHg concentrations (ng L^{-1}) plotted against cumulative *PAR* (m^2 E^{-1}) during field experiments in BYL24 on Bylot Island. Filtered samples that were exposed to the full solar spectrum produced a k_{PD} of 6.1 ± 0.7 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 95.5%); dark controls and filtered samples covered by a *UV* filter yielded no significant relationship (not shown).

In BYL24, PD only occurred in the presence of *UVs* (k_{PD} of 6.1 ± 0.7 × 10^{-3} m^2 E^{-1}) (Figure 1). This supports findings of other authors, identifying *UVs* as the main waveband responsible for PD (Black et al. 2012; Fernández-Gómez et al. 2013; Lehnherr and St Louis 2009; Lehnherr et al. 2012; Li et al. 2010). Field experiments lasting 48 hours conducted in BYL22 with 5.0 ng L^{-1} MeHg amendments yielded a k_{PD} of 9.3 ± 1.5 × 10^{-3} m^2 E^{-1} (Figure 2A). This resulted in the loss of 56.6% of initial MeHg (Figure 2A).

During the 9-day incubation experiment in low-*DOC* Char Lake, ambient MeHg levels were very low, and sometimes below the field detection limit (SI, Figure S2): no k_{PD} could

therefore be calculated. In spiked Char Lake water (5.0 ng L^{-1} MeHg), no significant k_{PD} was found after 48 hours (p-value > 0.05) (Figure 2B). However, final MeHg concentrations differed from the initial MeHg value and from the dark control (p-value < 0.05), and 16.4% of initial MeHg was lost over the course of the 9-day incubation: this is explained by the significant slope found in the first 12 hours of the experiment, (k_{PD} of 19.3 ± 5.9 × 10^{-3} m^2 E^{-1}) (Figure 2B inset). This underscores the importance of assessing early photo-mediated reactions in long-term incubations.

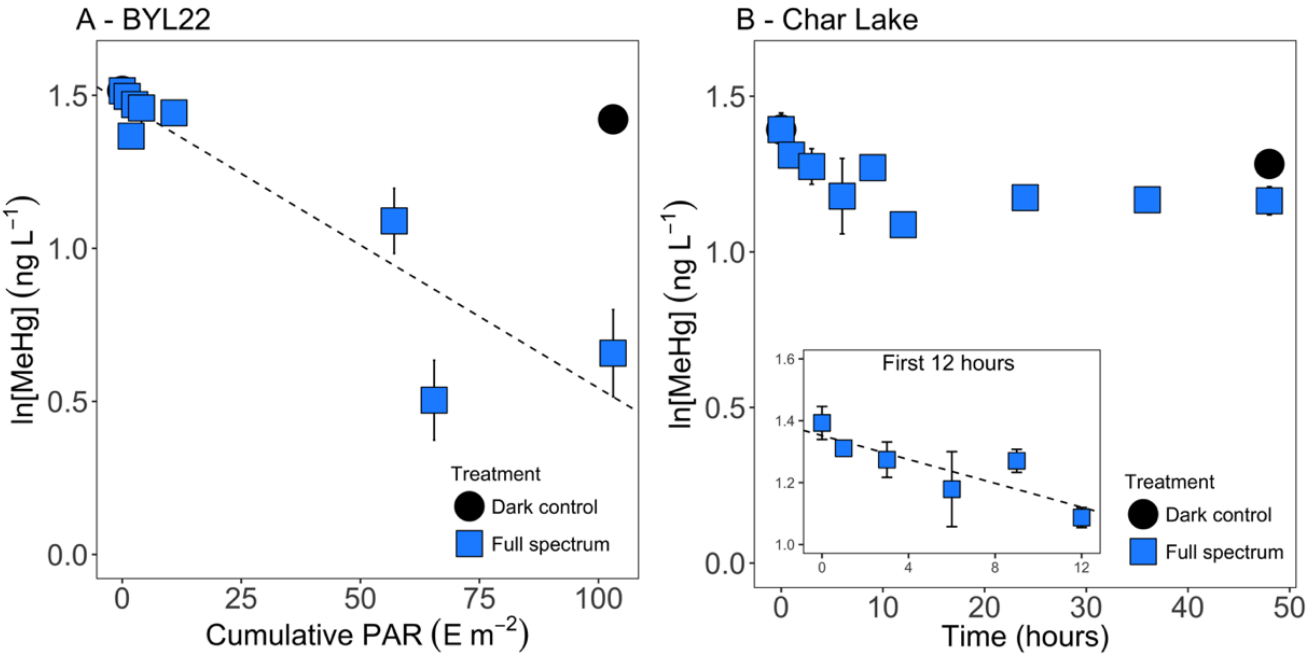

FIGURE 2. Natural logarithm of MeHg concentrations (ng L^{-1}) plotted against cumulative *PAR* (m^2 E^{-1}) during field experiments. A. In BYL22, MeHg amended samples that were exposed to the full solar spectrum produced a k_{PD} of 9.3 ± 1.5 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 82.0%). B. In Char Lake, no significant relationships were observed in any of the treatment series over 48 hours (regression p-values > 0.05). Inset: during the first 12 hours of incubation, samples exposed to the full solar spectrum yielded a k_{PD} of 19.3 ± 5.9 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 66.0%).

MeHg levels at both sites differed by one order of magnitude (SI, Table S1). Since PD has been found to be independent of MeHg concentrations (Black et al. 2012; Lehnherr and St

Louis 2009), differences in k_{PD} may be due to another contrasting characteristic of the BYL ponds and Char Lake, such as their *DOC* concentrations (respectively 6.20 to 9.60 and 0.94 mg L^{-1}) (SI, Table S1). We investigated if this contrast in *DOC* levels resulted in differences in MeHg speciation, using the thermodynamic model WHAM (Tipping et al. 2011). MeHg speciation was similar in both lakes with 75% of MeHg bound to fulvic acids in Char Lake, compared to 89% in BYL22. The potential effect of *DOC* on PD is therefore likely not achieved via differences in MeHg-*DOC* complexation in these two systems, but rather by other roles of *DOC* (e.g. generation of *ROS*, see below). It is also possible that differences in *DOC* quality may modify its complexation properties with an impact on PD (Chandan et al. 2015; Qian et al. 2014), as has been shown in the photo-production of another Hg species, dissolved gaseous Hg (Garcia et al. 2005). Information on changes in *DOC* fluorescence can be used as a proxy for *ROS* production (Garcia et al. 2005), and would provide valuable insight into *DOC*-mediated PD in these sites. Furthermore, allochtonous carbon from terrestrial surroundings, which has increased aromaticity and photoreactivity, has been found to increase PD efficiency in boreal lakes in Nova Scotia (Klapstein et al. 2016). Information on *DOC* quality (via specific ultraviolet absorbance, $SUVA_{254}$) and provenance could thus better our understanding of its relationship to MeHg and PD, especially in thaw ponds which receive high levels of allochtonous carbon from run-off and permafrost melt (Roiha et al. 2015).

Char Lake can be compared to another Arctic lake (Toolik Lake), which has a similar near-neutral pH and comparable MeHg concentrations in surface waters (respectively 0.02 ng L^{-1} and 0.05 ng L^{-1})[7] (SI, Table S1 and S2). However, *DOC* concentrations in Char Lake (0.94 mg L^{-1}) are lower than those measured in Toolik Lake (4.44 mg L^{-1}) (Hammerschmidt and Fitzgerald 2006). While no PD was measured in unspiked waters of Char Lake over 9 days, it has been observed in Toolik Lake over 6 day-incubations (2.6×10^{-3} m^2 E^{-1}) (Hammerschmidt and Fitzgerald 2006). This further points to organic matter as an important driver of PD, as has been reported by other authors (Black et al. 2012; Fernandez-Gomez et al. 2015; Zhang and Hsu-Kim 2010), and suggests that *DOC* could explain the discrepancy between PD rates in Char Lake and the BYL ponds.

Overall, these results clearly indicate that thermokarst thaw ponds are sites of PD whereas no significant PD could be measured in unspiked waters of the oligotrophic Char Lake.

To our knowledge, the results from the polar lake are the first instance of a freshwater system where PD is so limited, therefore providing boundaries to the presumed universal importance of this process.

Thiols and photodemethylation

Thiols can be found in natural waters in their low molecular weight (*LMW*) form or as a functional group of organic matter (Ravichandran 2004), and have previously been identified as promoters of PD in simulated waters (Zhang and Hsu-Kim 2010). In their *LMW* form, we measured at the study sites *GSH* and *TA* concentrations of 0.2 ± 0.1 nM and of 5.1 ± 1.6 nM, respectively. As functional groups of organic matter (assuming 7.3 x 10^{-5} moles reduced–S per gram of C) (Waples et al. 2005), thiols ranged from 70 nM (Char Lake) to 450 nM (BYL22), largely exceeding MeHg concentrations, as they do in most pristine environments (Ravichandran 2004). To determine if the action of *DOC* in mediating PD was limited by the concentration of thiol binding sites, incubation experiments were conducted in the field and in a solar simulator with thiol additions in the environmentally-relevant range of 10 to 1000 nM.

In an experiment conducted in natural sunlight in spiked BYL22 water (5.0 ng L^{-1} MeHg), 10 nM *TA* amendments (which doubled natural dissolved *TA* concentrations) slowed k_{PD} by 33.3%, from 9.3 ± 1.5 × 10^{-3} m^2 E^{-1} in the control series to 6.2 ± 1.0 × 10^{-3} m^2 E^{-1} (Figure 3A) (p-value < 0.05).

In field experiments in Char Lake, no significant relationship was observed between time and MeHg concentration (p-value > 0.05), and 10 nM *GSH* amendments (increasing natural dissolved *GSH* concentrations by 50-fold) did not induce PD (Figure 3B). However, when considering only the first 12 hours of the experiment (Figure 3B Inset), the control and *GSH* series both yielded significant k_{PD} (respectively 19.3 ± 5.9 × 10^{-3} m^2 E^{-1} and 19.3 ± 4.0 m^2 E^{-1}), that did not however differ from one another (p-value > 0.01).

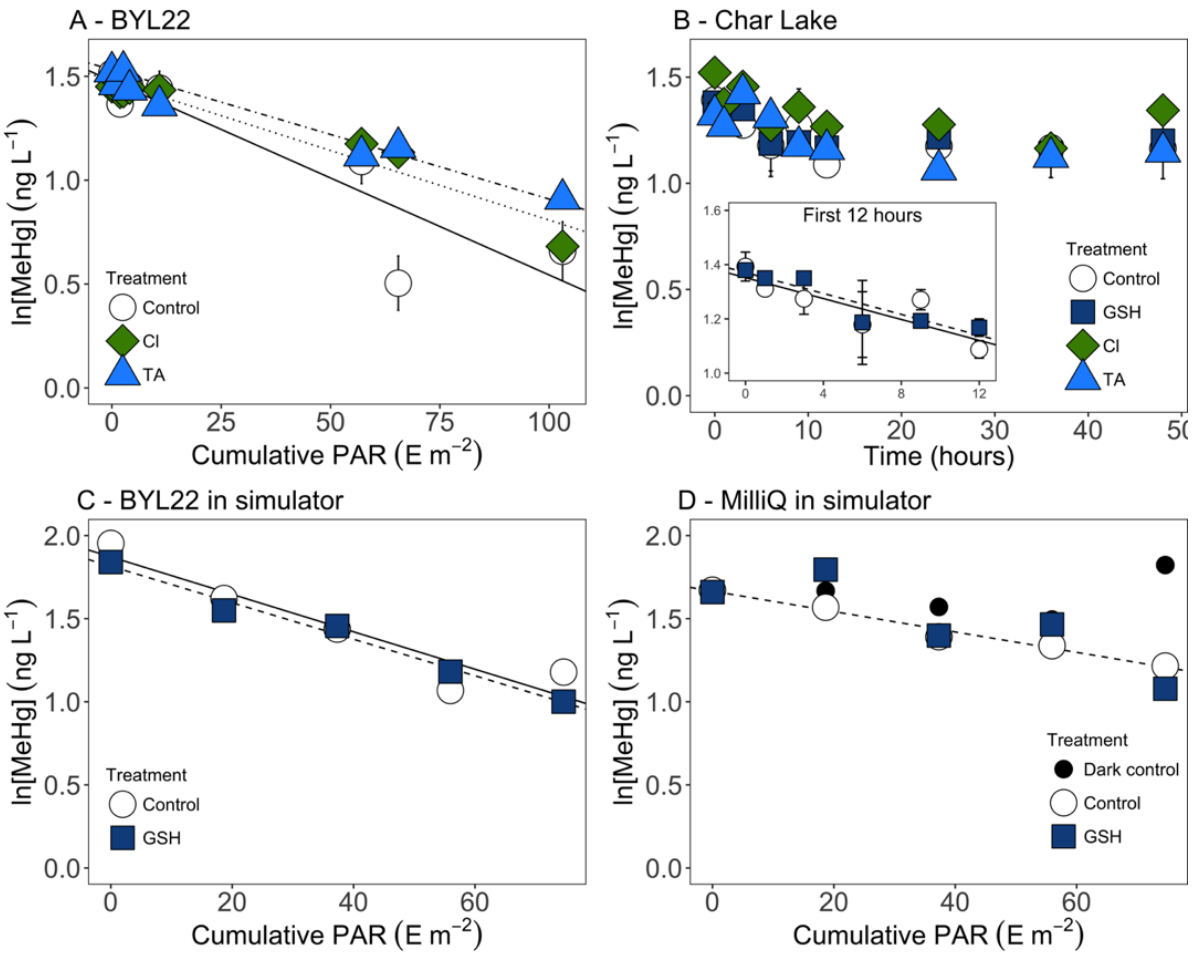

FIGURE 3. Natural logarithm of MeHg concentrations (ng L^{-1}) plotted against cumulative *PAR* (m^2 E^{-1}) received or incubation time (hours) in the field. A. In BYL22, the k_{PD} in spiked natural water was 9.3 ± 1.5 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 82.0%); in the Cl series, k_{PD} was 6.7 ± 0.6 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 93.5%); in the *TA* series, k_{PD} was 6.2 ± 1.0 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 82.1%). k_{PD} did not differ significantly from control rate (p-value > 0.05). B. In Char Lake, no significant relationships were observed in any of the treatment series over 48 hours (regression p-values > 0.05). Inset: first 12 hours of incubation, during which the control series yielded a k_{PD} of 19.3 ± 5.9 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 66.0%), while samples amended with *GSH* produced a k_{PD} of 19.3 ± 4.0 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 82.1%). No significant relationship was observed in the Cl and *TA* treatment series. C. In BYL22 water incubated in a solar simulator, the k_{PD} was 11.3 ± 2.4 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 84.0%); in the *GSH* series, k_{PD} was 11.0 ± 0.8 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 97.7%). *GSH* treatment did not differ from control rate (p-value > 0.05). D. In spiked MilliQ water incubated in a solar simulator, the k_{PD} was 6.1 ± 0.5 × 10^{-3} m^2 E^{-1} (p-value < 0.05, R^2 adjusted = 97.3%); and in the *GSH* series, no significant relationship was observed (not shown).

In incubations in a solar simulator with BYL22 water, *GSH* amendments had no significant effect on PD when compared to control rates (*GSH* k_{PD}: 11.0 ± 0.8 × 10^{-3} m^2 E^{-1}; control k_{PD}: 11.3 ± 2.4 × 10^{-3} m^2 E^{-1}) (p-value > 0.01 with Bonferroni's correction) (Figure 3C). Likewise, *GSH* had no impact in spiked MilliQ water in simulated light conditions (Figure 3D). Dark controls remained stable during incubations (Figure 3D). Both in BYL22 and Char Lake, thiols therefore had diverging effects on PD, with *TA* reducing k_{PD} and *GSH* having no impact.

Supplementary PD experiments conducted in water from a temperate lake showed that adding 10 nM *GSH* to this natural matrix lead to a k_{PD} similar to that of the control series, confirming results from BYL22 in simulated light (SI, Figure S5). Increasing *GSH* concentrations to 100 nM and 1000 nM increasingly slowed PD until it did not differ from dark controls (SI, Figure S5). Previous work has found that PD of MeHg bound to non-aromatic thiols (such as *GSH*) is slower than that of MeHg bound to aromatic thiols or *DOC* (Qian et al. 2014). Furthermore, in a study using simulated freshwater, PD was fastest when MeHg was bound to *DOC*, slowest for MeHg-thiol complexes only, and intermediate when both *DOC* and thiols were present (Jeremiason et al. 2015). These studies suggested that PD is possible through an intramolecular mechanism, where light is absorbed by *DOC* and energy is then transferred to break the Hg-C bond (Jeremiason et al. 2015; Qian et al. 2014). Adding thiols could thus remove some or all of the MeHg bound to *DOC*, preventing this intra-*DOC* process from occurring and thus slowing PD (Jeremiason et al. 2015). This could explain why increasing concentrations of *GSH* slowed and eventually halted PD in temperate waters (SI, Figure S5).

It is also important to consider *GSH* degradation during incubation experiments. In temperate lake water, *GSH* was also simultaneously degraded during exposure to light (approximately 30% after 5 hours) (SI, Figure S6), as has been previously reported (Moingt et al. 2010). *GSH* degradation can depend on the matrix and on other ions present. Indeed, some metals such as copper may affect the oxidation states of *GSH* and potentially lead to other degradation products (Moingt et al. 2010; Zhang and Hsu-Kim 2010). In BYL ponds, *GSH* may have been partly degraded before being able to fully intervene in PD.

Overall, thiol addition did not promote PD in different natural waters in these experiments. These results are in general agreement with the study of Tai et al. performed in the Florida Everglades (Tai et al. 2014).

Photodemethylation mediated by ROS radicals

Although they have highly contrasting *DOC* levels, WHAM simulations showed that Char Lake and BYL22 have similar *DOC* speciation, suggesting that MeHg-*DOC* complexation is not the cause of the difference observed in k_{PDs}. Furthermore, in both sites, thiol concentrations largely exceed those of MeHg, and while thiols complex MeHg efficiently and are likely implicated in photochemical reactions of Hg, MeHg-thiol complexation does not explain the difference in PD potential seen here. As *DOC* is consistently found to have an important role in mediating PD (Black et al. 2012; Fernandez-Gomez et al. 2015; Zhang and Hsu-Kim 2010), and is the main difference between Char and other Arctic lakes and ponds where PD was observed (Toolik Lake, BYL ponds), we propose that the discrepancy in observed k_{PDs} may be due to other roles of organic matter, such as the photoproduction of *ROS* radicals in the photic zone (Kieber et al. 2003; Zepp 1991). *ROS* are suspected of mediating PD, as quenchers for these molecules have been found to limit PD in sea water (Suda et al. 1993), in simulated waters (Chen et al. 2003; Zhang and Hsu-Kim 2010) and in an Arctic oligotrophic lake (Hammerschmidt and Fitzgerald 2010).

In a study conducted on lakes of a wide array of limnological characteristics, Char Lake was found to have the lowest formation rate of hydrogen peroxide (6.95 mg m^{-2} d^{-1}), another *DOC*-produced *ROS* that closely correlated to *DOC* concentrations (Scully et al. 1995). If Char Lake is equally poor in other *ROS*, it may be a non-conducive environment for PD. Early PD (Figure 2B inset) may have occurred before the rapid depletion of naturally-occurring *ROS* in experimental bottles.

We hypothesize that PD in Char Lake may be partially controlled by *ROS* and organic radicals produced during *DOC* photolysis (Fernández-Gómez et al. 2013). This supports observations by other authors that *DOC* may act as a catalyzer for PD, more than simply a light attenuator or complexation agent (Black et al. 2012), and the role of *ROS* should be further investigated. As no experiments in the present study measured the role of *ROS* on PD, we can only speculate on their potential role. Further field-based experiments are necessary to better understand the role of photo-produced *ROS* from *DOC* in natural waters.

Relationship between DOC and k_{PD}

A comparison of Arctic PD studies shows that up until a threshold, *DOC* exhibits a positive (yet not significant) relation with k_{PD} (Figure 4A), with polar desert Char Lake representing the lower limit of this relationship. When non-Arctic studies are included, no significant relationship is found, but the same general trend is observed (Figure 4B). There may be a breakpoint after which *DOC* inhibits PD. We propose that at lower *DOC* levels, PD is limited by *ROS* concentrations, while at *DOC* levels above this threshold, complexation or UV attenuation may be more important. Other studies on Hg and *DOC* in aquatic ecosystems have found comparable thresholds for Hg bioaccumulation/bioavailability (Driscoll et al. 1994; French et al. 2014) and for production of dissolved gaseous Hg (Garcia et al. 2005). A similar relationship may exist between *DOC* and PD. This observation is based on few studies and may be due to differences in experimental designs, and requires further investigation. More aquatic ecosystems should be sampled across a wider range of *DOC* values to assess this potential breaking point.

Another hypothesis for explaining the relationship between PD and *DOC* may be in competition of photoreactive processes. A recent study performed over multiple seasons in MeHg hotspots in Nova Scotia showed that k_{PD} were negatively correlated with *DOM*, on a range from 3.9 – 16.4 mg C L^{-1} (Klapstein et al. 2018). These authors attributed the inhibition of PD at high *DOM* levels to competition between photoreactions involving carbon bound to MeHg, and *DOM* that was not bound to it. At high *DOM* concentrations, *DOM* not bound to MeHg may dominate the absorbance of photos in the water column, and processes like photobleaching and photomineralization of C may be more prevalent than PD (Klapstein et al. 2018). This may explain the negative relationship we observe at higher *DOC* concentrations.

The low PD rates recorded in low-*DOC* Char Lake question the expectation of PD being the main degradation pathway for MeHg in low-*DOC* oligotrophic ecosystems with good light penetration (Hammerschmidt and Fitzgerald 2006; 2010; Lehnherr and St Louis 2009). Oligotrophic Arctic lakes like Char Lake are typically characterized by low *DOC* levels (Sobek et al. 2007), and permafrost-influenced regions hold the greatest number of lakes in the northern hemisphere (Smith et al. 2007). If climate change alters *DOC* input into these ecosystems, this could change PD potential and MeHg mass balances in Arctic aquatic ecosystems.

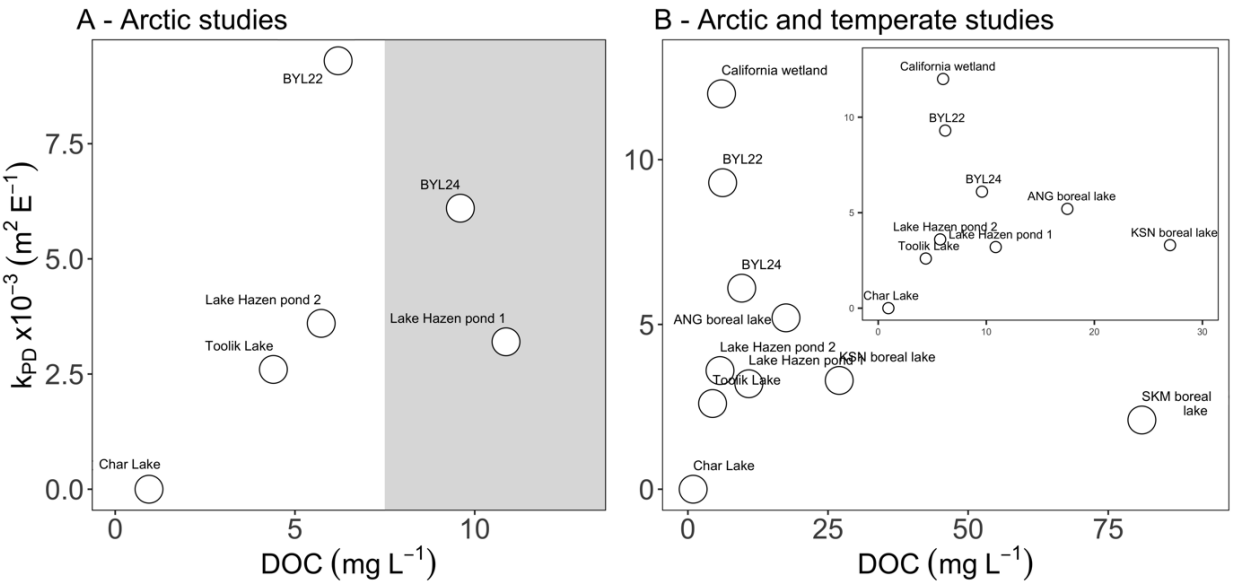

FIGURE 4. A. Relationship between k_{PD} (m^2 E^{-1}) and *DOC* (mg L^{-1}) from PD studies conducted in the Arctic only. Shaded area represents values above the potential threshold value. B. Relationship between k_{PD} (m^2 E^{-1}) and *DOC* (mg L^{-1}) from Arctic and temperate PD studies. Inset: *DOC* concentrations below 30 mg L^{-1}).

Photodemethylation mediated by chlorine

Previous authors have observed a negative relationship between PD rate and Cl concentrations (Black et al. 2012; Sun et al. 2013), and the complexation of MeHg to chloride ions has been used to explain lower k_{PD} values observed in the marine environment (Black et al. 2012; Kim et al. 2014; Whalin et al. 2007; Zhang and Hsu-Kim 2010). We therefore tested the effect of chlorides in spiked (5.0 ng L^{-1} MeHg) BYL22 water, which yielded a k_{PD} of 6.7 ± 0.6 × 10^{-3} m^2 E^{-1} (p-value < 0.05), which did not differ significantly from the control rate (p-value > 0.008 with Bonferroni's correction) (Figure 3A). In Char Lake, no PD was observed in control and Cl treatment series (Figure 3B).

At high Cl concentrations, the main species of MeHg is thought to be CH_3HgCl, with the strength of the Hg-Cl bond preventing MeHg decomposition (Sun et al. 2013). Indeed, in our field experiments, adding 0.6 M of NaCl led to a shift in MeHg speciation from the *DOC*-bound pool to the CH_3HgCl pool (with the *DOC*-bound pool decreasing from 89 to 8% of the

total in BYL22 and 75% to 29% in Char, according to calculations done with WHAM7). Therefore, adding Cl altered MeHg complexation, but did not slow PD. Chlorides have also been reported to accelerate PD by producing chlorine radicals capable of attacking the Hg-C bond. However, other authors have suggested that increasing salinity may also quench *ROS*, slowing PD (Black et al. 2012). These competing processes involving chlorides may explain why adding Cl did not impact PD in these experiments.

Ecosystem-scale observations of photodemethylation

To assess the impact of PD at the ecosystem level, BYL22 was monitored for 14 days, with a plastic tarpaulin covering the pond during Days 4-8. This blocked 98.7% of radiation from 290-895 nm, and 100% of radiation in the *UV* spectrum (400 nm $\geq \lambda$) (SI, Figure S7). On Day 7, gusts of wind ripped off the cover, exposing the pond for approximately three hours. Light exposure was minimal, as this occurred shortly after midnight, when the sun is at its lowest. This is nonetheless reflected in measurements taken at 6:00 a.m. on Day 7 (see dotted line, Figure 5).

Based on the rate previously measured in BYL22, PD was expected to remove up to 85% of MeHg at the surface during the 5 days following re-exposure to sunlight (Days 9-13) (See SI). However, MeHg concentrations did not decrease from Days 9-13: indeed, MeHg levels were higher on Day 13 than on Day 9 both in surface waters (Day 9: 0.14 ± 0.01 ng L-1; Day 13: 0.23 ± 0.02 ng L^{-1}) and at 30 cm (Day 9: 0.13 ± 0.03 ng L^{-1}; Day 13: 0.24 ± 0.03 ng L^{-1}) (*p*-value < 0.05). Free *LMW* thiol concentrations on the other hand decreased sharply following re-exposure of the pond to sunlight (from 16.56 to 6.85 nM over 18 h), suggesting a massive thiol photodegradation event (Figure 5C), with a slight lag potentially due to the persistence of these molecules in freshwater that ranges from hours to days (Moingt et al. 2010). This thiol degradation could have been caused by *ROS* produced from irradiated organic matter (Moingt et al. 2010), which could have contributed to PD. However, no decrease of MeHg was observed after surface waters were re-exposed. Over the course of the experiment, MeHg levels were higher after re-exposure to sunlight than when the experiment began, both at the surface (mean before cover: 0.16 ± 0.03 ng L^{-1}; mean after re-exposure: 0.22 ± 0.06 ng L^{-1}) and at 30 cm (0.15

± 0.04 ng L^{-1}; 0.21 ± 0.04 ng L^{-1}) (*p*-value < 0.05; Figure 5A). While temperatures remained stable during the covered period (9.33 ± 1.32 °C at the surface and 8.43 ± 1.54 °C at 30 cm) (Figure 5D), a chemical stratification was induced, which is exceptional for this type of shallow, normally well-mixed site (Figure 5E-G). Notably, near-hypoxic conditions developed at the water-sediment interface less than 24 hours into the dark treatment (3.68 ± 2.64 mg L^{-1}), while surface levels remained stable (9.07 ± 1.03 mg L^{-1}) (Figure 5E). This may have improved conditions for microbial methylation of inorganic Hg, leading to increased MeHg production during the covered period. Lack of mixing and the flocky nature of the sediments would likely have sequestered newly produced MeHg for some time, which became detectable only once it had diffused to 30 cm below the surface after the cover was removed (Figure 5A). The increase in MeHg following re-exposure of surface waters to sunlight may also be attributable to phototrophic blooms, which can create environments that can facilitate methylation of Hg (Grégoire and Poulain 2014). Therefore, if PD occurred, it may have been obscured by *in situ* MeHg production, resulting in a 43% net increase in MeHg concentrations (Figure 5A).

PD is frequently cited as a major degradation pathway of MeHg (Black et al. 2012; Hammerschmidt and Fitzgerald 2006; Lehnherr and St Louis 2009; Lehnherr et al. 2012; Sellers et al. 2001) and is often included in mass Hg budgets for aquatic ecosystems, where rates are extrapolated from bottle experiments to whole-lake levels. Following the observation of PD in BYL22 via bottle incubations, covering the entire pond with a tarpaulin was expected to have a significant impact on MeHg concentrations. Indeed, MeHg levels were expected to rise during the 4-day covered period, and to decrease following re-exposure to sunlight due to PD. However, no net decreases of MeHg were observed in the manipulated pond (Figure 5A).In lakes, substantial PD can occur in the photic zone before UV attenuation, making it a major pathway for MeHg degradation (Hammerschmidt and Fitzgerald 2006; Sellers et al. 2001). In contrast, in very shallow aquatic ecosystems such as the BYL ponds, UV radiation likely penetrates at least partially to the bottom of the short water column, allowing for interactions with bottom processes such as abiotic (Hammerschmidt and Fitzgerald 2001) and microbial methylation and demethylation (Pak and Bartha 1998). These overlapping sediment and photo-reactive processes could potentially blur the signal for PD, explaining why no PD was observed in this covered pond experiment.

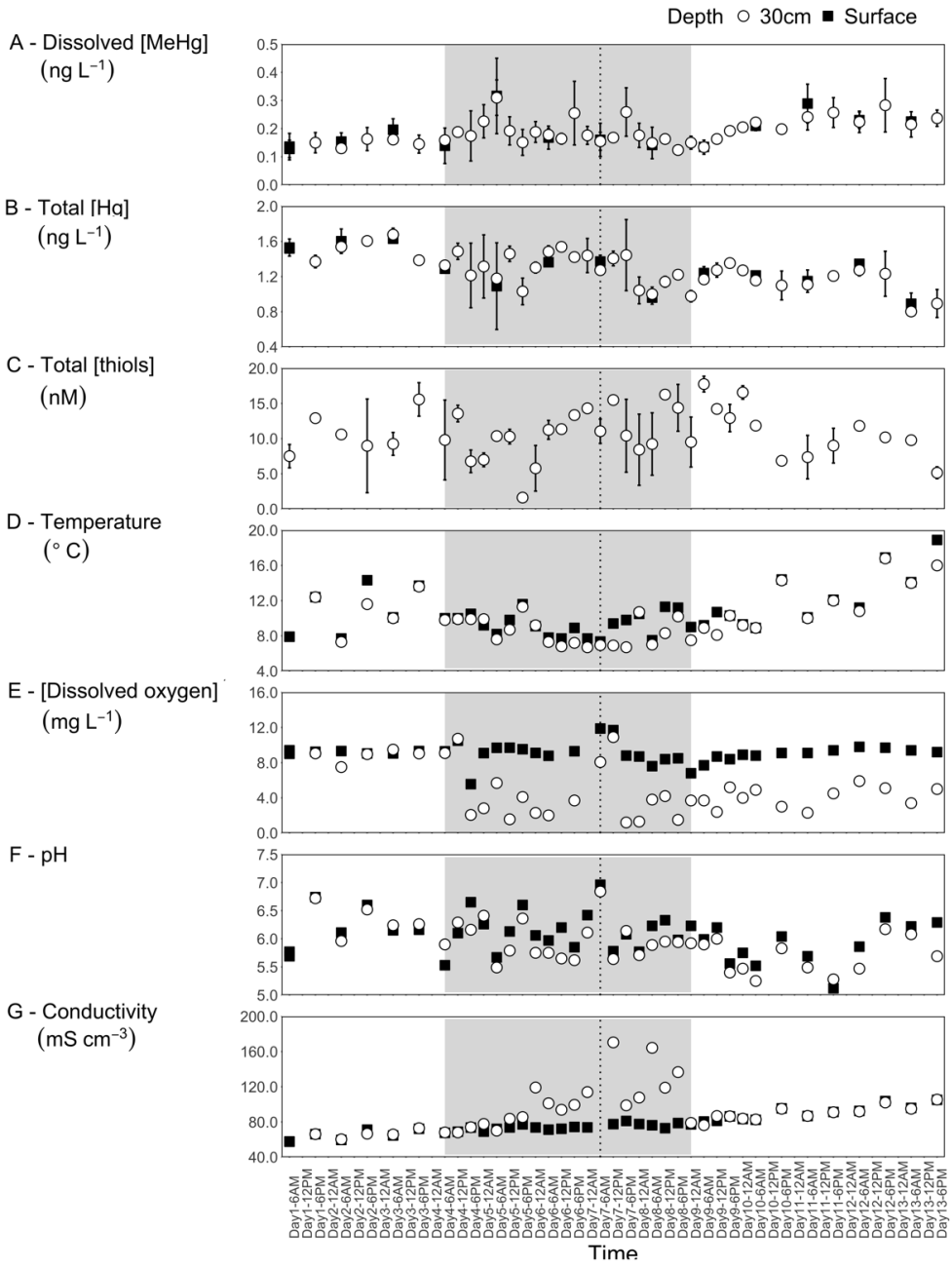

FIGURE 5. Monitoring results of BYL22 during the covered pond experiment, at the surface and at a 30 cm depth. Grayed section represents the period during which the pond was covered. Dotted line represents momentarily exposed pond surface to light shortly after midnight on Day 7. A. MeHg concentrations (ng L^{-1}) over time. B. Total Hg concentrations (ng L^{-1}) over time. C. Total thiol concentrations (nM) over time (sum of glutathione, cysteine, thioglycolic acid, 3-mercaptopropionic acid, cysteine-glycine). D. Temperature (°C) over time. E. Dissolved oxygen (mg L^{-1}) over time. F. pH over time. G. Conductivity (μS cm^{-3}) over time.

This interaction is of particular importance in the Arctic, where shallow thermokarst thaw ponds are the most abundant aquatic ecosystem (Pienitz et al. 2008). Studies on isotopic fractionation could help in better untangling sediment and surface processes, as they have previously been used to investigate PD (Chandan et al. 2015) and Hg photoreduction (Bergquist and Blum 2007).

While the design of this experiment could be improved upon, it constitutes a novel approach to study PD in shallow systems such as thermokarst thaw ponds, and emphasizes the importance of competing processes in the ecosystem. Alongside Lehnherr et al.'s extensive study of ponds in the Lake Hazen area (Nunavut) (Lehnherr et al. 2012), this represents one of the first studies on Hg PD in a northern system where surface and bottom processes are coupled. While bottle experiments provide essential insight into mechanistic reactions involved in PD, caution must thus be used when applying incubation rates to mass balance budgets across a range of aquatic ecosystems. The bottle effect may mask other reactions involved in the aquatic cycle of Hg, leading to an overestimation of the overall net impact of PD. Future PD experiments involving the manipulation of a pond should include full characterization of the *DOC* pool, tracking of microbial metabolism by molecular approaches and with gas fluxes, as well as replicate ponds for statistical power.

These results are important since they suggest that thermokarst thaw ponds recently shown to display high MeHg levels (Annex I, MacMillan et al. 2015) are unlikely to see their MeHg pool decrease significantly via PD. It is likely that, because of the shallow nature of these ponds, processes occurring in the surface waters are directly affected and in competition with processes occurring at the sediment/water interface. These ponds may therefore be sources of MeHg for other systems, including nearby streams, lakes and coastal areas in which native communities tend to fish. Results from Char Lake indicate that PD may not be a significant process in polar desert lakes, likely because of very low *DOC* levels.

Acknowledgments

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Supplementary information

Supplementary methods

Certified standard solutions

MeHg solution used for 5.0 ng L^{-1} amendments was made from a MeHg stock solution (1000 ppm, certified by Alfa Aesar). A 1 ppm MeHg solution was prepared by dilution in methanol (Fisher Scientific, HPLC grade). Intermediate and working solutions (10.0 μg L^{-1}, 600 ng L^{-1} and 10 ng L^{-1}) were prepared in MilliQ water (18.2 MΩ cm, EMD Millipore) and preserved with 0.3% acetic acid (Fisher Scientific, ACS-pur) and 0.2% HCL (EMD, Omni-trace ultra).

Sampling methods

Sampling and experimental solution bottles were rinsed with distilled water, then soaked in a 45% HNO_3, 5% HCl (Fisher Scientific, ACS-pur) bath overnight, before being rinsed 3 times with MilliQ water (18.2 MΩ cm, EMD Millipore).

The peristaltic pump used for sampling was flushed with a 10% HCl solution (Fisher Scientific, ACS-pur) for 10 minutes, then rinsed with site water for 10 minutes.

Vertical profiles in the water column for water conductivity, pH, temperature and dissolved oxygen were measured using a YSI 650 data logger (YSI Incorporated/Xylem Incorporated). Solar radiation data was collected during experiments with Hobo photosynthetic light (*PAR*) sensors and data loggers (Onset), and light measurements were collected with a Jaz spectrometer (Ocean Optics). Light attenuation coefficients at each study site were calculated with the Beer-Lambert law:

$$I_z = I_0 e^{-kz} \quad \text{(S1)}$$

where z is a given depth in the water column (m), I_z is the intensity of light (W m^{-2}) measured at depth z (Jaz; 290-895 nm), I_o is the intensity of light at the surface (W m^{-2}) and k is the light attenuation coefficient.

Analytical methods

DOC levels were quantified with an Aurora 1030 analyzer (OI Analytical) by persulfate heat oxidation, followed by conductiometric determination of released CO_2. Anions were analyzed via ionic chromatography (Waters), while cations were measured with flame atomic absorption spectroscopy (Varian, Agilent). Total (reduced and oxidized species) thiols (glutathione, cysteine, thioglycolic acid, 3-mercaptopropionic acid, cysteine-glycine) were analyzed by derivatization using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-sulfonate (SBD-F) (Fluka Analytical, Sigma-Aldrich) and high performance liquid chromatography with a fluorescence detector, as described in Moingt et al. (Moingt et al. 2010). Thiol detection limit was defined as three times the standard deviation calculated on 10 low concentration (< 5 nM) standards. These were 1.0 nM for glutathione, 1.0 nM for cysteine, 1.1 nM for thioglycolic, 2.2 nM for 3-mercaptopropionic acid, and 0.4 nM for cysteine-glycine.

Experimental design

Photodemethylation field experiments were conducted in natural sunlight. Water was collected in clear, acid-washed Teflon bottles with no headspace. On Bylot, bottles were incubated in floating rafts approximately 0.5 cm below the surface of the sampled pond. On Cornwallis, due to weather, incubations were conducted in basins on the shore, in water changed periodically to prevent warming. In all cases, bottles were placed horizontally with their caps pointing north to insure uniform irradiation (Poulain et al. 2004). At every time-point, a triplicate of every treatment series (or duplicate for dark controls) was removed from the incubation set-up and preserved with ultrapure HCl to a 0.4% final concentration. The bottles were then stored in the dark at 4 °C until analysis.

In experiments measuring natural rates conducted in BYL24 and Char Lake, water was collected and separated into three treatment series: one control series kept in the dark, a series exposed to the full spectrum of sunlight and a series exposed to the visible spectrum only using *UV* filters (transmission of approximately 16% for wavelengths (λ) <400 nm, and 100% for $400 \leq \lambda \leq 700$ nm) (Lee 226, Lee Filters).

Mechanistic experiments aiming to study the effect of chemical actors on photodemethylation were also conducted in the field at BYL22 and Char Lake. All bottles were spiked with 5.0 ng L^{-1} (± 0.4%) of MeHg, and treatments included thiols (10 nM glutathione (GSH), 10 nM thioglycolic acid (TA)) (formation constants for complexation to MeHg at pH 7.4: GSH = 11.55, TA = 11.47) (Reid and Rabenstein 1981; 1982) and chlorides (0.6 M of Cl). Incubations lasted 50 h. This experiment was repeated in the laboratory in a Suntest CPS+ solar simulator (Atlas Material Testing Technology) using BYL22 and MilliQ water. To insure comparability between field experiments and data generated in the laboratory, Hobo photosynthetically active radiation (*PAR*) sensors (Onset) were used to collect light measurements in the field, which were compiled to establish a total irradiance dose received during field experiments. The Suntest CPS+ (Atlas Material Testing Technology) was programmed to deliver constant total irradiance during artificial incubations, with a total dose equal to that received during field incubations but over a shorter period. This required using higher intensity irradiation than what naturally occurred on the field, with a lamp producing a slightly different wavelength spectrum (notably more *UV-A* radiation than sunlight on the field, and the absence of *UV-C* radiation) (SI Table S4). No replicates were done in this experiment, due to limited space in the solar simulator.

Finally, to assess the potential impact of photodemethylation on the MeHg budget of an aquatic ecosystem, we sampled BYL22 every 6 h (surface and 30 cm depth) over a 14-day period for total Hg and MeHg and water chemistry parameters. During days 4-8, the pond was covered with a clean opaque plastic tarpaulin anchored into the surrounding permafrost. Pump and sensor tubing were permanently installed at the desired depths to limit disturbances and light penetration associated with sampling. Water was pumped continuously during 5 minutes before each sampling, to thoroughly rinse system.

Supplementary results and discussion

Predicted MeHg losses in covered pond experiment

Theoretical MeHg losses following re-exposure of pond surface to sunlight were calculated using equation 1. This accounts for predicted photodemethylation occurring during Days 9-13, using BYL22 k_{PD} (9.3×10^{-3} m^2 E^{-1}), average daily *PAR* on Bylot Island (37.76 E d^{-1}) (SI, Table S1) and MeHg concentration on Day 9 (1.35 ng L^{-1}) (Figure 5).

$$\ln([MeHg]_t) = \ln([MeHg]_0) - k_{PD}PAR_t \quad \text{(S1)}$$

$$\ln([MeHg]_{Day13}) = \ln([MeHg]_{Day9}) - k_{PD}PAR_t$$

$$\ln([MeHg]_{Day13}) = \ln(0.14 \text{ ng } L^{-1}) - (9.3 \times 10^{-3} \text{ m}^2 \text{ E}^{-1}) \times (37.76 \text{ E m}^{-2} \text{ d}^{-1} \times 5 \text{ days})$$

$$\ln([MeHg]_{Day13}) = -1.97 - 1.76$$

$$\ln([MeHg]_{Day13}) = -3.73$$

$$MeHg_{Day13} = 0.02 \text{ ng } L^{-1}$$

$$\text{Predicted \% of MeHg loss} = 1 - MeHg_{Day13}/MeHg_{Day9} \times 100$$

$$\text{Predicted \% of MeHg loss} = 1 - 0.02 \text{ ng } L^{-1}/0.14 \text{ ng } L^{-1} \times 100$$

$$\text{Predicted \% of MeHg loss} = 85.71\%$$

Note that this loss is only applicable to the surface and should be compared to the surface data in Fig. 5. We only considered the surface here, and assumed that the water was not mixing well after the removal of the cover. This assumption is based on the oxygen data in Fig. 5 that indicated an oxygen stratification from Day 4 to Day 13. We consider the 85% loss as an upper limit.

Supplementary tables

TABLEAU SI. Surface water chemistry and Hg values measured at each site in 2010, where *Lat.* is latitude, *Long.* is longitude, *Temp.* is water temperature, *Cond.* is conductivity, *DO* is dissolved oxygen, z_{max} is maximum depth, *k* is light attenuation coefficient, *PAR* is photosynthetically active radiation measured during field experiments, *air temp* is average air temperature during field experiments on Bylot (Centre d'études nordiques) and Cornwallis Islands (Weather Canada) and *DOC* is dissolved organic carbon.

Site	Bylot Island		Cornwallis Island
	BYL22	BYL24	Char Lake
Lat. (°N)	73°9'29"	73°9'26"	74°42'16"
Long. (°W)	79°58'44"	79°58'42"	94°52'56"
Type	Pond	Pond	Lake
Temp. (°C)	15.37	10.5	5.59
Cond. (µS cm^{-1})	38	50	264
DO (mg L^{-1})	25.90	12.09	10.60
pH	7.24	7.22	7.92
z_{max} (m)	1.00	0.39	27.50
k	3.42	1.37	0.84
Average daily *PAR* (E d^{-1})	37.76 ± 16.13		24.62 ± 13.04
Average air *temp.* (°C)	5.71 ± 1.11		4.93 ± 1.70
[Hg_{tot}] (ng L^{-1})	2.38 ± 0.02	2.97 ± 0.06	0.44 ± 0.11
[MeHg] (ng L^{-1})	0.35 ± 0.04	0.70 ± 0.05	0.02 ± 0.004
DOC (mg L^{-1})	6.20	9.60	0.94
Cl (mg L^{-1})	2.88	3.99	21.54
NO_3 (mg L^{-1})	0.12	0.09	<0.62
NO_2 (mg L^{-1})	<0.004	<0.004	-
SO_4 (mg L^{-1})	1.81	1.62	26.35
F (mg L^{-1})	0.12	0.14	-
Ca (mg L^{-1})	-	-	33.59
Mg (mg L^{-1})	-	-	5.30
Na (mg L^{-1})	-	-	10.76
K (mg L^{-1})	-	-	1.07

TABLEAU SII. Comparison of photodemethylation first-order rates (k_{PD}) measured in recent studies against photosynthetically active radiation (*PAR*). Surface measurements in natural freshwater only. *Average over 3 years.

Study	Site	k_{PD} (m^2 E^{-1}) × 10^{-3} from *PAR*	*DOC* (mg L^{-1})	pH
Present study	Thaw ponds on Bylot Island, Nunavut	6.0 – 9.2	0.94 – 9.60	7.22 – 7.92
Black et al. 2012	Coastal wetlands near San Francisco, California	9.9 ± 2.0 (6.0 – 15.0)	6	6.50
Fernández-Gómez et al. 2013	Boreal lakes north of Umeå, Sweden	2.3 ± 0.2	17.50 – 81.00	3.80 – 6.60
Lehnherr and St Louis 2009	Lake 979 at the Experiment Lakes Area, Ontario	4.4	12.80	N/A
Lehnherr et al. 2012	Wetland ponds near Lake Hazen, Nunavut	3.2 – 3.6	5.73 – 10.87*	8.50 – 8.75
Hammerschmidt and Fitzgerald 2006	Toolik Lake, Alaska	2.6	4.40	7.60

TABLEAU SIII. All rates obtained in this study. k_{PDs} (m^2 E^{-1}) are presented with standard error (*St. err*), R^2 adjusted (R^2 *adj.*) and slope *p*-value (k_{PD} *p-value)*. For significant slopes, Bonferroni-corrected *ANCOVA* results are presented to identify within-experiment differences between treatment slopes in the *test* column.

Site	Treatment	k_{PD} $\times 10^{-3}$ m^2 E^{-1}	St.err x 10^{-3} m^2 E^{-1}	R^2 adj.	k_{PD} *p*-value	test
Figure 1 – k_{PDs} at ambient MeHg concentrations in natural waters						
BYL24	Dark control	0.6	0.9	-17.6%	Not significant	-
	Full spectrum	-6.1	0.7	95.5%	< 0.005**	a
	PAR only	0.0	1.0	-33.3%	Not significant	-
Figure 2 – k_{PDs} in spiked natural waters						
A. BYL22	Dark control	-0.9	-	-	-	-
	Full spectrum	-9.3	1.5	82.1%	< 0.0005***	a
Inset (first 12h)	Full spectrum	-3.1	5.3	-14.8%	Not significant	-
B. Char Lake	Dark control	-2.2	-	-	-	-
	Full spectrum	-3.2	1.7	24.6%	Not significant	-
Inset (first 12h)	Full spectrum	-19.3	5.9	66.0%	< 0.05*	a
Figure 3 – k_{PDs} in spiked natural waters according to treatment in the field or in a solar simulator						
A. BYL22	Control	-9.3	1.5	82.1%	< 0.0005***	a
	Cl	-6.7	0.6	93.5%	< 0.00005***	a
	TA	-6.2	1.0	82.1%	< 0.0005***	a
B. Char Lake	Control	-3.2	1.7	24.6%	Not significant	-
	GSH	-0.6	0.7	-4.3%	Not significant	-
	Cl	-3.3	1.6	28.9%	Not significant	-
	TA	0.2	1.0	-13.9%	Not significant	-
Inset (first 12h)	Control	-19.3	5.9	66.0%	< 0.05*	a
	GSH	-19.3	4.0	82.1%	< 0.01**	a
	Cl	-16.3	6.8	48.7%	Not significant	-
	TA	-15.2	7.2	40.8%	Not significant	-
C. BYL22 in solar simulator	Control	-11.3	2.4	84.0%	< 0.05*	a
	GSH	-11.0	0.8	97.7%	< 0.001***	a
D. MilliQ in solar simulator	Dark control	0.8	2.4	-28.4%	Not significant	-
	Control	-6.1	0.5	97.3%	< 0.005**	a
	GSH	-8.0	2.7	65.8%	Not significant	-

TABLEAU SIV. Comparison on *UV* spectrum delivered by Suntest CPS+ and sunlight on Bylot Island during experiments.

UV radiation	Irradiance (photons cm^{-2} s^{-1})	
	Suntest CPS+	Bylot Island sunlight
Total *UV*s (100 – 400 nm)	5.69×10^{15}	2.16×10^{15}
UV-A (320 – 400 nm)	5.61×10^{15}	2.08×10^{15}
UV-B (290 – 320 nm)	9.36×10^{13}	6.44×10^{13}

Supplementary figures

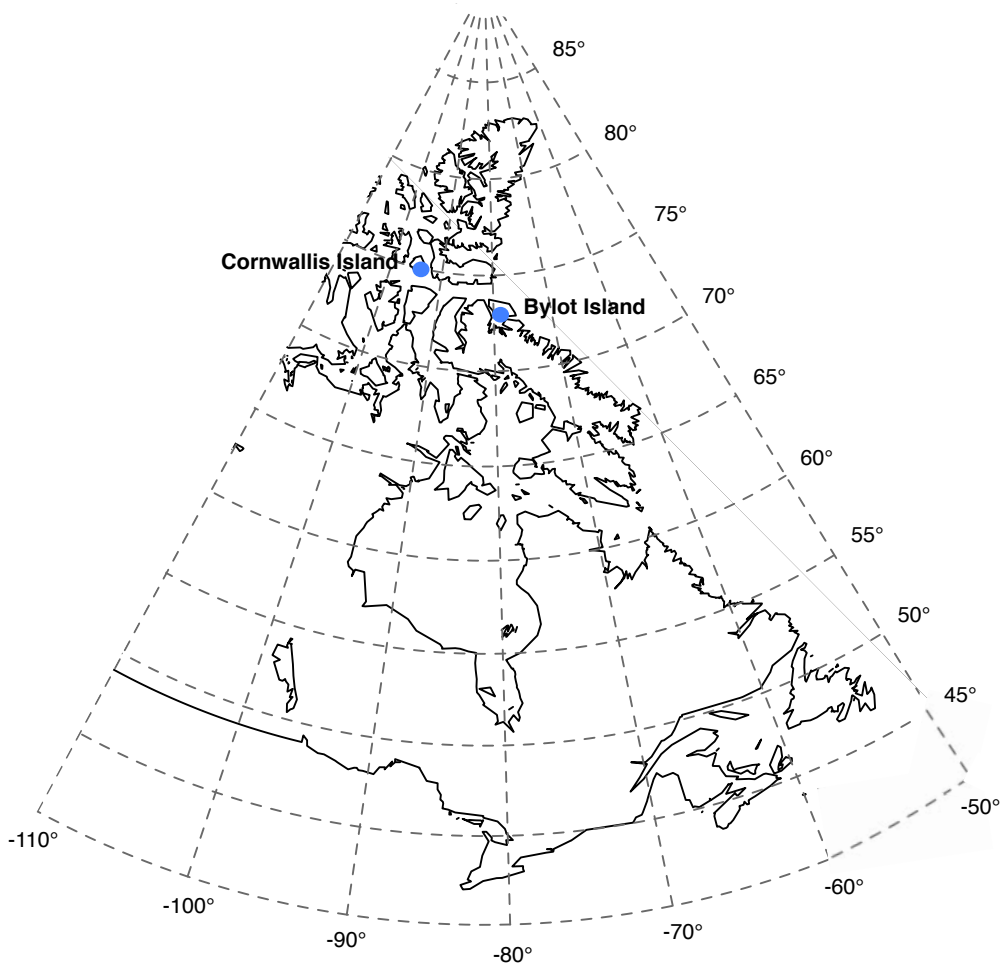

FIGURE S1. Map of Cornwallis and Bylot Islands in the Canadian Arctic Archipelago.

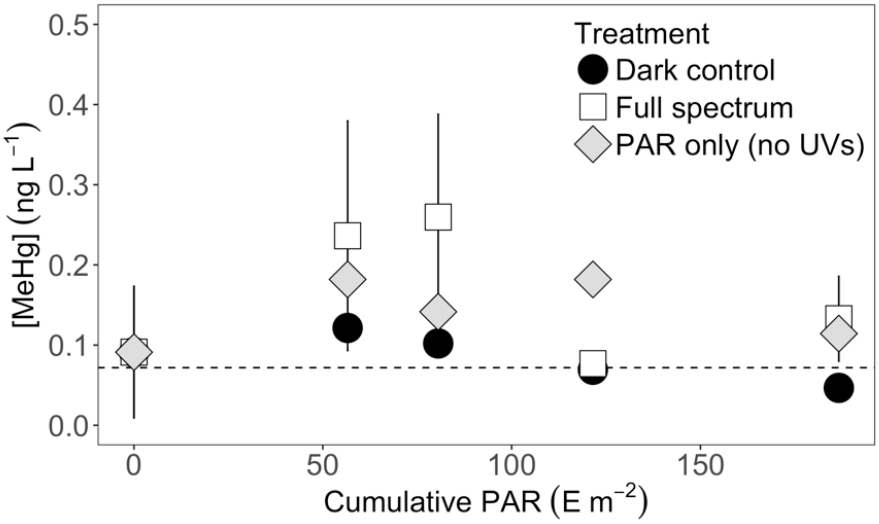

FIGURE S2. Raw MeHg data for experiment conducted in Char Lake. Some data points were below field detection limit, represented by the dotted line (3x standard deviation of triplicate control = 0.07 ng L^{-1}).

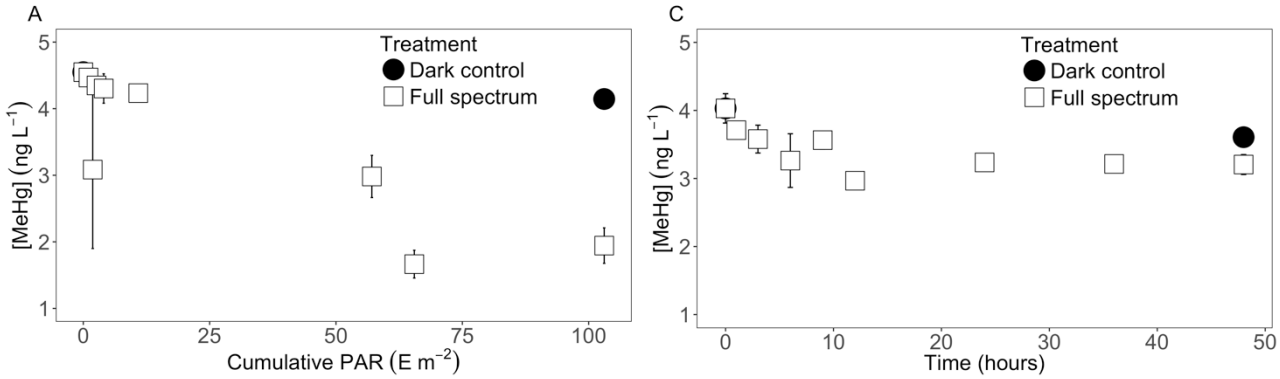

FIGURE S3. Raw MeHg data for photodemethylation experiments conducted in BYL22 and Char Lake, presented in Figure 2.

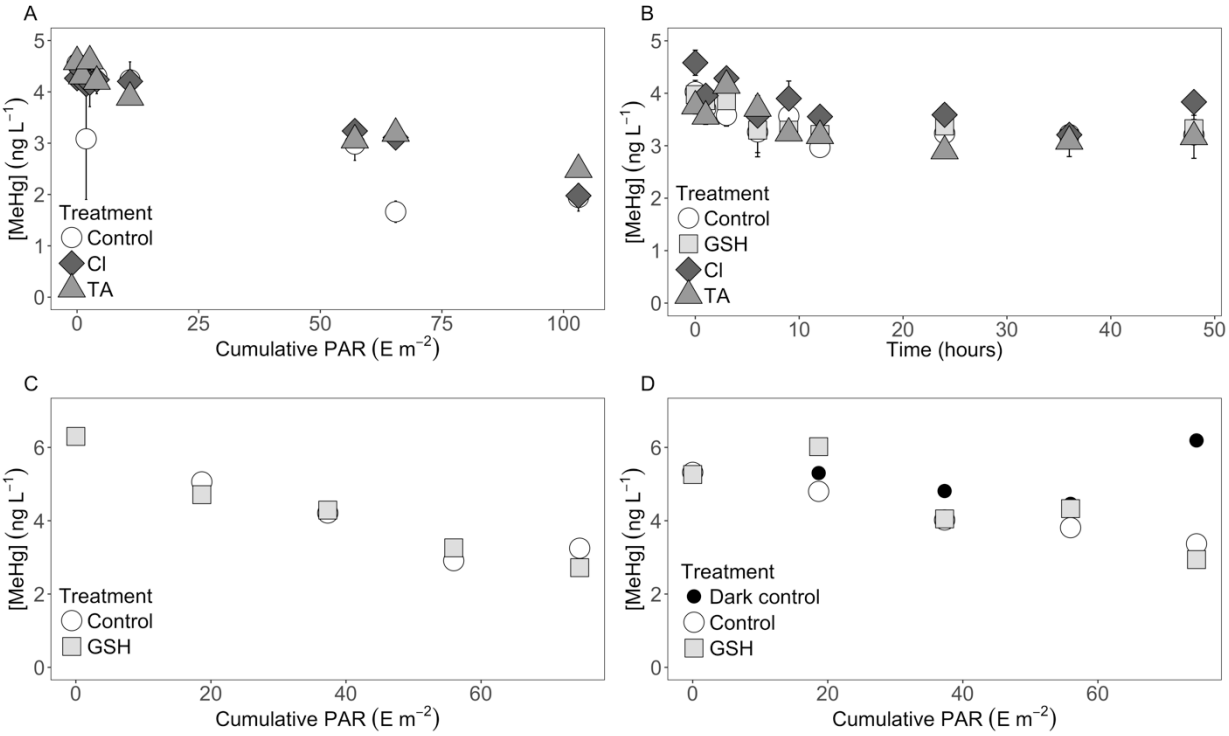

FIGURE S4. Raw MeHg data for experiment conducted on the field (BYL22 and Char Lake) and in the simulator with (MilliQ and BYL22 water), presented in Figure 3C & D.

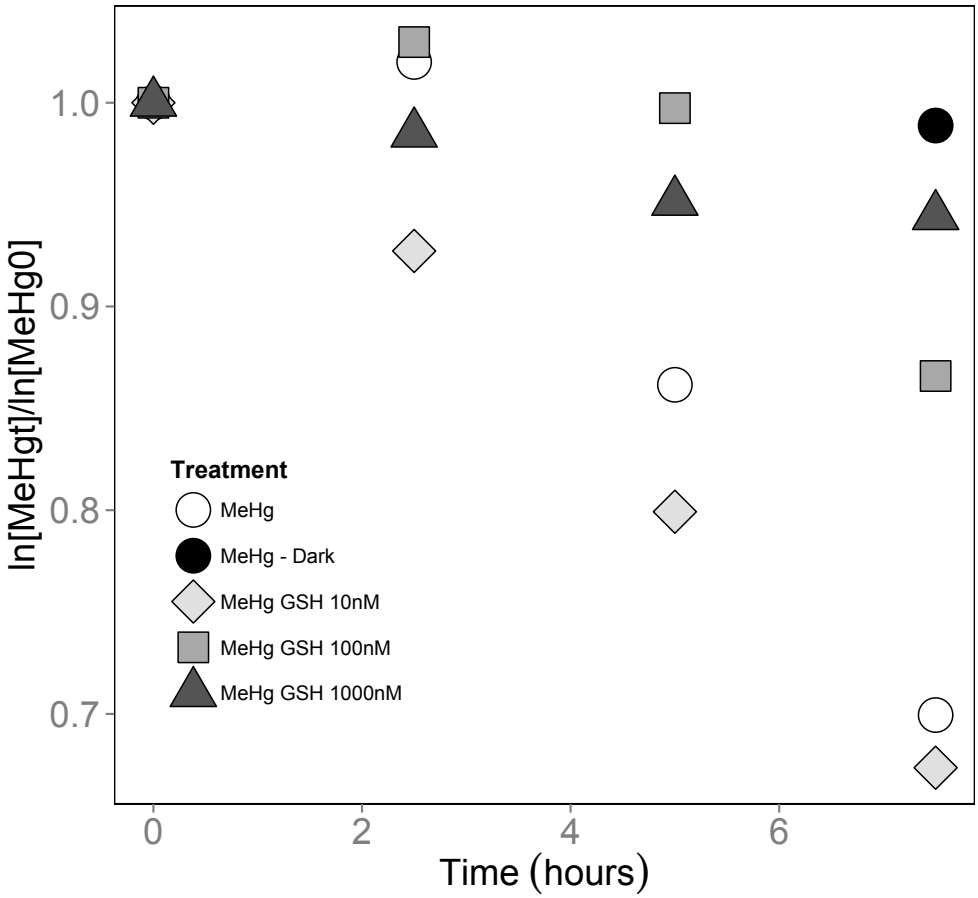

FIGURE S5. Photodemethylation experiment conducted in a solar simulator using water from a temperate lake (Lake Croche, described in Perron et al. 2014), in the presence of varying concentrations of *GSH* (10-1000 nM) over time (hours).

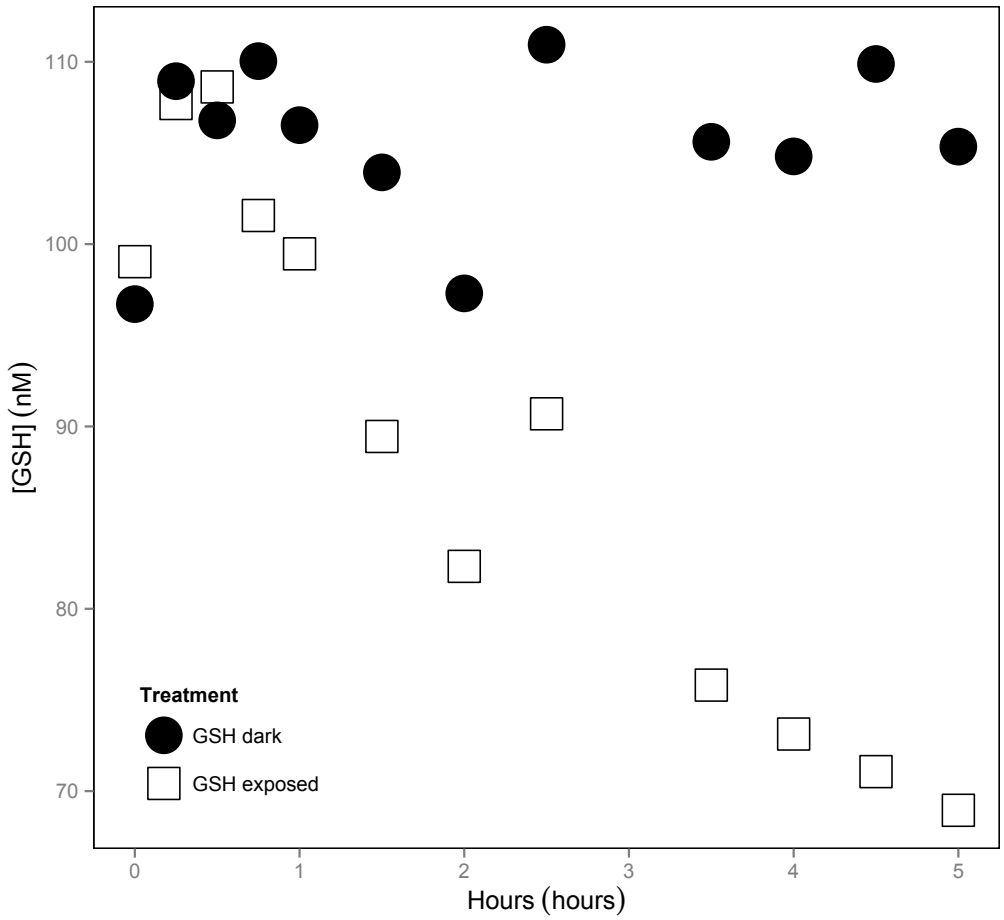

FIGURE S6. Degradation of *GSH* over a 5-hour period during irradiation in a Suntest solar simulator. This experiment was conducted using water from a temperate lake (Lake Croche, as described by Perron et al. 2014).

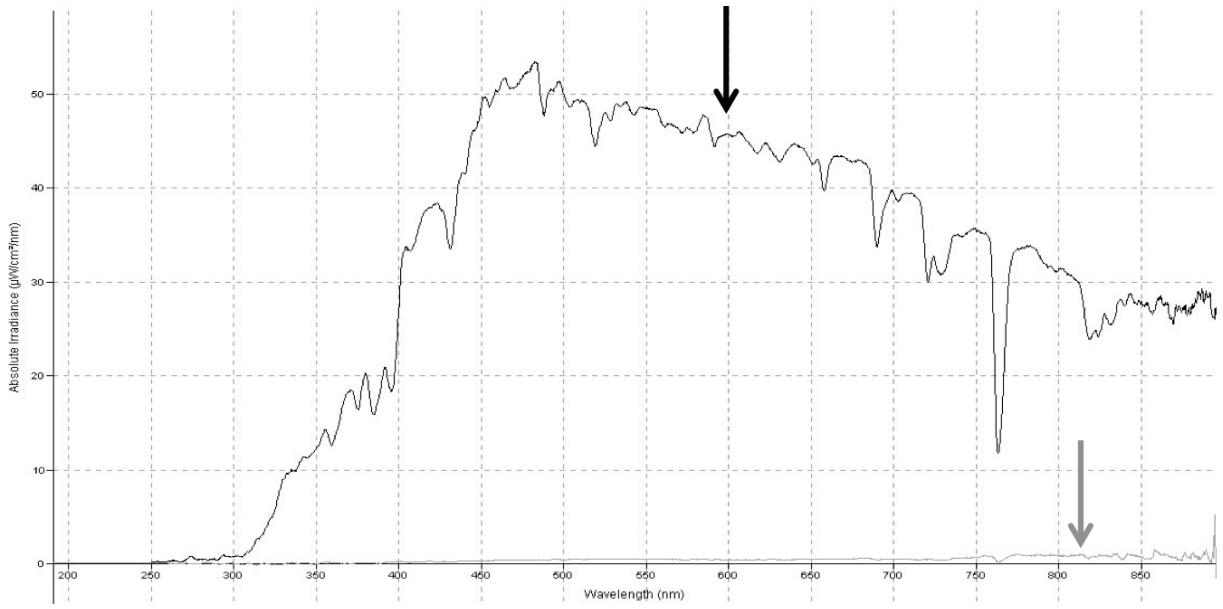

FIGURE S7. Absolute irradiance (µW cm^{-2} nm^{-1}) spectrum above the plastic tarpaulin (in black) and under the plastic tarpaulin (in gray) used in the covered pond experiment, showing radiation obstruction of 98.7% of radiation from 290-895 nm, and 100% of radiation in the UV spectrum (400 nm ≥ ʎ).

CHAPITRE 2. Effets de la cuisson et de la co-ingestion de polyphénols sur la bioaccessibilité *in vitro* du méthylmercure et conséquences sur l'exposition chez l'humain

Cooking and co-ingested polyphenols reduce *in vitro* methylmercury bioaccessibility from fish and may alter exposure in humans

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Abstract

Fish consumption is a major pathway for mercury exposure in humans. Current guidelines and risk assessments assume that 100% of methylmercury (MeHg) in fish is absorbed by the human body after ingestion. However, a growing body of literature suggests that this absorption rate may be overestimated. We used an *in vitro* digestion method to measure MeHg bioaccessibility in commercially-purchased fish, and investigated the effects of dietary practices on MeHg bioaccessibility. Cooking had the greatest effect, decreasing bioaccessibility on average to 12.5 ± 5.6%. Polyphenol-rich beverages also significantly reduced bioaccessibility to 22.7 ± 3.8% and 28.6 ± 13.9%, for green and black tea respectively. We confirmed the suspected role of polyphenols in tea as being a driver of MeHg's reduced bioaccessibility, and found that epicatechin, epigallocatechin gallate, rutin and caffeic acid could individually decrease MeHg bioaccessibility by up to 55%. When both cooking and polyphenol-rich beverage treatments were combined, only 1% of MeHg remained bioaccessible. These results call for *in vivo* validation, and suggest that dietary practices should be considered when setting consumer guidelines for MeHg. More realistic risk assessments could promote consumption of fish as a source of fatty acids, which can play a protective role against cardiovascular disease.

Introduction

A large proportion of the world's population depends on fish. Indeed, fish are estimated to provide 17% of animal proteins consumed by humans (and 6.7% of all proteins consumed worldwide) (Food and Agriculture Organization 2016), and are an important source of vitamins, minerals and fatty acids, which can protect from cardiovascular disease (Mahaffey et al. 2011). However, fish consumption is one of the major pathways of human exposure to mercury (Hg) (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States 2010), which in its organic form of methylmercury (MeHg) is a potent neurotoxin (Clarkson and Magos 2008). To protect at-risk populations, Hg blood guidelines have been established, derived from large-scale studies defining lowest adverse effect doses (Chapman and Chan 2000; Legrand et al. 2010).

However, there is also a growing body of evidence suggesting that our understanding of Hg absorption in the body is incomplete. Current recommendations on fish consumption consider that the ingested dose of Hg from fish is equal to MeHg's – this assumes that 100% of Hg in fish is in the form of MeHg, and that MeHg's absorption rate is 100% (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States 2010; Ha et al. 2016). This stems from older studies performed on human volunteers (Aberg et al. 1969) and on rats (Miettinen et al. 1971) with methylmercuric nitrate ($MeHgNO_3$). However, this may not be representative of MeHg speciation in fish, which is more likely bound to thiol groups included in proteins (Clarkson and Magos 2008; Harris 2003). Indeed, assuming that nearly all of Hg in fish is in the form of MeHg and entirely bioavailable may overestimate MeHg intake by 50% (Ha et al. 2016): while the absorption rate of solubilized MeHg may be high, not all MeHg is necessarily freed from the fish matrix into digestive fluids (i.e. made bioaccessible) and made available for absorption by the body following metabolism in the intestine by the gut microbiome or in the liver (bioavailable) (Afonso et al. 2015a). Thus, to postulate near total MeHg bioavailability overlooks processes that may occur before absorption and into systemic circulation. This is supported by studies reporting that Hg bioaccessibility is not positively correlated to concentration in the consumed food (Laird and Chan 2013; Laird et al. 2009a). While biomarkers like blood or hair Hg show robust relationships to Hg intake (Abdelouahab et al. 2008; Cole et al. 2004; Kosatsky et al. 2000; Legrand et al. 2005; Mahaffey and Mergler

1998), in most of these studies, Hg intake is estimated from food frequency questionnaires and the literature on the consumed fish species, rather than direct Hg measurements (Abdelouahab et al. 2008; Sunderland 2007), meaning that exact Hg intake is frequently unknown. Furthermore, there is evidence that populations exhibit toxicological responses to Hg in different ways (Canuel et al. 2006a; Chapman and Chan 2000). For example, a study of 3 groups of fish consumers in Canada found that individuals of indigenous descent had lower hair Hg concentrations than predicted, while Caucasians sampled in the study showed the expected Hg levels (Canuel et al., 2006a). Chapman & Chan, in their review of the effects of nutrition on MeHg toxicity, suggested that differences in diet may explain the variation in response across populations (Chapman & Chan, 2000). Genetic polymorphisms in genes involved in transport and elimination of MeHg have also been suggested as possible causes in varying excretion rates observed in humans (Caito et al. 2017). As Hg remains a contaminant of major concern (Mergler et al. 2007), it is critical we better understand its fate in the body. A cost-effective and non-invasive way of doing so is through *in vitro* bioaccessibility studies, to first investigate the fate of Hg in the gastrointestinal tract.

Many factors could be responsible for altering MeHg bioaccessibility from ingested food. Food matrix composition may affect the fate of MeHg in the body, with one study reporting that Hg from the flesh of a salmonid may be 6-fold more bioaccessible than that from marine mammalian organs (Laird et al. 2009a). Different levels of Hg bioaccessibility have also been reported for various fish species (Wang et al. 2013). Fish handling by industries and by consumers could also alter bioaccessibility. While freezing can induce physicochemical changes to meat (Farouk et al. 2004; Sanza et al. 1999), it is widely used in fish processing to prevent spoilage (George 1993), which could change MeHg bioaccessibility before fish become available on the market for purchase. Consumer-based food preparation can significantly transform meat, with cooking and drying reducing moisture, crude protein content and total lipids (Toyes-Vargas et al. 2016). Indeed, cooking has been found to reduce Hg and MeHg bioaccessibility (Afonso et al. 2015b; He and Wang 2011; Jadán Piedra et al. 2016; Ouédraogo and Amyot 2011; Torres-Escribano et al. 2010; 2011). *In vitro* studies have also suggested that foods rich in plant polyphenols (such as tea) may reduce MeHg bioaccessibility (He and Wang 2011; Ouédraogo and Amyot 2011; Shim et al. 2009). Dietary practices may thus alter the way

MeHg is solubilized from food (bioaccessibility), and ultimately change its bioavailability. A better understanding of these processes could lead to easily implementable guidelines and recommendations to reduce Hg loading in fish-consuming populations.

The goal of this study was to explore how dietary practices can alter MeHg bioaccessibility, using an *in vitro* digestion model. We explored how various cooking techniques and the co-ingestion of polyphenol-rich foods could alter MeHg solubilization from food. We also investigated the role of specific polyphenols in driving this effect which had been hypothesized in the literature, but never confirmed. We also assessed the potential effect of combined dietary practices on MeHg bioaccessibility. Finally, we report how these dietary practices can affect MeHg intake and loading in the body, and propose ways to use this information to inform future research and guidelines.

Methods

Food items, co-ingested foods and polyphenols

Experiments were performed on swordfish, grouper, tuna and salmon filets obtained from fish markets in Montreal. These species were selected to reflect fish readily available to Canadian consumers year-round. Mean MeHg concentrations in fish samples tested were below Health Canada guidelines (Food Directorate Bureau of Chemical Safety, Health Canada) (500 ng g^{-1} for retail fish, 1000 ng g^{-1} for swordfish and tuna), except for one grouper (835 ng g^{-1}) and one tuna (1244 ng g^{-1}) (Table S1).

Blueberries, coffee (Nescafé, Maxwell) and green and black teas of various brands (Twinnings, Stash, Green Sail, Salada) were purchased in Montreal supermarkets as were corn oil (Mazola) used for cooking treatments, and cornstarch (Ideal), used as a non-polyphenol control. Pure polyphenols (gallic acid (>97.5%), catechin (>98%), epigallocatechin gallate (>80%), theaflavin (>80%), rutin (>94%)) were obtained from Sigma-Aldrich.

Food preparation methods

Three cooking methods were tested: grilling, frying and boiling. Grilling was performed on a Teflon-coated pan, at 100 and 150 °C for 1 min. Frying treatments were conducted in 1 mL of corn oil, in glass vials heated on a burner for 1 min. Samples were boiled in 2 mL of ultrapure MilliQ water (> 18.2 MΩ cm-1) (EMD Millipore) in glass vials for 5 or 10 min. Temperature was monitored throughout cooking. For freezing, fish samples were subsampled immediately following their purchase and placed in glass vials, and kept at -20, -80 °C or flash frozen in liquid nitrogen (then kept at -80 °C). Glassware was rinsed with distilled water, soaked in a 45% HNO_3, 5% HCl (Fisher Scientific, ACS-pure) bath overnight and rinsed 3 times with MilliQ water before use.

For co-ingestion experiments, fish samples were digested simultaneously with either beverages or pure polyphenols. Beverages (tea, coffee, instant coffee) were prepared as per the manufacturer's instructions, and lyophilized overnight into a powder (Freezone6, Labconco). Powdered beverages were solubilized in 2 mL of MilliQ water, in two different doses: 40 mg or 120 mg, and were added to fish at the start of *in vitro* digestion experiments. In these experiments, controls were amended with 2 mL of MilliQ water to adjust the volume. Pure polyphenols were solubilized in 2 mL of dimethyl sulfoxide (DMSO), in amounts of 5 or 10 mg, and used in *in vitro* digestions. Controls with no polyphenols were also amended with 2 mL of DMSO, to account for volume increase.

Physiologically-based extraction test

Many *in vitro* digestion protocols exist to assess bioaccessibility of nutrients and dietary compounds (Dong et al. 2016; Minekus et al. 2014; Van de Wiele et al. 2007). We selected the Physiologically-based extraction test (PBET), adapted from Ruby et al. (1996) and Ouédraogo and Amyot (2011), to perform digestive simulations, as it has been used frequently for metals and Hg (Calatayud et al. 2012; Ouédraogo and Amyot 2011; Siedlikowski et al. 2016). All digestive simulations were performed on 1.0 ± 0.1 g of fresh fish sample (chopped into 15-20 smaller pieces), in triplicate. Experimental solutions were prepared in acid-washed Teflon

bottles prior to each PBET digestion. The gastric phase was prepared by combining 1.25 g porcine pepsin (>400 units/mg), 0.50 g sodium citrate (>99%), 0.50 g malic acid (>99%), 420 μL lactic acid (>85%) and 500 μL of acetic acid (99.7%) (purchased from Sigma-Aldrich and Fisher Scientific**)** in ultrapure MilliQ water in a final volume of 1 L, and pH was adjusted to 2 with HCl (OmniTrace Ultra, EMD). The intestinal phase contained 0.60 g bile salts and 0.15 g pancreatin (4 x USP grade, lipase >24 units/mg, protease >400 units/mg) (Sigma-Aldrich), in a final volume of 250 mL 1 M $NaHCO_3$.

Briefly, samples were placed in Falcon tubes with 40 mL of gastric solution, and were incubated at 37 °C with agitation (100 rpm) for 1 hour. pH was then adjusted to 7 using 5 M NaOH. Nine mL of intestinal solution were added to all samples, which were incubated at 37 °C with agitation (100 rpm) for 2 hours. Following incubation, samples were centrifuged for 15 minutes at 3,000 g. The supernatant, considered to contain the bioaccessible (solubilized) fraction of MeHg, was isolated and used for MeHg analyses.

Bioaccessibility

Bioaccessibility was calculated after PBET simulations with the following equation:

[Equation 1]

$$\%\ bioaccessibility = \frac{[MeHg]\ in\ PBET\ (ng\ g^{-1})\ x\ PBET\ volume\ (L)}{[MeHg]\ in\ fish\ \ (ng\ g^{-1})\ x\ fish\ mass\ (g)}\ x\ 100$$

with *[MeHg] in PBET* being MeHg measured in the extract of the simulated digestion, *PBET volume* being PBET digestive fluids volume, *[MeHg] in fish* is [MeHg] in initial fish sample, and *fish mass* is the mass of fresh fish used as input into the PBET simulation.

Multiple bioaccessibility experiments were also performed using the same commercially-purchased raw fish filet over different days (stored at 4 °C between experiments). Bioaccessibility values for controls were compared across runs, and we found no statistical

differences within a single fish filet (with the number of experimental days performed on each fish individual varying from 2 to 5) (Kruskal-Wallis, $P > 0.05$) (Figure S2). This showed us that we could use multiple samples from one individual fish filet over several days with minimal impact on bioaccessibility. To compare different sets of experiments, we normalized results within each experiment to raw untreated fish muscle that were digested as a control within each run (using Equation 2), giving a percent of bioaccessible MeHg compared to controls (now normalized to 100%).

[Equation 2]

$$\%\ bioaccessibility\ compared\ to\ control = \frac{average\ treated\ \%}{average\ control\ \%}\ x\ 100$$

where average treated % refers to the mean bioaccessibility obtained across triplicates of a given treatment (where one replicate is a fish sample digested in a PBET experiment), and average control % refers to mean bioaccessibility calculated in untreated triplicates of raw fish muscle from the same run. Non-normalized control values are presented in Table 1.

MeHg analyses

MeHg in fish was measured in freeze-dried samples (Freezone6, Labconco), while MeHg following simulated digestion was analyzed in PBET fluids. Prior to analysis, both dried fish samples and PBET fluids were extracted overnight in 5 mL of 4M HNO_3 (Fisher Scientific, ACS-pur) at 60 °C. MeHg in fish and PBET solutions was measured by gas chromatography and cold-vapor fluorescence spectrometry (CVAFS) (Tekran 2700, Tekran Instruments Corporation), according to U.S. EPA method 1630 (detection limit of 0.01 ng L^{-1}, defined as three times the standard deviation calculated on 10 ultrapure MilliQ blanks).

TABLEAU I. Total Hg and MeHg bioaccessibility (%) measured in freshly purchased, raw fish muscle in this study and from the literature. Values presented are averages and standard deviations (mean ± SD).

Fish species	*In vitro* digestion method	HgT bioaccessibility (mean ± SD)	MeHg bioaccessibility (mean ± SD)	Reference
Swordfish	PBET	-	32 ± 8%	this study (Fig. 3-4, S3, S5)
Grouper		-	55 ± 26%	this study (Fig. 1, S6)
Tuna		-	26 ± 10%	this study (Fig. 1, 2, S7)
Salmon		-	36 ± 2%	this study (Fig. 1)
Tuna	Versanvoort et al. 2005, Afonso et al. 2015b	78 ± 6%	78 ± 10%	Afonso et al. 2015b
Meagre	Versanvoort et al. 2005	87 ± 2%	100 ± 1%	Afonso et al. 2015a
Tuna	Luten et al. 1987	9%	-	Cabañero et al. 2004
Swordfish		17%	-	
Sardine		14%	-	
Sardine	Luten et al. 1987	10%	-	Cabañero et al. 2007
Anchovy	Laparra et al. 2003	77 - 86%	-	Calatayud et al. 2012
Anglerfish		57%	-	
Blue whiting		62 - 68%	-	
Hake		66 - 81%	-	
Salmon		102 - 106%	-	
Small hake		58 - 89%	-	
Sole		67 - 105%	-	
Swordfish		42 - 66%	-	
Salmon	Afonso et al. 2015b	90 ± 0.1%	-	Costa et al. 2015
Rabbitfish	Versanvoort et al. 2005	-	27%	He and Wang 2011
Grouper		-	64%	
Cod	Shim et al. 2009	-	28 ± 19%	Kwasniak et al. 2012
Perch		-	48 ± 21%	
Herring		-	62 ± 25%	
Flounder		-	38 ± 22%	
Turbot		-	26 ± 0.2%	
Arctic char	SHIME	33 - 94%	-	Laird et al. 2009a
Chinook salmon	SHIME	49 ± 22.1%	-	Laird and Chan 2013
Sockeye salmon		46 ± 21.3%	-	
Blue shark	Versanvoort et al. 2005, Afonso et al. 2015b	94 ± 3%	98 ± 5%	Matos et al. 2015
Black scarbard	Hur et al. 2009	56%	-	Maulvault et al. 2011
Tuna	PBET	73 ± 12%	-	Ouédraogo and Amyot 2011
Cat shark		79 ± 12%	-	
Spanish mackerel		78 ± 2%	-	
Cod	PBET	-	65%	Siedlikowski et al. 2016
Halibut		-	86%	
Salmon		-	71%	
Tilapia		-	66%	
Tuna		-	74%	
Swordfish	Luten et al. 1987	73 ± 14%	-	Torres-Escribano et al. 2011
Tope shark		57 ± 13%	-	
Bonito		21 ± 3%	-	
Tuna		16 ± 3%	-	
Swordfish	Jovani et al. 2001, Laparra et al. 2003	64 ± 14%	-	Torres-Escribano et al. 2010
Tilapia	Wang et al. 2010, Wang et al. 2011	42%	56%	Wang et al. 2013
Spotted snakehead		37%	50%	
Snakehead		33%	43%	
Rice field eel		39%	38%	
Mud carp		34%	42%	
Mandarin fish		40%	50%	
Grey mullet		41%	47%	
Grass carp		37%	48%	
Catfish		49%	56%	
Bigheadcarp		35%	36%	
Yellow seafin		21%	29%	
Yellow croaker		22%	20%	
Tongue sole		25%	26%	
Snubnose pompano		37%	39%	
Orange-spotted grouper		52%	58%	
Golden threadfin bream		44%	59%	
Goldspotted rabbitfish		34%	35%	
Bleeker's grouper		41%	53%	
Bigeye		19%	43%	
Bartail flathead		47%	47%	

Fish matrix characterization

Lipids were quantified by gravimetry, using a method adapted from Folch et al. 1957. Nitrogen was used as a proxy for protein content and was quantified with a CHN Element Analyzer 1108 (Thermo Fisher). Moisture content in fish muscle was quantified by subtracting sample dry weight from wet weight after drying. Full methods on fish matrix characterization are presented in Supplementary Information.

Protein, lipid and moisture content did not vary across fish species (Kruskal-Wallis, $P > 0.05$) (Table S1), and we found no correlation between MeHg bioaccessibility and lipid content in fish ($P > 0.05$) (Figure S2).

Polyphenol analyses

Polyphenols were quantified by ultra-performance liquid chromatography with tandem mass spectrometer (UPLC-MSMS) at the Institute of Nutrition and Functional Foods (Quebec, Canada) using a Waters Acquity Ultra-Performance LC system (Waters). The full method is presented in Supplemental Information. The seven polyphenols that were quantified in co-ingested foods and subsequently tested in their purified form are presented in Table S2. Complete profiles of the 56 polyphenols analyzed in co-ingested foods are presented in Table S3.

Risk assessment

We estimated a probable daily intake (PDI) (μg kg^{-1}) for an average adult for each of the fish tested in this study, using the following formula:

[Equation 3]

$$PDI = \frac{[MeHg\ in\ fish\ ]\ (ug\ g^{-1}) x\ average\ daily\ fish\ intake\ (g)}{average\ adult\ body\ weight\ (kg)}$$

where [MeHg in fish] is the MeHg concentration measured in the fish sample tested; average daily fish intake is based on fish consumption values from the Bureau of Chemical Safety of Canada (22 g for an adult) (Bureau of Chemical Safety Health Canada 2004); and the average adult body weight is based on values from Nutrition Canada (60 kg for an adult) (Health Canada 2004). PDIs represent average exposure to MeHg, if an adult consumed each fish daily over a long period of time. We then calculated a bioaccessibility-corrected PDI (PDI_{BA}):

[Equation 4]

$$PDI_{BA} = PDI\ x\ \%\ bioaccessibility$$

where the % bioaccessibility is the soluble fraction calculated from our experiments. This PDI_{BA} accounts for the bioaccessibility of MeHg in the *in vitro* model and the effect of food preparation and co-ingestion treatments.

Statistical analyses

Statistical analyses were performed with R software (R Development Core Team) using nonparametric methods, as normality and homoscedasticity were not respected (tested with shapiro.test () and bartlett.test () functions). Differences in bioaccessibility across treatments were compared with Kruskal-Wallis analysis of variance (kruskal () in the agricolae{} package) (De Mendiburu 2012), and Bonferroni corrections were used to correct for multiple hypothesis testing. Linear regressions were used to model bioaccessibility and lipid content, and ordinations were calculated on log-transformed data. Plots were prepared using ggplot2{} (Wickham 2009) and ggbiplot{} (Vu 2011) packages. Letters on plots denote significantly different treatments ($P < 0.05$), bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Results

Fish sample characterization and inter-fish and inter-simulation variation

Mean MeHg concentrations in fish samples tested were below Health Canada guidelines (Food Directorate Bureau of Chemical Safety, Health Canada) (500 ng g^{-1} for retail fish, 1000 ng g^{-1} for swordfish and tuna) (average concentration in swordfish = 439 ± 237 ng g^{-1}; in grouper: 612 ± 315 ng g^{-1}; in tuna = 694 ± 778 ng g^{-1} and in salmon = 20.1 ng g^{-1}), except for one grouper (835 ng g^{-1}) and one tuna (1244 ng g^{-1}) (Table S1). Protein, lipid and moisture content did not vary across fish species (Kruskal-Wallis, $P > 0.05$) (Table S1), and we found no correlation between MeHg bioaccessibility and lipid content in fish ($P > 0.05$) (Figure S1).

Multiple fresh filets from different individual fish were tested for each species. For grouper and tuna, we observed significant differences in MeHg bioaccessibility among individual fish (Kruskal-Wallis, $P < 0.05$) (Figure S2). Differential lipid content in the muscles tested did not account for this variation, as we observed no relationship between lipids and raw bioaccessibility percentages when considering the four fish species tested in this study (Figure S1). The use of only one salmon in this study may mask potential variation between fish, and inter-individual absolute bioaccessibility may vary in ways similar to grouper and tuna. However, this study does not aim to report absolute bioaccessibility values, and trends from treatments experiments were robust across individuals for all species.

Effects of cooking and freezing on MeHg bioaccessibility

Cooking had a significant impact on MeHg bioaccessibility compared to raw controls in all fish species tested. In swordfish (Kruskal-Wallis, $P < 0.05$) (Figure 1A), on average, MeHg bioaccessibility was reduced to 12.6 ± 5.6% of control values across all cooking treatments. Grilling and frying at maximal temperatures (150 and 160 °C) reduced bioaccessibility to 18.0 ± 5.6 and 7.1 ± 1.2% respectively, while boiling decreased bioaccessibility to 8.4 ± 3.0% of control values (Figure 1A). Lower temperatures or cooking times did not yield significantly greater bioaccessibility losses ($P > 0.05$). The effect of cooking was consistent for grouper (18.6

± 10.3%), tuna (12.2 ± 5.5%) and salmon (10.9 ± 6.7%) ($P < 0.05$) (Figure 1B-D). In all fish species, frying tended to be the most effective at reducing MeHg bioaccessibility, but this trend was not significant ($P > 0.05$) (Figure 1).

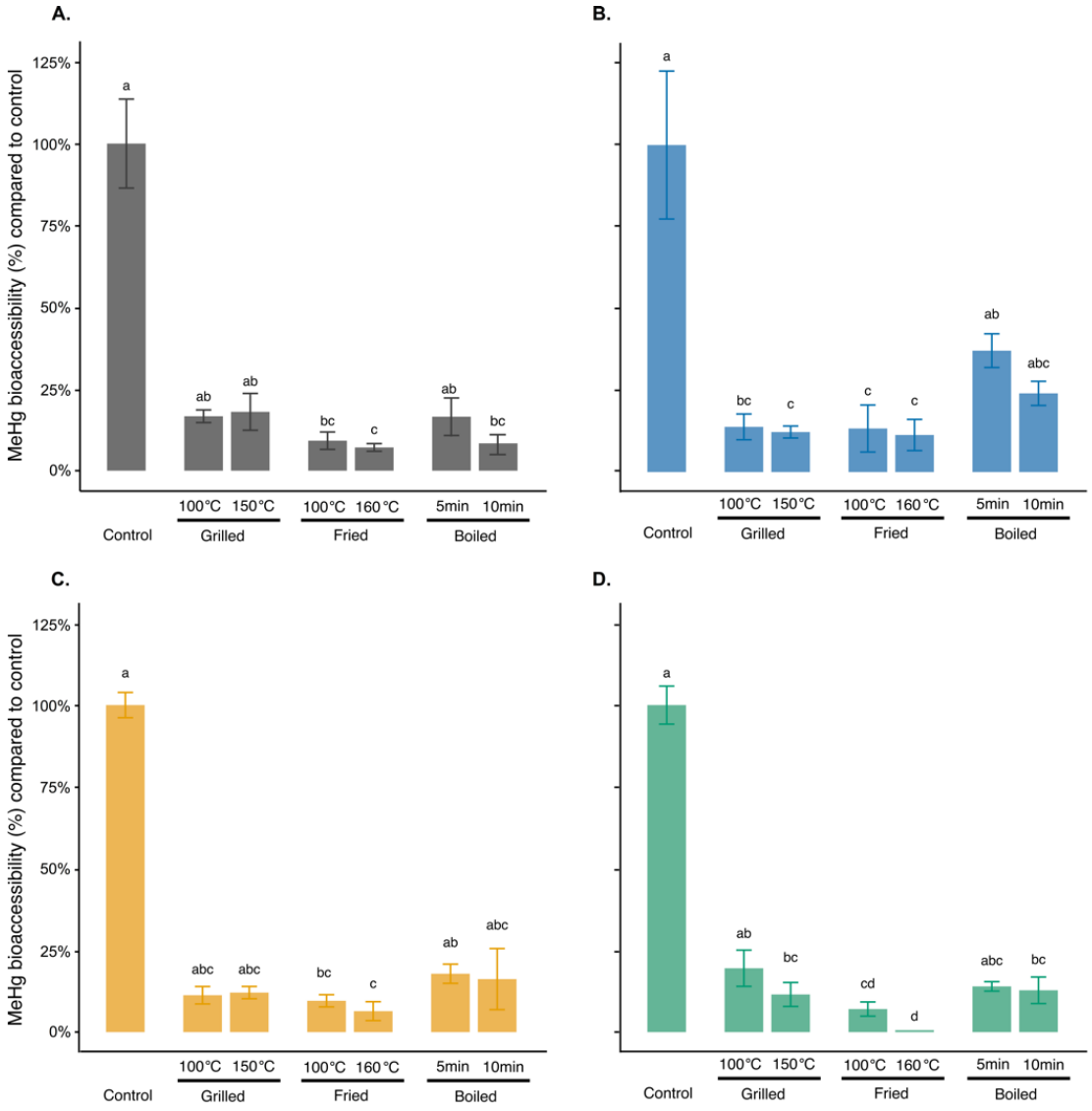

FIGURE 1. Effect of cooking on MeHg bioaccessibility in A. swordfish, B. grouper, C. tuna and D. salmon. Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

When swordfish samples were frozen, decreasing freezing temperatures, from -20 to -80 °C and flash freezing did not lead to significantly different MeHg bioaccessibility levels ($P > 0.05$) (Figure S3).

Effects of co-ingested foods and polyphenols on MeHg bioaccessibility

We tested the effects of co-ingested foods on MeHg bioaccessibility in swordfish and in tuna compared to unamended raw controls (Figure 2). As previous reports have suggested that polyphenols were responsible for altered MeHg bioaccessibility from fish (He and Wang 2011; Ouédraogo and Amyot 2011; Shim et al. 2009), we selected several polyphenol-rich foods and beverages, and one food not enriched in polyphenols (cornstarch). Our results show that green tea led to the lowest MeHg bioaccessibility values in swordfish (22.7 ± 3.8% when 120 mg was used) (Figure 2A-B) and in tuna (34.8 ± 5.6% with 120 mg of tea) ($P < 0.05$) (Figure 2C). Black tea also significantly decreased MeHg bioaccessibility in swordfish (28.6 ± 13.9% with 120 mg of tea) ($P < 0.05$). The effect of tea was greater when 120 mg of dried tea (approximately 375 mL of prepared tea) was used compared to 40 mg (approximately 125 mL). As expected, cornstarch, which contained negligible amounts of polyphenols (Table S2) had no effect on MeHg bioaccessibility (Figure 2B). Coffee and instant coffee had a slight, yet non-significant effect, while blueberries had no detectable impact ($P > 0.05$) (Figure 2C). Therefore, it appears only certain polyphenol-rich beverages can alter MeHg bioaccessibility.

To identify which specific polyphenols may be responsible for this effect, we quantified 56 polyphenols in all foods that were used in bioaccessibility experiments. Of these, we found that gallic acid and flavonoids (including flavanol quercetins such as rutin, and flavon-3-ol catechins and theaflavins) were abundant in green and black tea, and in small or undetectable concentrations in the other tested co-foods (Table S2). Multivariate analysis showed that treatments with the greatest decreases in bioaccessibility in this experiment were positively associated with certain polyphenol groups such as catechins, quercetins, thearubigins and kaempferols (Figure S4).

To verify the hypothetical role of these polyphenols on MeHg bioaccessibility, we repeated PBET simulations using individual purified polyphenol compounds. First, we observed that the effect of polyphenols increased with the amount added to digestion experiments, from 5 to 10 mg (Figure 3). However, while catechin is cited in the literature as a hypothetical driver of reduced MeHg bioaccessibility (He and Wang 2011), we found that its purified form did not lead to significant decreases ($P > 0.05$) (Figure 3A). Other forms of catechin, including cis-configuration epicatechin and epigallocatechin gallate (EGCG) did significantly limit MeHg bioaccessibility to 61.7 ± 4.5 and 47.0 ± 15.5% of unamended control, respectively ($P < 0.05$).

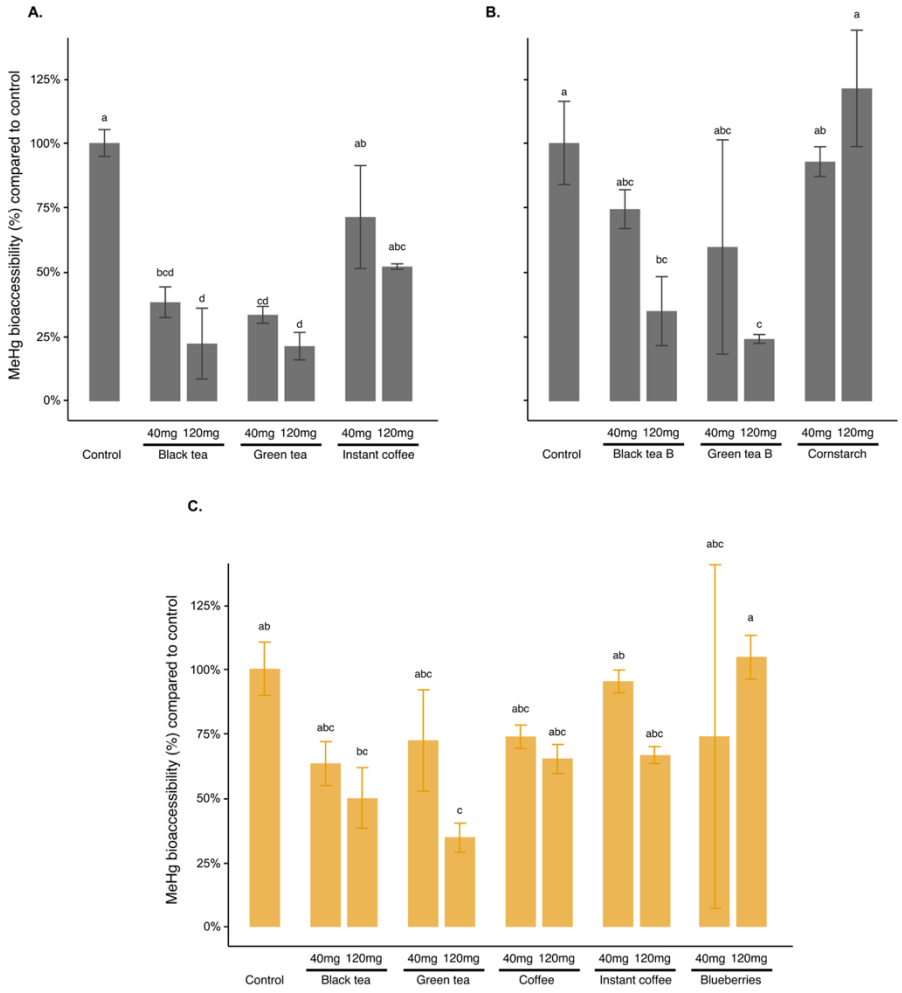

FIGURE 2. Effect of polyphenol-rich beverages and foods on MeHg bioaccessibility in A. & B. swordfish and C. tuna. Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

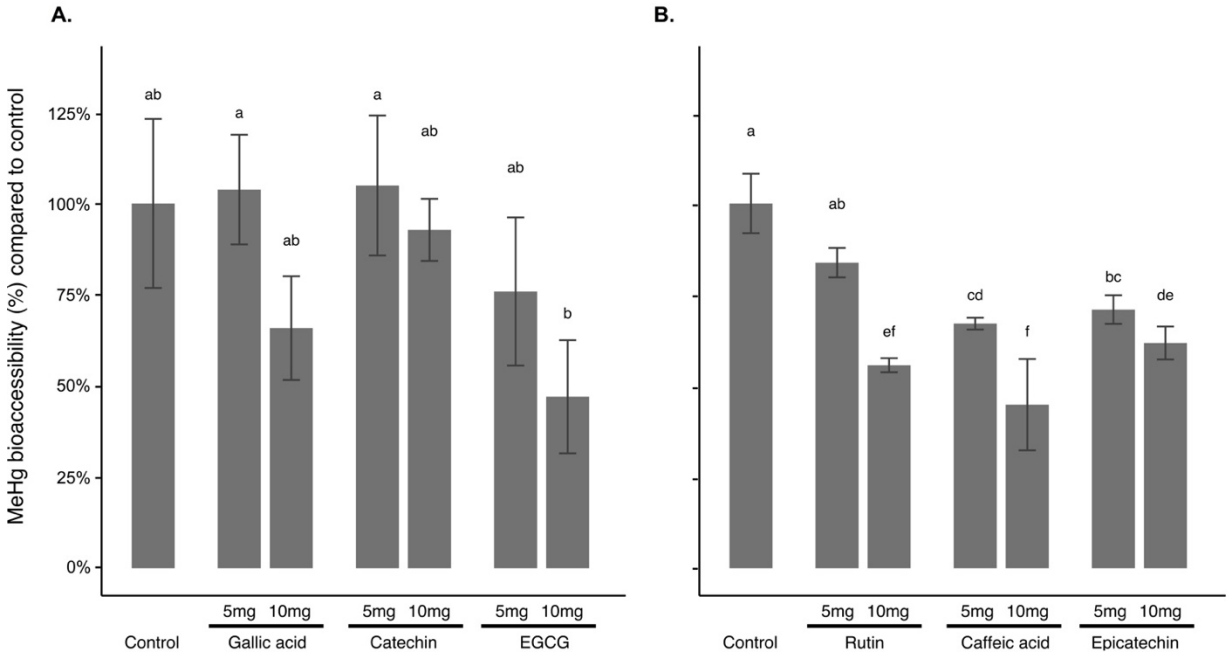

FIGURE 3. Effect of pure polyphenol compounds on MeHg bioaccessibility in swordfish. A & B. Epicatechin, epigallocatechin gallate, rutin and caffeic acid lead to significant decreases in MeHg bioaccessibility (Kruskal-Wallis, $P < 0.05$). Results were normalized to controls at 100%, to allow comparison across experiments (see description in Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Another flavon-3-ol (rutin) and a hydroxycinnamic acid (caffeic acid) also significantly reduced bioaccessibility to 55.6 ± 1.9%, and 44.8 ± 12.5%, respectively ($P < 0.05$) (Figure 3).

Effects of multiple dietary practices on MeHg bioaccessibility

Applied separately, cooking and polyphenols (as beverages or purified compounds) both impacted MeHg bioaccessibility compared to raw, unamended controls (Figures 1-3). We then tested these treatments together within the same experiment, to assess their combined effects. We found that cooking swordfish (by grilling, frying or boiling) and digesting it with 120 mg of green or black tea led to less than 1% of MeHg remaining bioaccessible ($P < 0.05$, Figure 4A

and B). Combining cooking and 10 mg of purified gallic acid or catechin also significantly decreased MeHg bioaccessibility, to 2-17% of bioaccessible MeHg compared to raw unamended controls (Figure 4C and D) ($P < 0.05$).

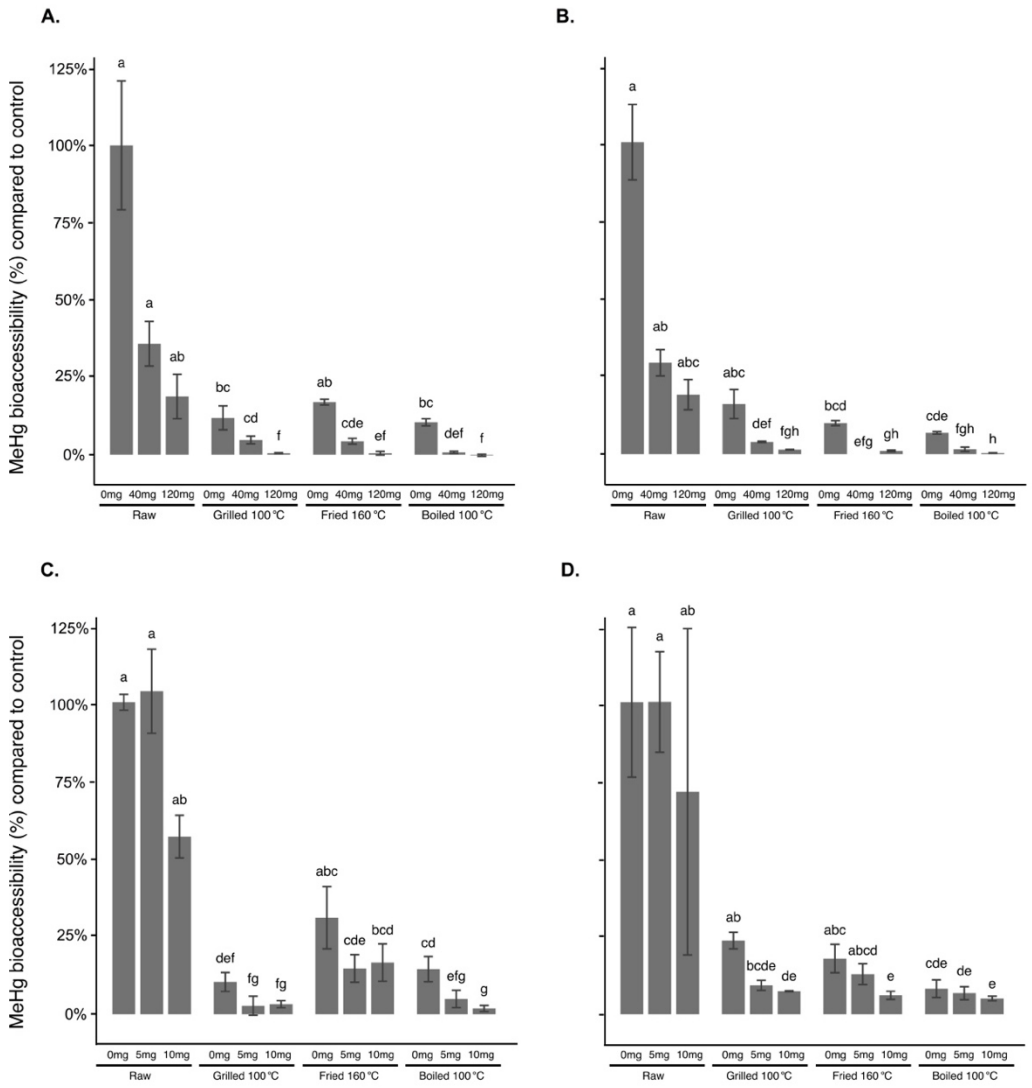

FIGURE 4. Mixed effect of cooking and polyphenol compounds and polyphenol-rich beverages on MeHg bioaccessibility in swordfish for polyphenol-rich beverages and foods (A. black tea, B. green tea) and for pure polyphenols (C. gallic acid, D. catechin). Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

The combined effects of cooking and co-ingested polyphenols (as beverages or purified extracts) were also observed in grouper ($P < 0.05$, Figure S6) and tuna ($P < 0.05$, Figure S7). In all cases, boiling combined with green or black tea were the most effective combination to reduce MeHg bioaccessibility, decreasing bioaccessibility by 99% compared to raw, unamended controls ($P < 0.05$, Figure 4, S6 and S7).

Bioaccessibility and risk assessments

Finally, we compared PDIs for an average adult, calculated with the MeHg concentration measured in tested fish, to corrected PDI_{BAs} considering bioaccessibility and the effect of dietary practices. This is a theoretical exercise in the absence of *in vivo*-validated results, and is presented here only to compare the impact of different treatments, rather than to estimate realistic PDIs. Table S4 shows that the estimated daily intake for an adult is greatly reduced when bioaccessibility is considered. All values calculated from MeHg concentrations were below the provisional tolerable daily intake (the maximum amount of MeHg that can be ingested daily over a lifetime without increasing risk of health effects) established by the World Health Organization ($0.23\ \mu g\ kg^{-1}\ d^{-1}$) (Joint FAO/WHO Expert Committee on Food Additives 2007).

Discussion

Cooking reduces MeHg bioaccessibility from fish; freezing has no effect

Several studies have shown that cooking increases Hg concentrations in fish (Burger et al. 2003; Morgan et al. 1997; Perelló et al. 2008). It has been suggested that this may be due to weight loss due to moisture and fat loss during cooking (Morgan et al. 1997). When considering bioaccessibility however, studies consistently show a reduction of solubilized Hg (Ouédraogo and Amyot 2011; Torres-Escribano et al. 2011) and MeHg (He and Wang 2011) from cooked fish muscle. Our results on MeHg bioaccessibility were consistent with these earlier studies (Figure 1). Here we expanded on these previous studies by comparing multiple temperatures and cooking times for each cooking treatment, and we found no significant differences between

100 and 150 °C for grilling, 100 and 160 °C and frying, nor for 5 and 10 minutes for boiling (Figure 1). All temperatures tested were greater than 70 °C, the safe internal cooking temperature recommended by Health Canada (Health Canada First Nations Branch, Government of Canada 2016). However, while fish cooked by consumers may reach this safe internal threshold, higher temperatures are typically used when preparing meals. Indeed, temperatures greater than 100 °C are typically used in studies measuring the effects of cooking on metals (Devesa et al. 2001; Ersoy et al. 2006) and heterocyclic amines (Oz et al. 2007). It is likely that the impact of heat on MeHg solubilization increases until a given temperature (below 100 °C), and further increases have no effect on MeHg bioaccessibility within the range of temperatures commonly used in cooking. Protein aggregation induced by high temperatures, leading to lowered pepsin digestibility, may explain these results. Cooking has also been found to induce the formation of disulfide bonds in proteins, which may further limit digestibility (Duodu et al. 2002; He et al. 2010; Kulp et al. 2003). This is supported by observations that heat induces structural changes to meat: at temperatures greater than 100 °C, oxidation can cause protein aggregation, slowing enzymatic digestion by pepsin (Bax et al. 2012).

It is important to note that these experiments were performed on small (1 g) sub-samples of fish muscle, which were thoroughly cooked during treatments. It is likely that in a thicker and larger fish filet more representative of an adult portion (150 g as per Health Canada) (Bureau of Chemical Safety Health Canada 2007), cooking has more heterogeneous effects: temperature may not be even throughout the portion, and structural protein changes that might control MeHg bioaccessibility may vary across filet thickness. Nonetheless, these experiments on small test meals do provide insight into the effect of temperature on MeHg solubilization from proteins. Future experiments on cooking and MeHg should include portion-sized fish for a more realistic portrait of bioaccessibility.

Freezing can induce protein denaturation and physicochemical changes to meat (Farouk et al. 2004; Sanza et al. 1999). In a study of 27 samples of frozen swordfish, Torres-Escribano et al. (2010) suggested that variations in Hg bioaccessibility from 38-83% (64 ± 14%) may be attributable to protein denaturation caused by different freezing and thawing rates, or varying storage temperatures. In this study, we observed no relationship between bioaccessibility and colder temperatures. However, since all the fish tested in this study were purchased

commercially, they had all likely been frozen before, as freezing is widely used in fish processing to prevent spoilage (George 1993). This was the case for swordfish, which was confirmed to have been frozen before purchase by the vendor and was used in our freezing experiment. While this industrial freezing performed after catch to conserve fish may alter bioaccessibility, our results suggest that a second freezing has no impact. Therefore, consumers who freeze commercially-purchased fish are unlikely to further affect MeHg bioaccessibility (Figure S3).

Plant polyphenols found in tea can limit MeHg solubilization

As plant metabolites present in all plant organs, polyphenols are an important component of human diets (Bravo 2009), and dietary intake of flavonoids alone (which include the different catechins) has been estimated at 187 mg d^{-1} in the US (Chun et al. 2007). While they are of great interest in nutrition due to their ability to bind and precipitate certain molecules, and for their antioxidant effects in humans (Bravo 2009), it is likely their role in metal chelation (Graham 1992; Hider et al. 2001; Ragan et al. 1979; Wang et al. 2009) that drives their impact on MeHg bioaccessibility. We found that certain polyphenol-rich foods, such as green and black tea, could significantly reduce MeHg bioaccessibility, and that the effect increased with the amount of tea added (Figure 2). However, not all polyphenol-rich foods limited bioaccessibility: blueberries (rich in anthocyanidins) (Table S3), had no significant impact on MeHg bioaccessibility.

The amounts of dried tea used in our experiments roughly reflect the ratio of one cup of tea consumed with one portion (150 g) of fish. The polyphenol molecules found in tea are suspected to reduce MeHg and Hg bioaccessibility (He and Wang 2011; Ouédraogo and Amyot 2011; Shim et al. 2009) but the causal role of polyphenols had not been directly investigated. Here, we tested a wide range of purified polyphenols to confirm their role in decreasing MeHg bioaccessibility, and to identify which compounds may be responsible for the effects of tea. Our results suggest that EGCG, rutin, epicatechin and caffeic acid likely have the greatest chelating properties (Figure 3), possibly forming insoluble complexes with MeHg and decreasing bioaccessibility. Indeed, metals chelated to polyphenols are considered to have low bioavailability: in humans, consumption of large amounts of tea or polyphenols is associated

with poor iron absorption and anemia (Baynes and Bothwell 1990). Our findings from fish and polyphenols support the results of Jadan Piedra et al., who tested the effects of catechin and tannic acids on bioaccessibility in standard MeHg aqueous solutions (Jadán Piedra et al. 2016).

Other polyphenols that were measured in co-foods (Table S3) but that were not tested in their purified form could also contribute to the effect of tea. Indeed, kaempferols, thearubigins or quercetins other than rutin may have a similar effect as EGCG for example (Figure S4). Their role should be investigated, by testing more compounds in bioaccessibility assays. Furthermore, tea may include other compounds that could limit MeHg bioaccessibility. Tea leaves growing in certain areas of China has been found to be enriched in selenium (Molan et al. 2009), and selenium can interfere with Hg toxicity and bioaccessibility (Cabañero et al. 2004; 2006). While our results from purified polyphenols suggest that these compounds are the main drivers of the effects of tea on MeHg bioaccessibility, other molecules in the beverage may also have a role to play.

Tea polyphenols may also have other impacts on the fate of MeHg in the body: *in vivo* assays have suggested that tea could accelerate enterohepatic cycling of MeHg in the body (Canuel et al. 2006b) or that tea extracts may limit oxidative stress induced by MeHg in rats and alter its pharmacokinetics (Black et al. 2011). Green tea has also been found to increase Hg load in blood after consumption of MeHg-contaminated fish (Janle et al. 2015). These mechanisms should be further investigated through controlled *in vivo* experiments.

Bioaccessibility studies can be used to inform current guidelines

Studies performed on Hg and MeHg bioaccessibility in fish report values that range from 9-100%, with large variations often seen within a single species (Table 1). However, over half of the values reported in Table 1 are below 50%, suggesting that only a fraction of mercury is solubilized (bioaccessible) during simulated digestion, and thus potentially available for absorption (bioavailable) and metabolism in the intestine and liver (bioavailable) (Afonso et al. 2015a). Current guidelines and recommendations assume that 100% of MeHg in fish is readily absorbed by the gastrointestinal tract (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States 2010; Ha et al. 2016). This value is based on

early work on the excretion of an orally administered radio-labelled MeHg nitrate solution from the human body (Aberg et al. 1969). However, mercury in fish is more typically bound to sulfur-rich groups such as thiols (Clarkson and Magos 2008; Harris 2003), and may behave differently. This suggests that the form of Hg and its complexation to food or other elements may reduce its absorption, and that the 100% MeHg absorption rate likely overestimates what is bioavailable from fish. When considering raw tuna, our PDI_{BA} estimate which accounts for incomplete bioaccessibility is 71.7% of the PDI value (Table S4).

These results are amplified when considering not only bioaccessibility, but also dietary practices. Indeed, when the effects of cooking are included in PDI calculations, bioaccessibility-corrected PDI_{BAs} are reduced by 70.6 – 98.1% across all fish species (Table S4). As most fish consumed in North America is cooked (with the exception of sushi as well as traditional practices in indigenous communities), not including this factor in guidelines leads to overestimating MeHg exposure in most populations. If PDIs are corrected for green and black tea consumption, the bioaccessibility-corrected PDI_{BA} is reduced by 74.6 – 94.4% (Table S4). Finally, when correcting PDI for bioaccessibility data from a meal that is both cooked and consumed with a cup of tea, PDI_{BA} drops by 99%, to less than 1 ng kg^{-1} (Table S4). All PDIs presented here, calculated from commercially-purchased fish, were below the WHO's maximal provisional tolerable daily intake (PTDI) (0.23 μg kg^{-1} d^{-1}) (Joint FAO/WHO Expert Committee on Food Additives 2007).

It is important to note that these PDI_{BAs} are calculated from the results of a simplified *in vitro* system. While these estimates do not replace *in vivo* or epidemiological studies, they provide valuable insight into the ways Hg is solubilized from food in the gut, and may explain population-based variations in Hg toxicological responses (Canuel et al. 2006a; Chapman and Chan 2000). These bioaccessibility-based results can be used to guide more informative, but more expensive *in vivo* studies, and *in vitro* studies may be of particular relevance to populations who frequently consume fish that are more heavily contaminated than commercially-monitored species. This includes recreational fishermen, coastal populations who often depend heavily on fish (Cisneros-Montemayor et al. 2016), and indigenous groups like the Inuit, who's reliance on marine mammals and fish exposes them to higher dietary MeHg intake (Laird et al. 2013). If validated by *in vivo* studies, cooking and polyphenol-rich foods could thus be easily

implementable recommendations to reduce exposure to Hg. This could promote safe consumption of fish as a source of fatty-acids, which could protect from cardiovascular disease (Mahaffey et al. 2011; Rideout and Kosatsky 2017).

While in vitro bioaccessibility assays suggest that guidelines may overestimate MeHg exposure from fish, it is important to consider that these guidelines are designed to be overly conservative, in order to to protect especially vulnerable groups. These individuals, including children and pregnant women, are particularly sensitive to MeHg-induced health risks (Committee on the Toxicological Effects of MeHg). Criticism of guidelines should thus keep in mind the specific needs of vulnerable sub-groups.

Other factors may alter MeHg bioaccessibility

Other factors may also impact MeHg bioaccessibility, that were not taken into account here. Most bioaccessibility studies performed on MeHg in fish use commercially-purchased filets, hence the type of muscle and the area of the fish sampled are unknown. Fish muscles have diverse physiological function and cellular composition (Sänger and Stoiber 2001). Furthermore, other elements found in fish could influence bioaccessibility, such as selenium and its ratio to Hg (Cabañero et al. 2004; 2006). Future research into within-individual variations of MeHg bioaccessibility should include gradients along muscle tissues, and perform full characterizations of muscles, to assess how Hg binds fish muscle tissue in fish.

MeHg bioaccessibility has also been found to be limited by co-ingested plant products such as dietary fibers (Shim et al. 2009) and plant cell walls compounds (lignin, methylcellulose, pectin) (Jadán Piedra et al. 2016). Studies conducted on rats fed with intrinsically MeHg-contaminated food matrices (*ie.* grown in contaminated soils) also show that plant compounds can limit absorption (Yannai and Sachs 1993). Current guidelines do not take into consideration complex diets, accounting only for MeHg measured in fish muscle. Our results combined with others from the literature suggest that ignoring plant-based co-foods, which contain polyphenols and dietary fiber may overestimate Hg intake from fish. Risk assessments also overlook cooking and the combined effect of different processes involved in preparing a meal, contributing to this lack of realism.

Host genetics, including the gut microbiome could also potentially alter the fate of Hg in the body, as has been observed with other metals. For example, toxic species of arsenic and bismuth can be produced by gut bacteria prior to absorption by the epithelial lining (Diaz-Bone and Van de Wiele 2010; Van de Wiele et al. 2010), and Laird et al. observed increased arsenic bioaccessibility in the presence of a simulated gut microbiome community, compared to sterile conditions (Laird et al. 2009b). While *in vivo* methylation would increase Hg toxicity, this pathway has not yet be observed in primates (Gilmour et al. 2013; Martín-Doimeadios et al. 2017). Evidence for MeHg demethylation by the microbiome has been reported from mice models, producing poorly-absorbed inorganic Hg (Rowland 1988). Meanwhile the *mer* operon, responsible for mercury resistance and cell membrane transport, is frequent: a study of 800 antibiotic-resistance plasmids from Gram-negative bacteria have been found to carry the operon (Schottel et al. 1974). This may allow it to alter Hg cycling in the gut. Lactic acid bacteria have been found to reduce Hg bioaccessibility in mushrooms and aqueous solutions, but not in seafood (Jadán-Piedra et al. 2017a; 2017b). Interactions between dietary Hg and the microbiome are thus unclear, and should be further explored.

In vitro findings must be validated to improve current risk assessments

In vitro gastrointestinal models are useful to understand the fate of food components and contaminants in the human body, as they are inexpensive and easy to use. They allow for screening high numbers of samples in a controlled setting, can be validated with reference materials, and avoid ethical considerations of using model animals, which can be more or less relevant to humans (Fernández-García et al. 2009).

Bioaccessibility assays have been suggested as a way to improve risk assessments (Cardoso et al. 2014) and guidelines (Ángeles García et al. 2016). However, *in vitro* models are rarely validated because of the lack of *in vivo* experiments using consumer products in contaminant studies (Brandon et al. 2006; Hur et al. 2011). *In vitro* studies could be improved by integrating cell cultures such as Caco-2, to account for membrane transport and thus bioavailability estimates (Hur et al. 2011; Moreda-Piñeiro et al. 2011), but this does not take into account whole-body processes that could alter the fate of Hg, such as stimulation of the

enterohepatic cycle (Canuel et al. 2006b) or interactions with the gut microbiome, which are also determinants of bioavailability. Future work should involve *in vivo* experiments, to validate the effects of dietary practices and co-foods on Hg bioaccessibility. *In vitro* investigations remain useful, as they offer insight into the structural and complexation changes that Hg undergoes during food preparation and digestion, and provide evidence that it would be worthwhile to embark on costly and ethically-loaded *in vivo* assays. We recommend that further work on the fate of MeHg in the body should be performed in animal models such as swine, which are considered appropriate for human health risk assessments (Moreda-Piñeiro et al. 2011).

Culturally-specific guidelines may also be necessary: for example, current pharmacokinetic models are poor predictors of Hg burden in Canadian indigenous populations (Canuel et al. 2006a), who have a different genetic background, but who also have specific dietary practices regarding food preparation and co-foods. This supports observations made by a European study on MeHg risk assessment, which showed that guidelines should be population and country-specific (Jacobs et al. 2016). While smaller, low-trophic level fish could be recommended over more contaminated species to promote fish intake, this solution may not be implementable in developing countries or coastal populations who rely on high-trophic level organisms. Risk assessments based on validated bioaccessibility data could provide specific recommendations for various populations, who are exposed to dietary MeHg in different ways. This may lead to easily applicable, non-invasive guidelines that allow a population to adapt its food preparation to limit exposure to Hg in culturally important foods. This could be of critical importance for coastal indigenous populations, which have a per capita fish consumption 15 times greater than non-indigenous peoples (Cisneros-Montemayor et al. 2016). Altering MeHg bioaccessibility through dietary practices in these populations could have important consequences on Hg absorption and health. Since fish are an excellent source of protein, vitamins, fatty acids and minerals which feed a significant portion of the world's population and are associated with cardiac health, it is critical to better understand the risk that Hg in fish represents to human health.

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Supplemental Material

Supplementary Methods

MeHg analyses

Calibration curves were prepared using certified MeHg solutions. In brief, a MeHg stock solution (1000 ppm, certified by Alfa Aesar) was diluted in methanol (Fished Scientific, HPLC grade) to prepare a 1 mg L^{-1} MeHg solution. Intermediate working solutions (10.0 μg L^{-1}, 600 ng L^{-1} and 10 ng L^{-1}) were freshly prepared in MilliQ water and preserved with 0.3% acetic acid (Fisher Scientific, ACS-pur) and 0.2% HCl (EMD, Omni-trace ultra).

Fish matrix characterization

Lipids were quantified by gravimetry, using a method adapted from Folch et al. 1957. Briefly, 5 mL of a 2:1 chloroform:methanol solution (>99.8 and 99.9%, Fisher Scientific) were added to a Falcon tube containing 0.5 – 1 g of lyophilized and grinded fish sample. Tubes were vortexed for 15 seconds, then left under a fumehood overnight. Samples were vortexed again for 15 seconds, and centrifuged for 5 minutes at 1,500 g. Supernatant was poured into pre-weighed aluminum vessels, the pellet was washed with 2:1 chloroform:methanol solution and centrifuged once more, and washing solution was added to the vessel. Solutions were air-dried under a fumehood overnight, and were weighed. Percent lipid content was obtained with the following equation:

[Equation S1]

$$\%\ lipid\ content = \frac{mass\ of\ vessel\ with\ sample\ (g) - mass\ of empty\ vessel\ (g)}{mass\ of fish\ extracted\ (g)}$$

Nitrogen was used as a proxy for protein content, and was quantified using a CHN Element Analyzer 1108 (Thermo Fisher). Samples were weighed in tin capsules and then burnt

in a combustion column at 1,040 °C and reduced in a reduction column at 650 °C, converting nitrogen into N_2. N_2 was separated from other gases generated during reduction by gas chromatography, then quantified by a thermo-electric detector. Calibrations were performed with acetanilide. Atropine and sulfanilamide (Elemental Microanalysis Limited) were used as standards.

Moisture content in fish muscle was quantified by subtracting sample dry weight from wet weight after drying.

Polyphenol analyses

Polyphenols were quantified by UPLC-MSMS using a Waters Acquity Ultra-Performance LC system with a quaternary pump system and an Acquity high-strength silica T3 column (120 mm x 2.1 mm, 1.8 mm particle size) (Waters). The stationary phase was 100% silica particles. Compounds were separated with a mobile phase of 0.2% acetic acid (eluent A) and acetonitrile (eluent B), with a flow rate of 0.4 mL min^{-1}. The gradient elution was as follows: initial 5% B, 5-20% B (0 – 4.5 min), isocratic 20% B (4.50 – 6.45 min), 20-45% B (6.45 – 13.50 min), 45-100% B (13.5 – 16.5 min), isocratic 100% B (16.5-19.5 min), 100-5% B (19.5 – 19.52 min), isocratic 5% B (19.52 – 22.5 min). MS analyses were performed on a TQD mass spectrometer (Waters) with a Z-spray electrospray interface in negative mode, with data acquisition carried out by multiple reactions monitoring (MRM). Ionization source parameters were as follow: capillary voltage at 2.5 kV, source temperature at 140 °C, cone gas flow rate of 80 L h^{-1}, desolvation gas flow rate of 900 L h^{-1} and desolvation temperature of 350 °C. Nitrogen (>99%) was used as a nebulizing gas, and argon (>99%) as a collision gas. Data acquisition was performed with MassLynx 4.1 software. Results were quantified as gallic acid equivalents, and detection limits were calculated at 0.9 μg g^{-1} using standard gallic acid.

Supplementary Tables

TABLEAU SI. Composition of fish matrix from the four species tested in bioaccessibility experiments. Values are reported for an individual fish, which was used in numerous subsequent analyses. Moisture was calculated from wet weight, while proteins and lipids were quantified in dry matter). Means are shown with standard deviation, with range in brackets and number of individual fish per species (*n*).

Fish	MeHg (ng g^{-1})	Protein (%)	Lipids (%)	Moisture (%)
Swordfish	439.20 ± 237.47 ng g^{-1} (152.48-679.8) *n*=4	86.35 ± 3.41% (13.25-14.33) *n*=3	11.85 ± 2.36% (8.79-14.89) *n*=4	70.06 ± 1.18% (69.13-72.23) *n*=4
Grouper	612.42 ± 315.32 ng g^{-1} (389.45-835.39) *n*=2	86.35 ± 0.36% (13.77-13.88) *n*=3	8.55 ± 1.20% (6.40-9.09) *n*=2	64.43 ± 20.35% (50.04-78.82) *n*=2
Tuna	694.00 ± 777.80 ng g^{-1} (144.01-1243.98) *n*=2	87.42 ± 0.34% (13.93-14.04) *n*=3	10.05 ± 0.13% (9.91-10.16) *n*=2	60.66 ± 11.88% (52.26-69.06) *n*=2
Salmon	20.16 ng g^{-1} (-) *n*=1	n/a	26.54% (-) *n*=1	70.00% (-) *n*=1

TABLEAU SII. Polyphenol content in different beverages and foods used in MeHg bioaccessibility, measured by UPLC-MS/MS. Polyphenols presented here are those we subsequently tested in their purified form. EGCG: epigallocatechin gallate; ND: undetected (detection limit = 0.9 μg g^{-1}).

Food item	Gallic acid (μg g^{-1})	Catechin (μg g^{-1})	Epicatechin (μg g^{-1})	EGCG (μg g^{-1})	Theaflavin (μg g^{-1})	Rutin (μg g^{-1})	Caffeic acid (μg g^{-1})
Black tea A	8218.01	1338.73	4428.45	17505.91	153.97	41138.98	58.21
Black tea B	7892.87	1226.50	3816.15	25131.52	52.71	47043.56	104.86
Green tea A	1431.36	1770.37	25154.20	96256.16	167.06	38485.35	30.29
Green tea B	3376.76	2269.17	16921.85	110499.05	133.59	15277.43	75.13
Coffee	ND	ND	ND	ND	ND	ND	331.34
Instant coffee	ND	ND	ND	ND	ND	ND	497.10
Blueberries	11.52	33.49	ND	84.35	ND	147.57	50.89
Cornstarch	ND	ND	ND	31.31	ND	ND	ND

TABLEAU SIII. Raw polyphenol content in beverages and foods tested in bioaccessibility experiments, measured by UPLC-MSMS ($\mu g\ g^{-1}$).

Polyphenolic compound	Black tea A	Black tea B	Green tea A	Green tea B	Coffee	Instant coffee	Blueberries	Cornstarch
Quercetin	536.98	302.52	135.76	126.82	ND	ND	112.34	ND
Quercetin glucoside	3,067.32	7,732.63	2,759.58	1,669.35	ND	ND	5,266.82	ND
Quercetin galactoside	7,994.12	16,543.78	3,716.84	3,605.19	ND	ND	ND	ND
Quercetin rhamnoside	178.88	149.41	248.81	16.63	ND	ND	ND	ND
Quercetin-3-xyloside	396.15	682.85	252.01	121.80	ND	ND	787.78	ND
Quercetin-3-arabinoside	35.29	324.25	ND	9.53	ND	ND	ND	ND
Quercetin diglucoside	ND	ND	ND	ND	ND	ND	ND	ND
Kaempferol	16.03	11.01	ND	26.19	ND	ND	ND	ND
Kaempferol galactoside	14.65	34.50	56.24	63.04	ND	ND	ND	ND
Kaempferol glucoside	644.11	2,377.26	131.32	1103.18	ND	ND	ND	ND
Myricetin	9.42	ND	5.73	39.92	ND	ND	34.37	ND
Myricetin glucoside galactoside	ND	7.37	2.99	27.33	ND	ND	ND	ND
Isohamnetin	ND	ND	ND	ND	ND	ND	2.78	ND
Rutin	41,138.98	47,043.56	38,485.35	15,277.43	ND	ND	147.57	ND
Fisetin	ND	ND	ND	ND	ND	ND	ND	ND
Phlorizin	ND	ND	ND	ND	ND	ND	ND	ND
Ellagic acid	ND	ND	ND	ND	ND	ND	ND	ND
Catechin	1,338.73	1,226.50	1,770.37	2,269.17	ND	ND	33.49	ND
Epicatechin	4,428.45	3,816.15	25,154.20	16,921.85	ND	ND	ND	ND
Gallic_acid	8,218.01	7,892.87	1,431.36	3,376.76	ND	ND	11.52	ND
4-Hydroxybenzoic acid	215.80	49.55	16.22	22.22	41.51	43.76	ND	ND
3-Hydroxybenzoic acid	ND	3.95	7.96	ND	41.51	43.76	ND	ND
p-Coumaric acid	571.89	317.55	63.55	220.62	ND	ND	ND	43.35
m-Coumaric acid	96.71	60.04	ND	28.70	ND	ND	ND	ND
o-Coumaric acid	ND	ND	ND	ND	ND	ND	ND	ND
Vanillic acid	ND	ND	ND	ND	ND	ND	ND	ND
Caffeic acid	58.21	104.86	30.29	75.13	331.34	497.10	50.89	ND
Ferulic acid	15.95	30.63	ND	1.73	56.74	48.27	1.83	ND
Protocatechuic acid	770.17	86.79	83.75	83.03	196.94	116.46	20.83	ND
Gentisic acid	4.89	ND	ND	ND	ND	ND	ND	ND
b-Resorcyclic acid	4.02	43.42	44.80	17.46	50.49	11.50	ND	ND
Hypogallic acid	52.08	43.42	44.64	13.58	50.49	ND	ND	ND

y-Resorcyclic acid	ND	ND	ND	ND	5.92	ND	32.10	7.72
Syringic acid	ND	ND	ND	ND	ND	ND	11.42	ND
Sinapic acid	ND	ND	ND	ND	ND	ND	ND	ND
Shikimic acid	209.20	174.16	191.49	186.32	59.73	32.36	37.41	ND
Syringaldehyde	ND	ND	ND	ND	ND	ND	ND	ND
Hydrocaffeic acid	ND	ND	ND	ND	ND	ND	ND	ND
Homovanillic acid	ND	ND	ND	ND	ND	ND	ND	ND
2,5-Dimethoxybenzoic acid	ND	ND	ND	ND	ND	ND	ND	ND
4-Hydroxy pheylacetic acid	ND	ND	ND	ND	ND	ND	ND	ND
3-Hydroxy phenylacetic acid	ND	ND	ND	ND	ND	ND	ND	ND
4-Methoxycinnamic acid	ND	4.38	ND	ND	4.64	ND	ND	ND
Ferruloyl glucoside	ND	ND	ND	ND	ND	ND	ND	ND
Caffeoyl glucoside	68.10	81.34	129.16	163.77	24.65	16.49	411.63	ND
Coumaroyl glucoside	344.54	141.79	194.19	723.58	3.09	3.19	170.91	ND
Resveratrol	ND	ND	ND	ND	ND	ND	ND	ND
Pterostilbene	ND	ND	ND	ND	ND	ND	ND	ND
Neochlorogenic acid	186.59	1,833.31	46.43	746.91	32,260.45	21,900.79	28.86	ND
Chlorogenic acid	926.65	4,975.39	454.36	1,847.19	100,554.22	60,444.60	17,177.49	ND
Cryptochlorogenic acid	33.22	425.32	60.27	116.45	1,430.06	1,267.85	1,511.85	ND
EGCG	17,505.91	25,131.52	96,256.16	110,499.05	ND	ND	84.35	31.31
Luteolin	ND	ND	ND	ND	ND	ND	ND	ND
Apigenin	ND	ND	ND	ND	ND	ND	ND	8.30
Theaflavin	153.97	52.71	167.06	133.59	ND	ND	ND	ND
Thearubigin	8.11	23.92	68.27	94.35	ND	ND	ND	ND

TABLEAU SIV. Provisional daily intake (PDI) and bioaccessibility-corrected PDI (PDI_{BA}) for all fish and treatments tested in this experiment.

Treatment	Swordfish		Grouper		Tuna		Salmon	
	PDI $\mu g\ kg^{-1}$	PDI_{BA} $\mu g\ kg^{-1}$	PDI $\mu g\ kg^{-1\ 1}$	PDI_{BA} $\mu g\ kg^{-1}$	PDI $\mu g\ kg^{-1}$	PDI_{BA} $\mu g\ kg^{-1}$	PDI $\mu g\ kg^{-1}$	PDI_{BA} $\mu g\ kg^{-1}$
Control (raw, unamended)	0.126	0.037	0.143	0.096	0.053	0.015	0.007	0.003
Grilling		0.007		0.012		0.002		<0.001
Frying		0.003		0.011		0.001		<0.001
Boiling		0.003		0.023		0.002		<0.001
Control (raw, unamended)	0.126	0.032	0.306	0.072	0.456	0.075	-	-
Green tea		0.007		0.017		0.026		-
Black tea		0.007		0.025		0.037		-
Control (raw, unamended	0.249	0.084	-	-	-	-	-	-
EGCG		0.040		-		-		-
Control (raw, unamended)	0.126	0.050	-	-	0.456	0.082	-	-
Grilling + green tea		0.001		-		<0.001		-

Supplementary Figures

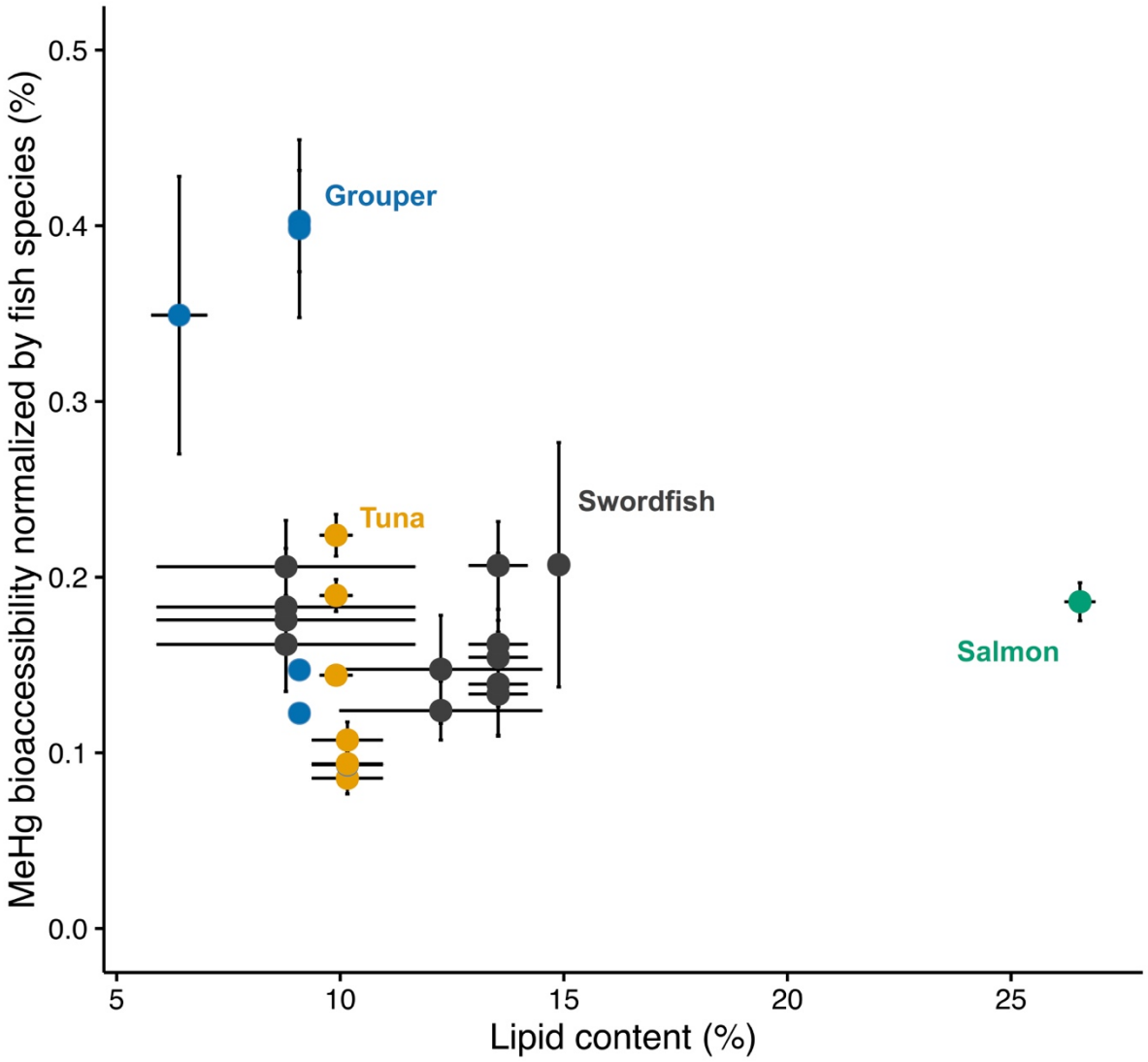


FIGURE S1. MeHg bioaccessibility in relation to lipid content in different fish species (linear regression, $P > 0.05$). Points present averages from individual fish, and error bars show standard deviation for lipid content (x-axis) and MeHg bioaccessibility (y-axis).

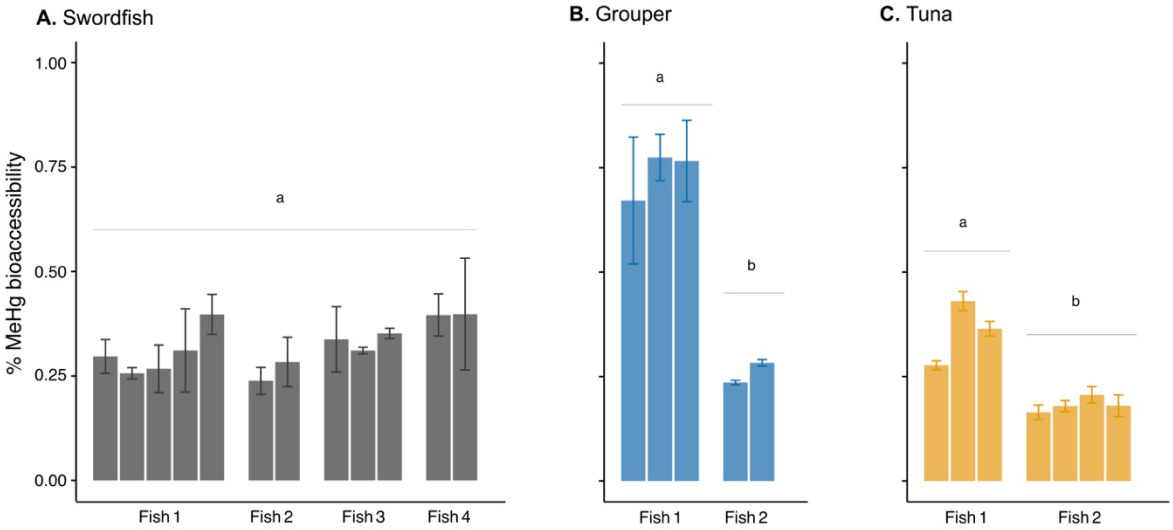


FIGURE S2. MeHg bioaccessibility in raw fish from different swordfish, grouper and tuna individuals. Each bar shows the average MeHg bioaccessibility in 3 replicate samples of a fish filet, digested by PBET simulations on the same experimental day. Multiple bars for a single fish show between-run variation in PBET results. Within a given individual, bioaccessibility did not vary significantly between experimental days (Kruskal-Wallis, $P < 0.05$). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

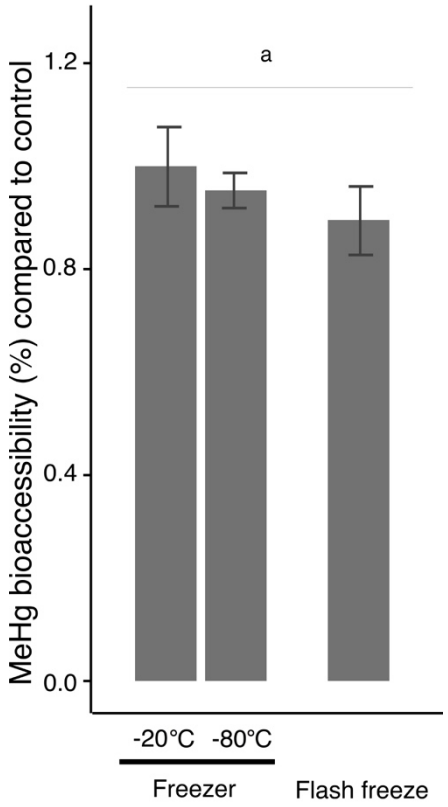


FIGURE S3. Effect of freezing on MeHg bioaccessibility in swordfish, which had already been frozen by the vendor prior to purchase. Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

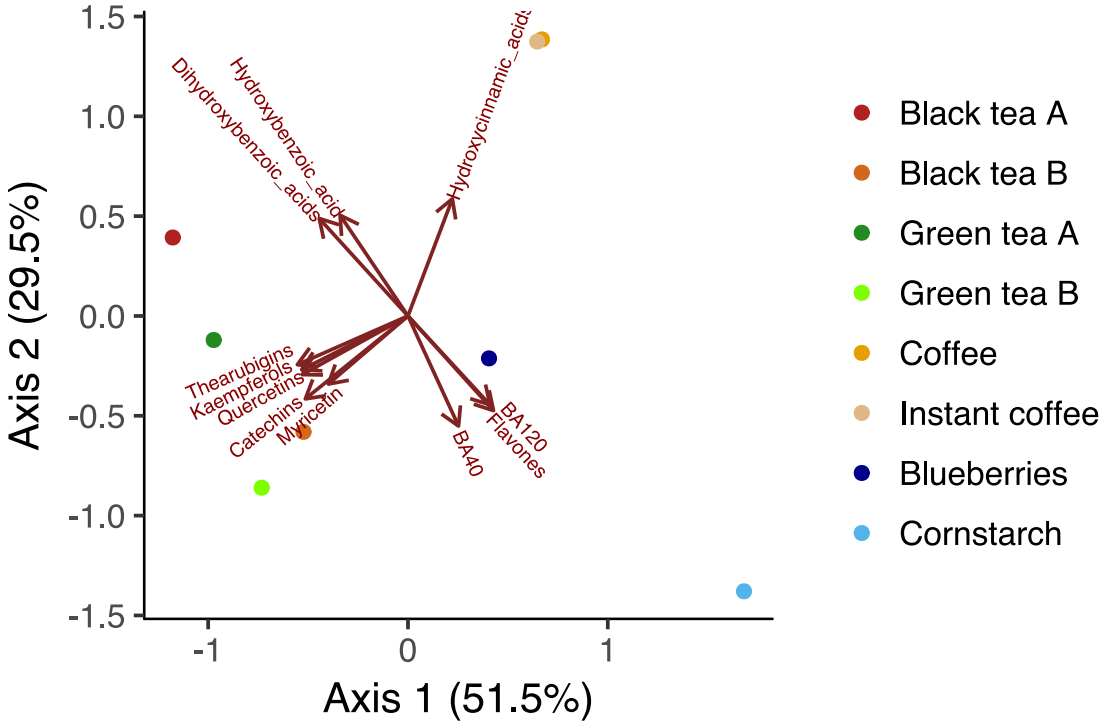


FIGURE S4. Principal components analysis (PCA) correlation biplot showing bioaccessibility from experiments performed with polyphenol-rich treatments (colored points) and polyphenol content, broken down into 9 categories (red arrows). The PCA accounts for 81% of total variation from Axes 1 and 2.

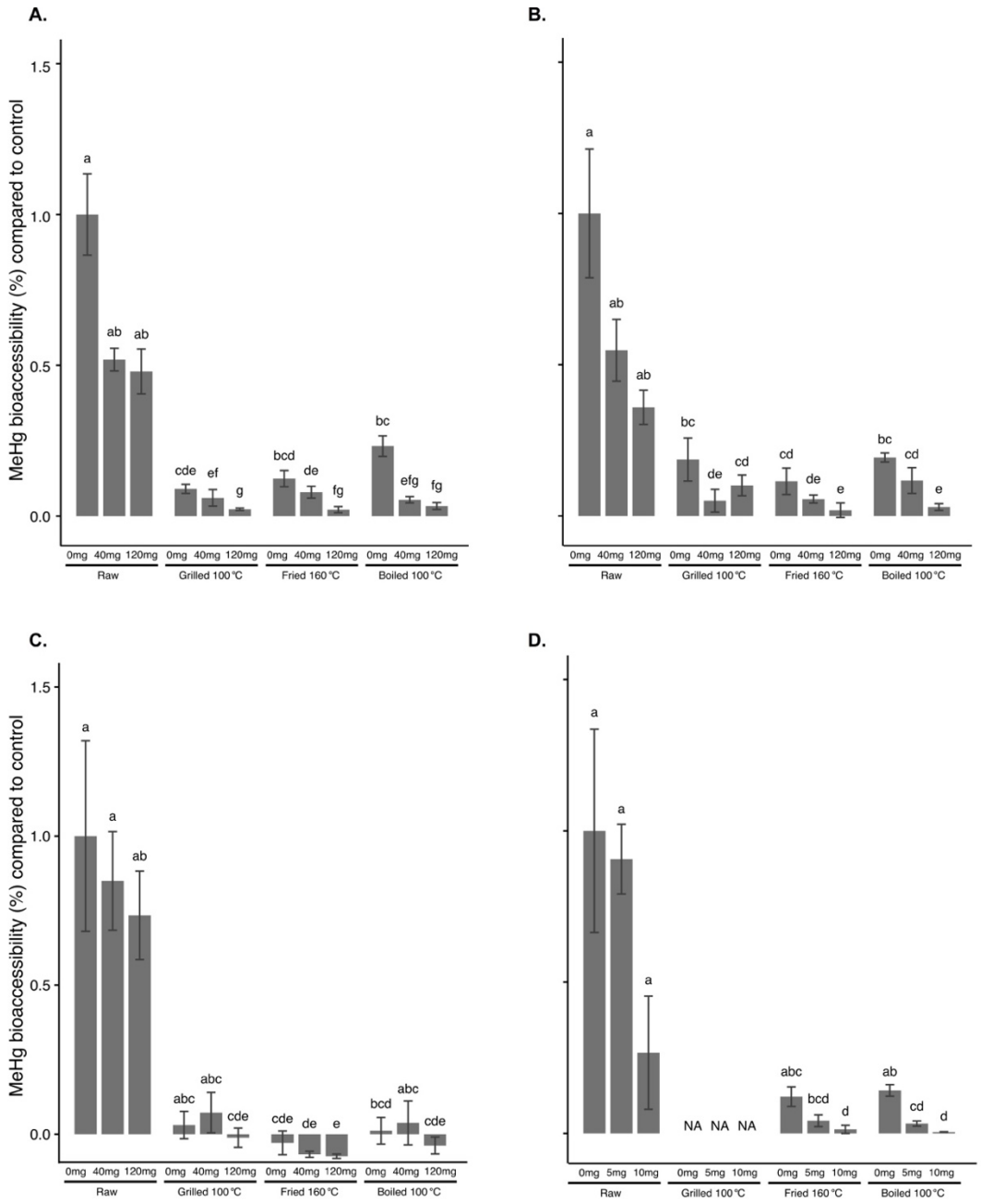


FIGURE S5. Mixed effect of cooking and polyphenol/beverages on MeHg bioaccessibility in swordfish for polyphenol-rich beverages and foods (A. coffee, B. instant coffee and C. blueberries) and for pure polyphenols compounds (D. epigallocatechin gallate). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

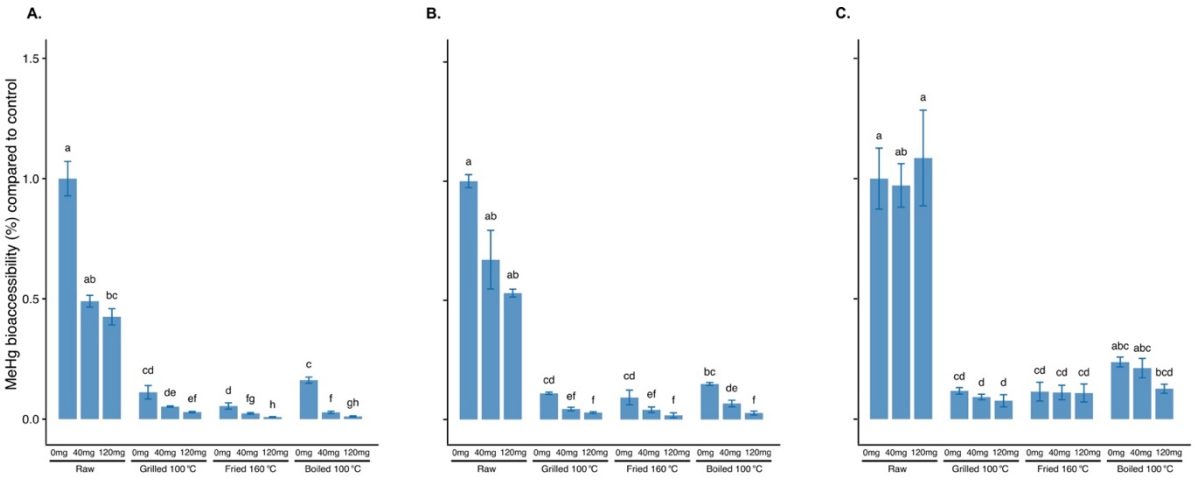


FIGURE S6. Mixed effect of cooking and polyphenols-rich beverages on MeHg bioaccessibility in grouper (A. coffee, B. instant coffee and C. blueberries). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

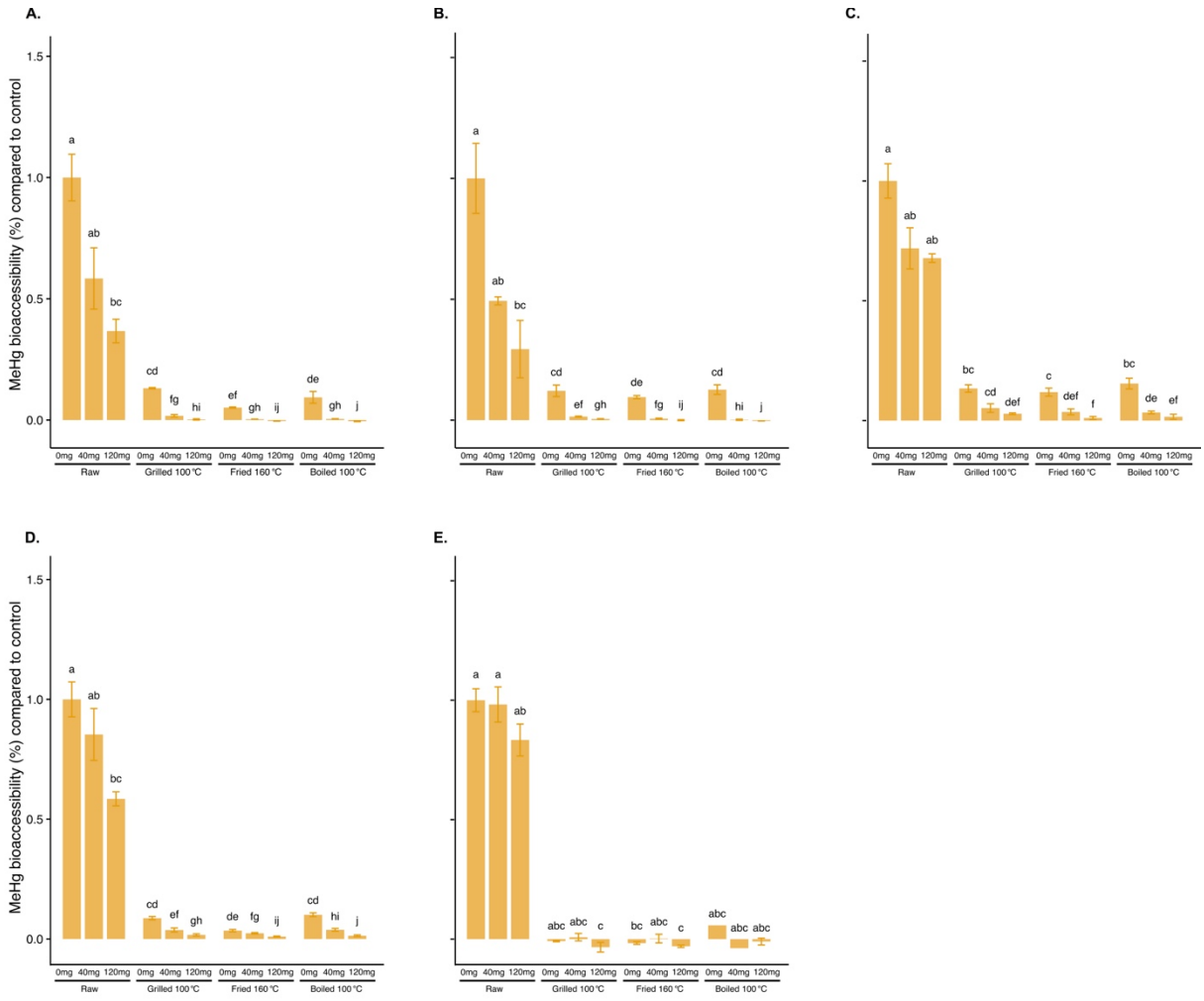
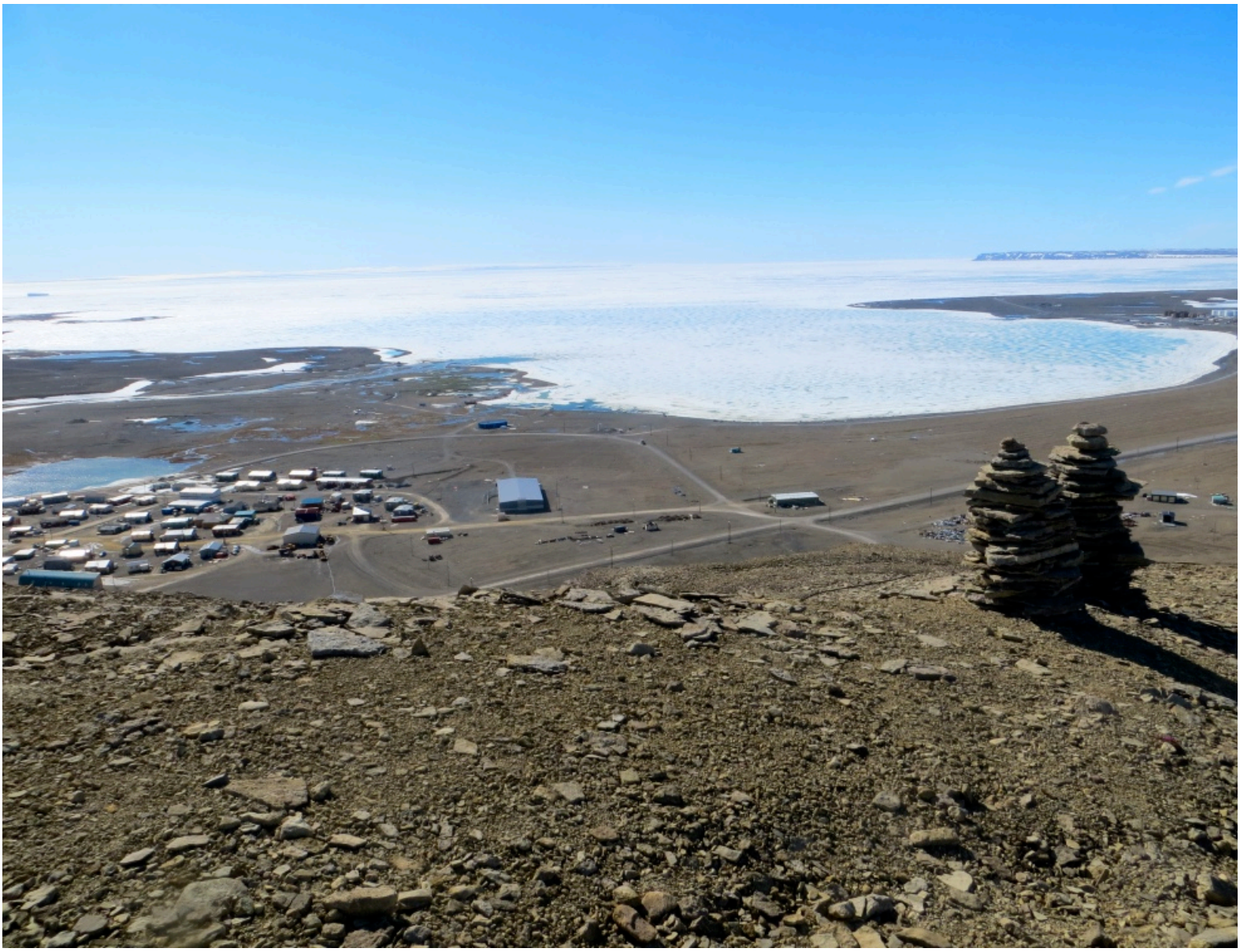


FIGURE S7. Mixed effect of cooking and polyphenols/beverages on MeHg bioaccessibility in tuna. A. Black tea, B. green tea, C. coffee, D. instant coffee and E. blueberries. Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.



CHAPITRE 3. Microbiome intestinal des Inuits de l'Arctique canadien

Gut microbiome of the Canadian Arctic Inuit

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Abstract

Diet is a major determinant of community composition in the human gut microbiome, and "traditional" diets have been associated with distinct and highly diverse communities, compared to Western diets. However, most traditional diets studied have come from agrarians and hunter-gatherers consuming fiber-rich diets. In contrast, the Inuit of the Canadian Arctic have been consuming a traditional diet low in carbohydrates and rich in animal fats and protein for thousands of years. We hypothesized the Inuit diet and lifestyle would be associated with a distinct microbiome. We used deep sequencing of the 16S rRNA gene to compare the gut microbiomes of Montrealers with a Western diet to Inuit consuming a range of traditional and Western diets. At the overall microbial community level, Montrealers and Inuit were indistinguishable and contained similar levels of microbial diversity. However, we observed significant differences in the relative abundances of certain microbial taxa, down to the sub-genus level using oligotyping. For example, *Prevotella*, which has been previously associated with high-fiber diets, was enriched in Montreal and among Inuit consuming a Western diet. Inuit consuming a traditional diet also had significantly less genetic diversity within the *Prevotella* genus, suggesting that low fiber might not only select against *Prevotella*, but also reduce its diversity. Other microbes, such as *Akkermansia*, were associated with geography as well as diet, suggesting limited dispersal to the Arctic. Our study provides a snapshot of the Inuit microbiome as Western-like in overall community structure, but distinct in the relative abundance and diversity of certain genera and strains.

Importance

Non-western populations have been shown to have distinct gut microbial communities shaped by traditional diets. The until now uncharacterized microbiome of the Inuit may help better understand health risks specific to this population like diabetes and obesity, which increase in prevalence as many Inuit transition to a Western diet. Here we show that even Inuit consuming a mostly traditional diet have a broadly Western-like microbiome. This suggests that similarities between the Inuit and Western diet (low fiber, high fat) may lead to the convergence

of community structure and diversity. However, certain species and strains of microbes have significantly different abundance and diversity in the Inuit, possibly driven by differences in diet. Furthermore, the Inuit diet provides an exception to the correlation between traditional diets and high microbial diversity, potentially due to their transitioning diet. Knowledge of the Inuit microbiome may provide future resources for interventions and conservation of Inuit heritage.

Introduction

The human gut microbiome is a complex ecosystem of microbes that contribute to host immunity, nutrition and behavior (Cryan and Dinan 2012; Nicholson et al. 2012; Turnbaugh et al. 2006) and varies with diet, lifestyle and disease (David et al. 2014; 2013; Morgan et al. 2012; Zimmer et al. 2011). The gut microbiome is an important source of genetic and metabolic variation across human populations (Mueller et al. 2006; Yatsunenko et al. 2012). Diet is one of the main drivers of community structure of the intestinal microbial community (David et al. 2013), and much effort has been put into characterizing the microbiome of populations with contrasting dietary habits. Studies comparing microbiomes of Westerners to agrarian or hunter-gatherer populations found significant differences associated with their contrasting diets (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012). For example, *Prevotella* and *Xylanibacter* were associated with a diet rich in indigestible polysaccharides due to their fermentative abilities (De Filippo et al. 2010). Short-term consumption of an extreme animal-rich diet (composed entirely of animal products) was experimentally shown to significantly alter the human gut microbiome, increasing the relative abundance of *Bacteroides*, *Bilophila* and *Alistipes*, while reducing polysaccharide-degrading *Firmicutes* (David et al. 2013). However, animal-rich diets have yet to be explored in more realistic, natural contexts, such as the Inuit inhabiting the Arctic regions of the world (Cordain et al. 2000).

The Inuit are a traditionally nomadic culture based around the hunt and gathering of food from the environment (Sharma 2010). The Arctic environment has shaped the traditional Inuit diet, which includes many land and marine mammals, such as caribou, muskox, seal, whale and fish, and this traditional diet has been consumed for hundreds, if not thousands of years (Raghavan et al. 2014). Meat is often consumed raw, and occasionally frozen, dried or cooked. Like many other indigenous peoples around the world, the Inuit are undergoing a rapid transition away from their traditional diet toward a more Western diet (Kuhnlein et al. 2004; Sharma 2010). For the Inuit, major lifestyle changes in the last hundred years (including settlement into permanent communities) have favored a shift toward processed store-bought foods shipped from the South, and away from traditional food, leading to lower micronutrient intakes (Kuhnlein et al. 2004; Sharma 2010; Sharma et al. 2010). This shift could impact the gut microbiome of the

Inuit, with potential health consequences. The modern Inuit diet is therefore different from the ancestral diet, with Western food becoming more and more popular. The Inuit may thus be an example of a population nearing the end of their transition towards the Western diet (Kuhnlein et al. 2004; Sharma 2010).

The Inuit have a unique set of health risks, many of which could be modulated by the microbiome. For instance, obesity rates in Northern Canada currently exceed the national average (Kuhnlein et al. 2004). However, the consequences of obesity may be different for the Inuit: a study comparing the Inuit to Europeans and Southern Canadians found that at every BMI level, the Inuit had lower blood pressure and lipid levels than their Western counterparts (Young et al. 2007). It is currently unclear how the Inuit microbiome might contribute to these different clinical manifestations of obesity, and other Inuit-specific health risks.

We hypothesized that modern Inuit harbor a distinct gut microbiome, associated with traditional diet. We compared the gut microbiome of an Inuit population with a range of traditional and Western diets to individuals from Montreal (Canada), adhering to a typical Western diet. We found that at the broad scale of the entire gut microbiome community, the Inuit resemble Montrealers in both community composition and diversity. However, we identified subtle but significant differences in the relative abundances of several microbial taxa, driven by a combination of diet and environmental factors.

Results

Study populations

To compare gut microbial diversity and community composition, we deeply sequenced 16S amplicons from stool samples. We sequenced samples from 19 adults from an Arctic community in the Canadian territory of Nunavut (16 Inuit, 2 individuals of European descent and 1 person of mixed-heritage), and 26 adults of European descent from Montreal – a metropolitan area at a temperate latitude (Materials and Methods; Tables S1A & B). The majority of participants from Nunavut adhered to a modern traditional Inuit diet, and frequently

consumed raw game, especially sea mammals (Fig. S1; Table S1A). The Inuit diet is limited in plant-derived foods and is enriched in animal protein (Hopping et al. 2010; Kuhnlein et al. 2004) and is an excellent source of vitamins, minerals and micronutrients (Kuhnlein et al. 2004). For the purposes of this study, we defined a traditional diet when traditional meats were consumed daily or multiple times a week (Table S1C). This definition does not exclude individuals who also consumed imported or packaged foods. However, three Nunavut participants who never or only occasionally consumed the traditional Inuit diet (once per week or less) were classified as having a fully Western diet, along with participants from Montreal. The average body mass index (BMI) of participants in Nunavut (28.1 ± 7.0 kg m^{-2}) was significantly higher than in Montreal (23.4 ± 3.5 kg m^{-2}) (t-test, $P < 5 \times 10^{-3}$; Table S1B).

The small cohort sampled from a single isolated community in this study cannot represent all Inuit. For the sake of clarity, the term "Inuit" here is used to refer to our small cohort, but should not be inferred as representing all Inuit worldwide.

Similar levels of alpha diversity between Inuit and Western microbiomes

In previous studies of the gut microbiome, greater diversity has consistently been observed in agrarians and hunter-gatherers compared to Western industrialized populations (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012). We therefore asked whether the Nunavut population or the traditional Inuit diet were associated with high or low microbiome diversity. Using a variety of diversity indices, we found no significant differences in the observed number of bacterial operational taxonomic units (OTUs, defined at 97% nucleotide identity; Materials and Methods) or other alpha diversity metrics between Montreal and Nunavut (Mann-Whitney test, $P > 0.05$) (Fig. 1A; Fig. S2). No differences in diversity were observed by diet (Fig. 1B; Fig. S2). When restricting the dietary comparison to Nunavut residents only, thereby controlling for geography, there was a tendency toward lower diversity in participants consuming an Inuit diet, although not statistically significant (Fig. S2C). Ethnicity did not have a measurable impact on alpha diversity (Fig. S2E).

On average, overweight individuals (BMI > 25) had lower Shannon and Simpson diversity (Manny-Whitney test, $P < 0.05$; Fig. S2D). They also contained slightly fewer OTUs than lean individuals (BMI < 25), although this difference was not statistically significant (Mann-Whitney test, $P > 0.05$; Fig. 1C). The same trend toward lower diversity in overweight individuals was observed in the Montreal and Nunavut populations analyzed separately, and also in a much larger dataset of North Americans (Fig. 2). Within a given BMI bin, there is a slight (not statistically significant) tendency for individuals from Nunavut to have higher diversity than Montrealers (Fig. S3).

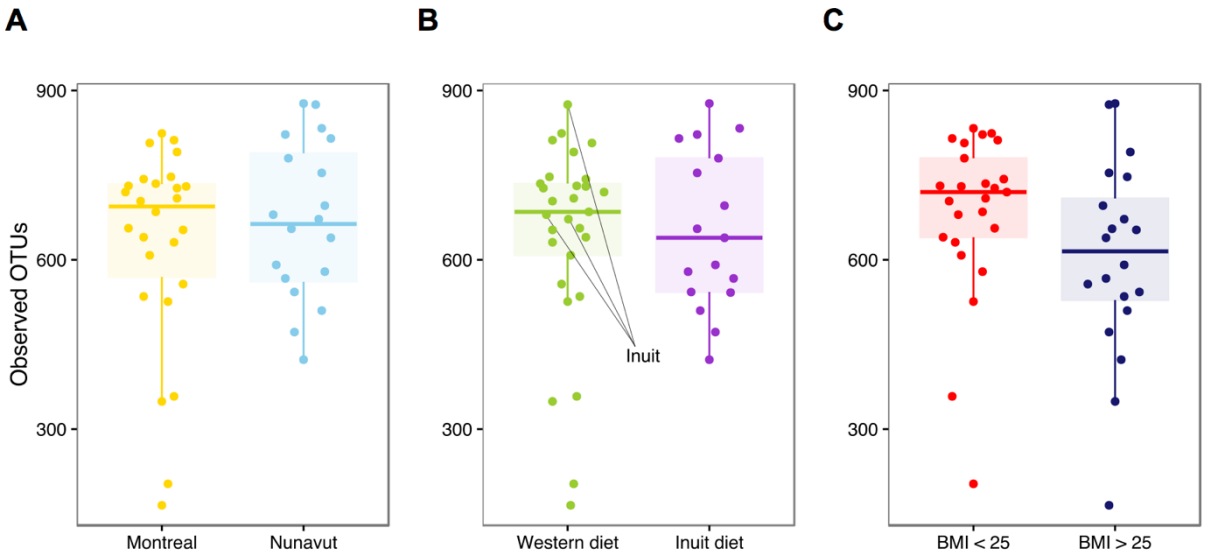

FIGURE 1. Similar microbiome diversity across diet and geography. We observed no significant differences in microbial taxonomic diversity in samples compared by A. geography, B. diet, or C. BMI (Mann-Whitney test, $P > 0.05$). See Fig. S2 for other diversity indices. OTUs were counted using open-reference (Methods). Boxplots show the median and whiskers show 25% and 75% quartiles.

The Inuit microbiome is broadly similar to the Western microbiome

Previous comparisons of Western and traditional diets have also found significant differences in overall gut community composition, attributable to genetics, cultural practices, diet or geography (Clemente et al. 2015; De Filippo et al. 2010; Mueller et al. 2006; Schnorr et

al. 2015; Yatsunenko et al. 2012). In contrast, we found that Montreal and Nunavut community compositions were similar, and did not cluster according to geographic location or diet (Fig. 3A & C). This lack of clustering was observed regardless of the distance metric used to compare microbiomes (Fig. S4A & B). The Montreal cohort contains two possible outliers (visible in the bottom-left quadrant of Fig. 3A) which also contain relatively few OTUs (two lowest points in Fig. 1A). However, these samples likely lack rare OTUs, as they cluster with other samples in the weighted UniFrac PCoA (Fig. 3C). Montreal and Nunavut gut microbial community structures are therefore broadly similar, and both cohorts cluster near other Western populations and away from agrarian and hunter-gatherer groups from Burkina Faso, Tanzania, and Venezuela (Fig. 3B and D, adonis adjusted R^2 = 6.5 and 10.5% for unweighted and weighted UniFrac respectively, $P < 0.001$). While there is methodological bias involved in such a comparison (with study effects explaining 15.5% of variation in unweighted UniFrac and 17.1% in weighted UniFrac; adonis, $P < 0.001$), agrarian and hunter-gatherer populations clearly cluster apart from Western groups along PCoA axis 1, and Nunavut samples overlap with other Western populations. Genetic relatedness and ethnicity did not significantly explain clustering (Fig. S4C& D) consistent with a relatively minor effect of human genetics on overall microbiome composition, as has been observed in another indigenous population (Sankaranarayanan et al. 2015). We did not identify any other factors (age, gender, or BMI) that could explain the variation in gut microbial community structure (adonis, $P > 0.05$). Consistent with broadly similar microbial communities, we found no significant differences between populations in stool short chain fatty acid profiles (Fig. S5).

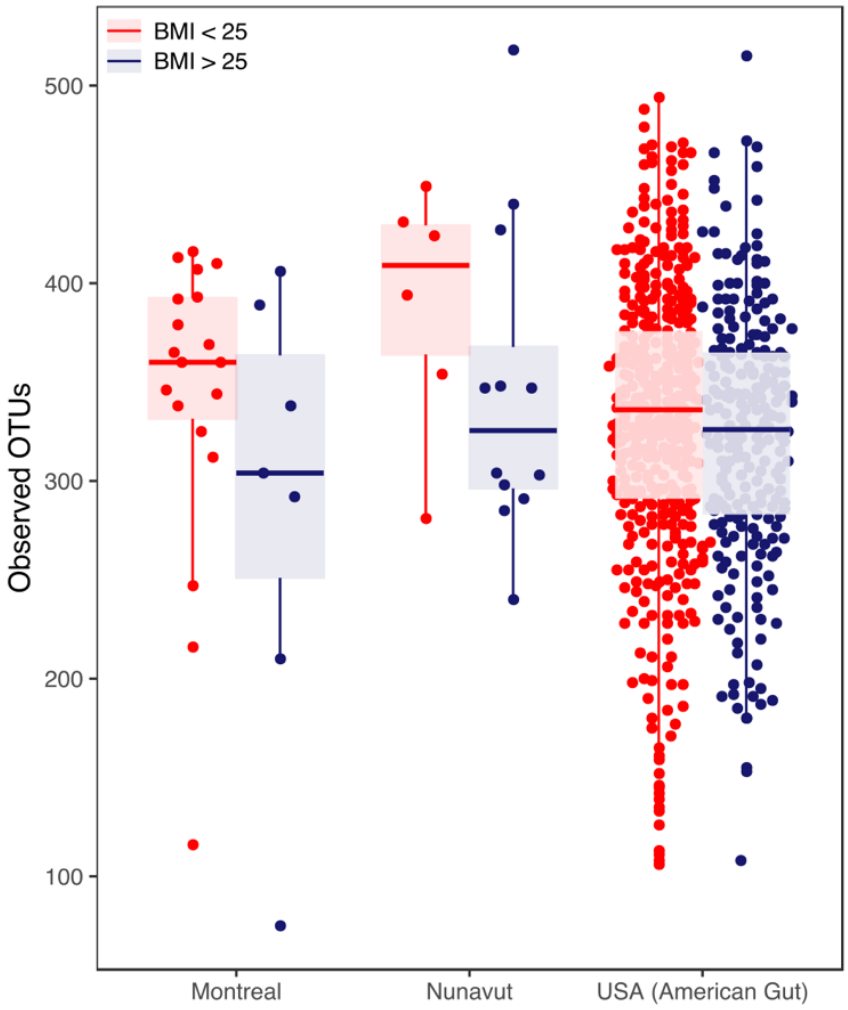

FIGURE 2. Comparison of microbiome diversity by BMI, stratified by geography. To put our data in the context of a larger study, we performed closed-reference OTU picking to compare OTU counts across our dataset and 1000 random samples from the American Gut project. In all geographic regions (Montreal, Nunavut or USA), lean individuals (BMI<25) had slightly higher diversity (number of observed OTUs) than overweight individuals (BMI>25), but the differences were not significant for any of the comparisons (Mann-Whitney test, $P > 0.05$). Boxplots show the median and whiskers show 25% and 75% quartiles.

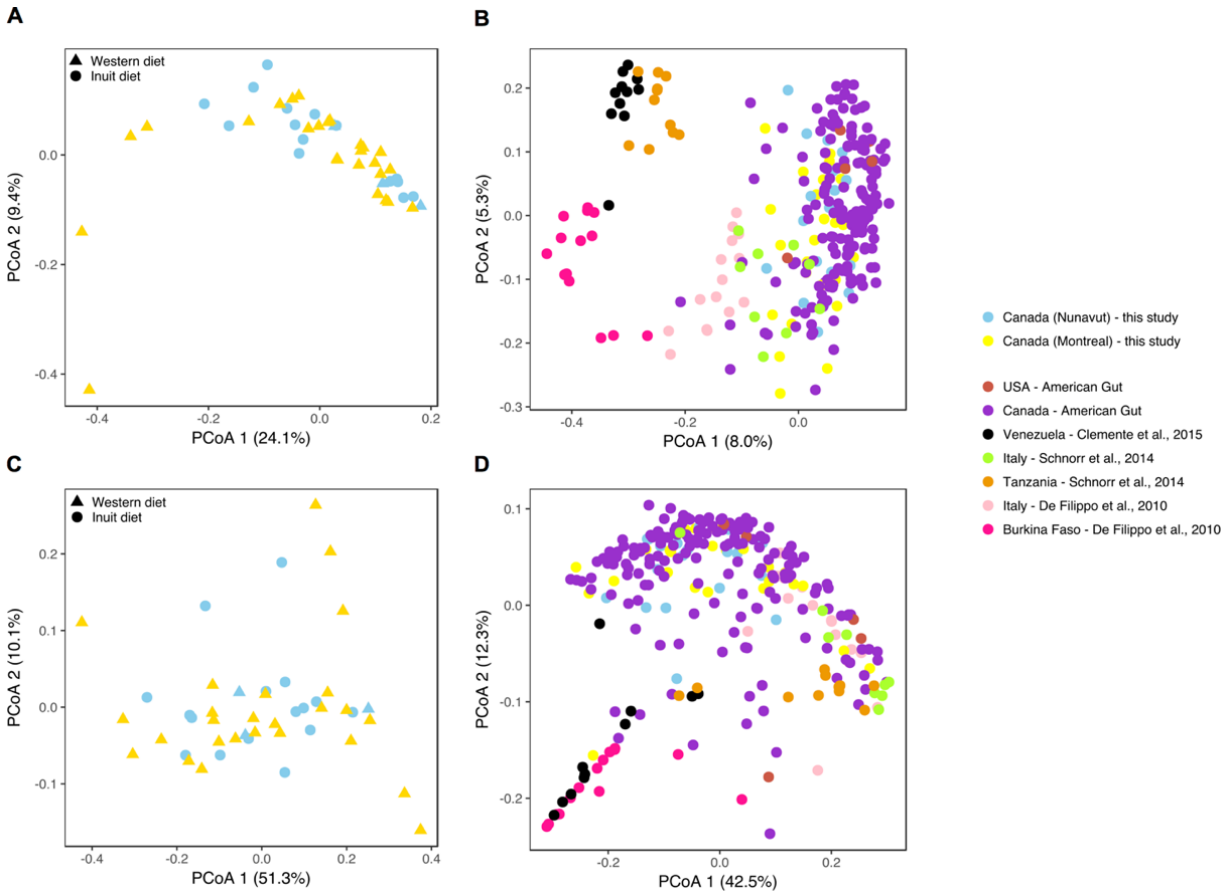

FIGURE 3. The Inuit microbiome has similar community composition as the Western microbiome. A & C. Montreal and Nunavut microbiomes cluster together, regardless of diet, based on principal coordinate analysis of A. unweighted and C. weighted UniFrac distances computed from open-reference OTUs (Methods). Gap statistic analyses identified only one cluster, showing that both populations overlap at the overall microbial community level. (See Fig. S4A-B for additional distance metrics.) B & D. Montreal and Nunavut microbiomes cluster with other Western microbiomes sampled in other studies. Comparisons between studies were performed with B. unweighted and D. weighted UniFrac distances were computed from closed-reference OTU tables to limit inter-study variability. Binning samples by traditional agrarian/hunter-gatherer (Burkina Fason, Tanzania, Venezuela) and Western populations (USA, Italy, Montreal, Nunavut) explains 6.5% and 10.5% of the variation in the combined datasets (adonis, $P < 0.001$) for unweighted and weighted UniFrac respectively.

Subtle differences distinguish the Inuit microbiome

Despite the broad, community-level similarity of Nunavut and Montreal microbiomes, they could still differ in the relative abundances of certain microbial taxa. Such subtle differences might not affect overall community structure (Fig. 3A & B and Fig. S4), particularly if they involve relatively rare taxa or small changes in relative abundance. To identify microbial taxa that varied between cohorts, we compared all samples by geography (Montreal vs. Nunavut; Fig. S6A and Tables S1D & G), diet (Inuit vs. Western diet; Fig. S6B and Tables S1E & G) and BMI (BMI < 25, BMI > 25; Fig. S6C and Tables S1F & G). To disentangle diet from geography and genetics, we also compared the Inuit and Western diets among Nunavut participants only, who shared the same geographic location and were of mostly Inuit ancestry. Comparisons at higher taxonomic levels (phyla through family) were performed with linear discriminant analysis (to identify biomarkers, as defined by LEfSe) (Segata et al. 2011) and differentially abundant OTUs were identified using the negative binomial Wald test in DESeq2 (Anders and Huber 2014; Love et al. 2014).

Comparing samples across geography, Lactobacillales and Bacilli were identified by LEfSe as the top two biomarkers for Montreal microbiomes (Fig 4A, Table S1G), while Ruminococcaceae were found to be associated with the Western diet (Fig 4C, Table S1G). Bacteria were also identified as a biomarker for Montreal, because there were more unassigned reads in the Nunavut cohort (0.019% of reads per individual, compared to 0.015% in Montreal). DESeq2 analyses found Montreal samples to be enriched in *Faecalibacterium prausnitzii* (OTUs 147702 and 339494) and three *Prevotella copri* OTUs (OTUs 326482, 4410166 and 4436552) (Fig. 4B and Table S1D). Meanwhile, the archaeal methanogen *Methanosphaera* was enriched in Nunavut (Table S1D). A different *P. copri* (OTU 184464) as well as *Akkermansia muciniphila* (OTU593043) were more abundant in Nunavut samples (Fig. 4B, Table S1D).

OTUs of *P. copri* and *A. muciniphila* were also identified as differentially abundant between Inuit and Western diets (Fig. 4D). The effects of diet and geography co-varied substantially because all Montrealers consumed a Western diet and most participants from Nunavut consumed an Inuit diet. Consistent with this covariation, all biomarkers of the Inuit diet were also biomarkers of Nunavut (identified by asterisks in Table S1G). Of the 80 OTUs associated with Montreal, over half (43) were also associated with the Western diet (Fig. S6D

and Tables S1D & E). Only one of the 80 Montreal-associated OTUs was also associated with the Inuit diet. Of the 212 OTUs associated with Nunavut, 104 were significantly enriched in the Inuit diet. Four of the Nunavut OTUs were associated with the Western diet (Tables S1D & E), including one associated with BMI > 25 (an unclassified OTU in the family Barnesiellaceae, Table S1F). These results suggest that roughly 50% of geographic associations (43/80 and 104/212) are due to co-variation between diet and geography.

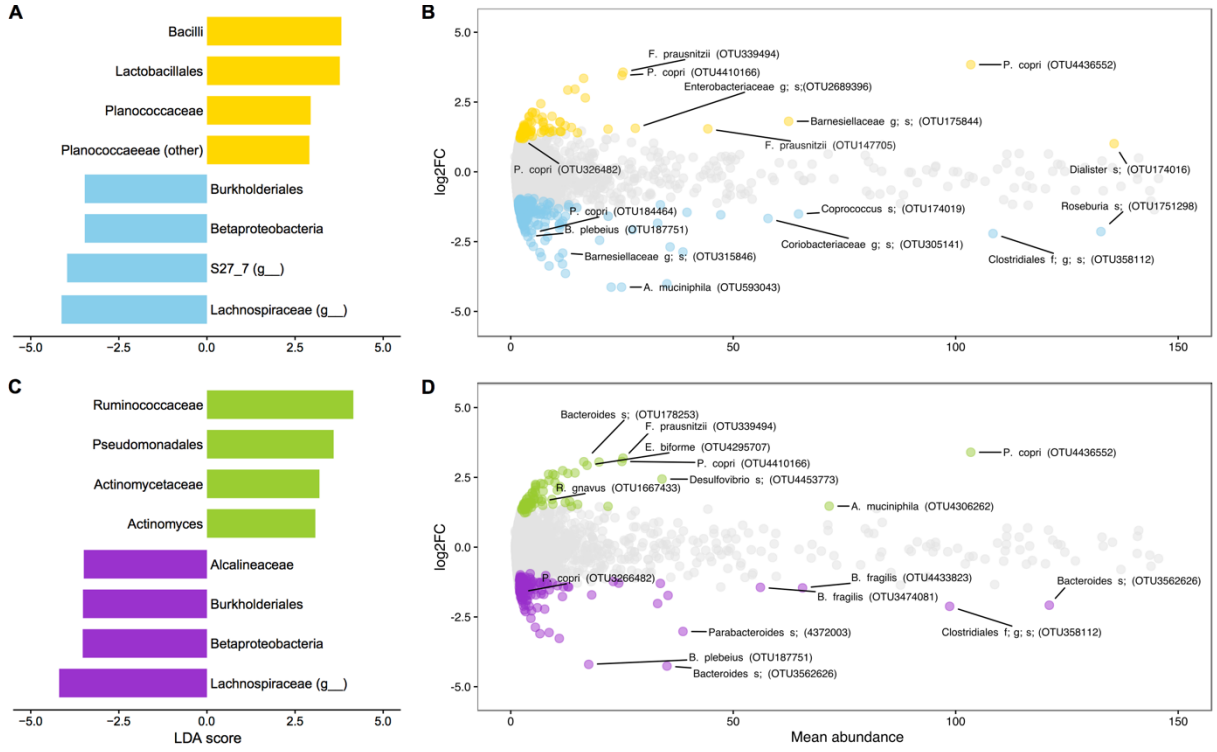

FIGURE 4. Differentially abundant OTUs and higher taxonomic units across geography and diet. A, C. Linear discriminant analyses (LDA) using LEfSe were used to identify biomarkers at higher taxonomic levels (from phylum to genus level). B, D. Differentially abundant OTUs were identified using DESeq2 (as described in Methods). Samples were compared across geographic regions (Montreal *n=26* in yellow, Nunavut *n=19* in blue) for A. LEfSe biomarkers and B. differentially abundant OTUs identified by DESeq2. Samples compared by diet (Western diet *n=29* in green, Inuit diet *n=19* in purple) for C. LEFSe biomarkers and D. differentially abundant OTUs identified by DESeq2. All association *P* values < 0.05 after correction for multiple testing. Only the top four LEfSe biomarkers (LDA score > 2.5) for each category are presented here. For full LEfSe and DESeq2 results, see Supplementary information (Tables S1D-G and Fig. S6A-C). Differentially abundant OTUs named in panels B and D focus on those discussed in the main text.

Low abundance and diversity of *Prevotella* in the Inuit diet

To disentangle the effects of geography and diet, we attempted to identify biomarkers of diet within the Nunavut participants only. Probably due to the reduced power afforded by this limited sample size (n=19), DESeq2 did not identify any OTUs associated with diet within Nunavut. However, LEfSe identified 14 taxonomic biomarkers of the Western diet in Nunavut; the top two biomarkers were the family Prevotellaceae and the genus *Prevotella* (Table S1G). *Prevotella* OTUs are present in both Montreal and Nunavut (Fig. 4, Table S1D) but there are more OTUs (7) associated with the Western diet than the Inuit diet (1) (Table S1E). We therefore hypothesized that the Western diet harbored a greater diversity of *Prevotella* strains.

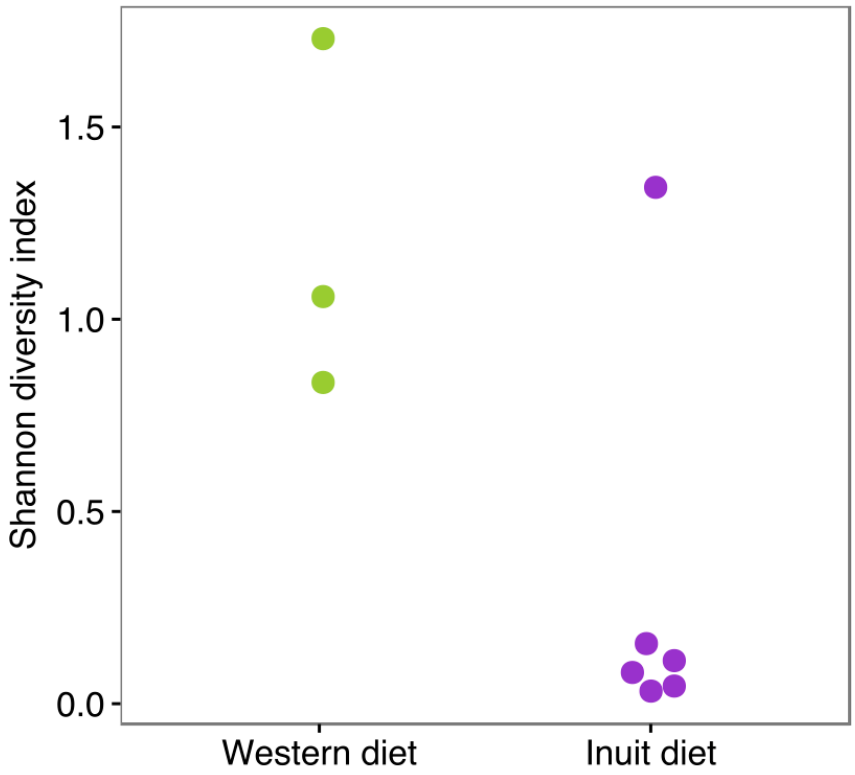

FIGURE 5. Inuit diet is associated with low diversity within the *Prevotella* genus. A. Nunavut participants consuming a Western diet had a significantly greater diversity of *Prevotella* strains (Shannon diversity of oligotypes) than those adhering to the Inuit diet (Mann-Whitney test, $P < 0.05$).

To test this hypothesis, we defined 48 strains of *Prevotella* using unsupervised oligotyping (Materials and Methods) and compared strain diversity across diets within Nunavut (Fig 5). We observed significantly lower *Prevotella* strain diversity in Nunavut participants consuming an Inuit diet compared to those consuming a Western diet (Mann-Whitney test, $P < 0.05$). The five individuals with low diversity and an Inuit diet (Fig. 5) were unrelated and did not share a household, suggesting that *Prevotella* diversity was associated with diet rather than household transmission or human genetics. No effect of the Inuit diet was observed on strain diversity within either *Akkermansia* or *Bacteroides* (Mann-Whitney test, $P > 0.05$), suggesting the effect is *Prevotella*-specific. We divided Nunavut participants into four diet categories instead of two bins, which qualitatively confirmed that a more traditional Inuit diet was associated with reduced *Prevotella* strain diversity (Fig S7).

Geographic and dietary associations of *Akkermansia* strains

OTUs within the *Akkermansia* genus, which is of interest for human health and obesity (Everard et al. 2013), exhibited associations with both geography and diet (Fig. 4B&D, Tables S1D & E). We identified 8 strains of *Akkermansia* by unsupervised oligotyping, which we refined to 7 strains using supervised oligotyping. Two of these strains were associated with Montreal and Western diet and three with Nunavut and/or Inuit diet (Fig 6A; Table S1H). We hypothesized that these strains might be phylogenetically grouped into two lineages – one corresponding to Montreal and one to Nunavut. However, inconsistent with this hypothesis, we constructed a phylogenetic tree of oligotypes and found two lineages ("Abundant" and "Rare"), each containing representatives in both Montreal and Nunavut (Fig 6B). One lineage was always found at low abundance (~1% of *Akkermansia* oligotypes), and one at variable abundance, sometimes near 100% (Fig 6).

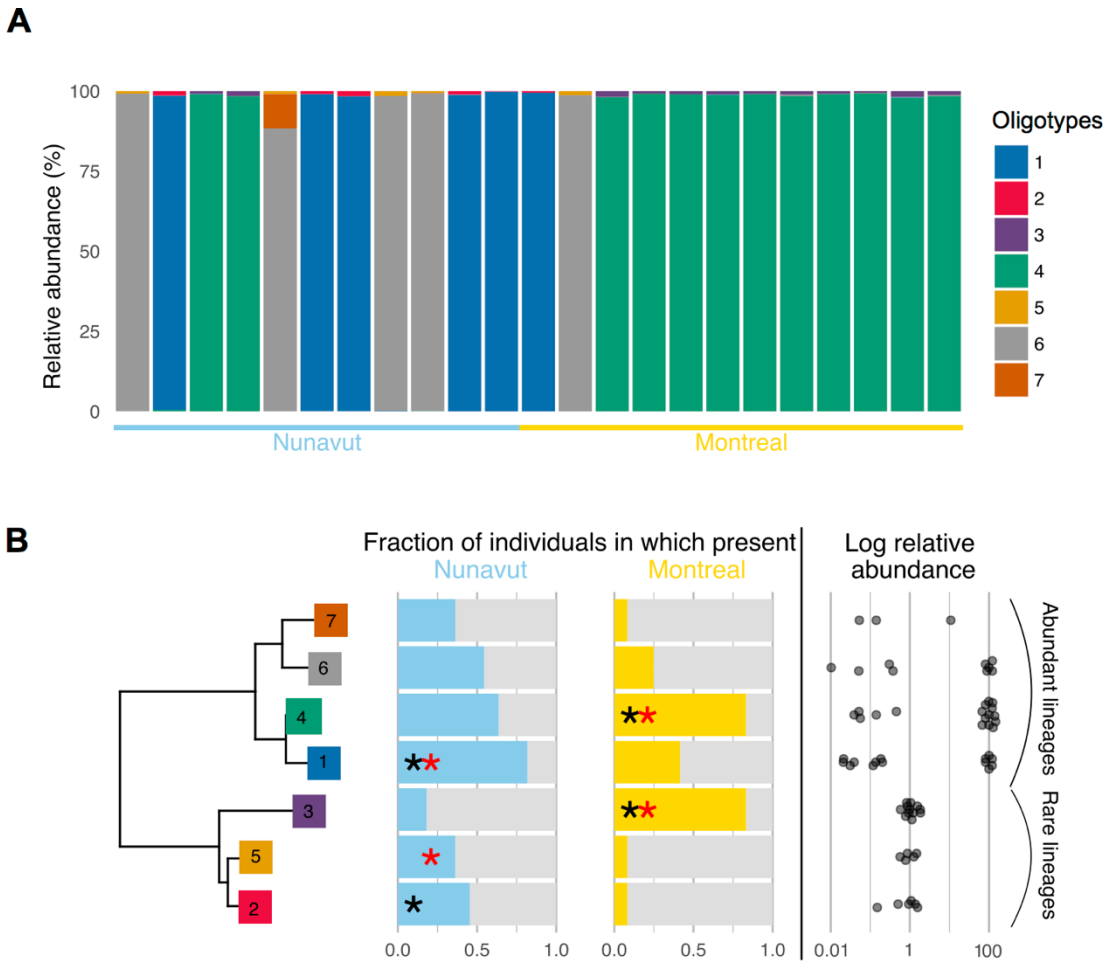

FIGURE 6. Two distinct *Akkermansia* lineages, each containing strains associated with geography and diet. A. *Akkermansia* strains (oligotypes 1-7) across samples (individuals). Only individuals with at least 100 *Akkermansia* reads are included. Percentages are relative to the total number of *Akkermansia* reads in the individual. Most individuals were dominated by one single strain (representing >88% of reads) out of 7 strains identified. B. Neighbor-joining tree (left) of oligotype sequences, with the fraction of individuals in which the oligotype is present, and its mean abundance within individuals (right). Stars indicate significant associations of oligotypes with geography (Nunavut vs. Montreal; black stars) and diet (Western vs. Inuit diet; red stars) (LEfSe, $P < 0.05$ after correction for multiple tests; Table S1H).

Discussion

In previous studies of the gut microbiome, greater diversity has consistently been observed in agrarians and hunter-gatherers compared to Western industrialized populations (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012). Our study in Nunavut thus provides a contrasting example of a modern traditional diet associated with approximately equal diversity compared to a Western diet (Fig. 1), and which clusters with other Western populations (Fig. 3). These previous studies found large differences between Western and traditional microbiomes, despite relatively small sample sizes (Clemente et al. 2015; De Filippo et al. 2010; Schnorr et al. 2015). Using a similar sample size, we failed to detect large differences between Inuit and Western microbiomes. This suggests that modern Inuit and Western microbiomes are broadly similar, although additional sampling might detect subtle but significant differences in alpha and beta diversity. The broad similarity between Inuit and Western microbiomes could arise because even Inuit frequently consuming traditional foods are also exposed to at least some Western market food. This is consistent with an Inuit population nearing the end of their transition to a Western diet. More specifically, there are at least four factors that might explain the similarity of Inuit and Western microbiomes: BMI, fiber, meat consumption, and seasonality.

First, we observed that Inuit microbiomes tended to be slightly (but not significantly) more diverse than their Southern Canadian counterparts, when binned by geography and BMI (Fig 2, Fig. S3). The Inuit of the Canadian Artic are undergoing a dietary transition, like many other indigenous populations, from a highly traditional to a westernized diet (Kuhnlein et al. 2004; Sharma 2010). This transition has been concurrent with increased prevalence of obesity in the Inuit (Sharma et al. 2010; Sheikh et al. 2016), which in turn has been linked with slightly (~2%) reduced gut microbiome alpha diversity (Sze and Schloss 2016). Consistently, we observe slightly reduced Shannon and Simpson diversity with higher BMI (Fig. S2D). The small effect of BMI could potentially mask a slightly elevated diversity in the Inuit microbiome. The ancestral state of the Inuit microbiome may thus have been of high diversity (like other traditional populations) (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012), as suggested by the higher diversity in Nunavut once the effects of BMI are removed (Fig. 2, Fig. S3). However, increasingly westernized diets

and increasing obesity may have led to reduction in diversity, leading to a Western-like diversity in the Inuit (Fig. 1). Further studies with much larger sample sizes of Inuit will be needed to detect potentially small effects of traditional diet, BMI, and their interaction on microbiome diversity.

Second, high gut microbiome diversity in traditional populations has frequently been associated with high fiber intake (Schnorr et al. 2015; Sonnenburg et al. 2016), which is typically high in non-Western diets. Here however, the difference in daily fiber intake between the Inuit (13.1-14.4 g for the Inuit of Nunavut (Sharma et al. 2013)) and the average Western diet (15.1g) (Cordain et al. 2005) (both below daily recommended intakes) is negligible compared to the difference with agrarian populations with high-fiber diets (De Filippo et al. 2010; Schnorr et al. 2015). Lack of diversity in the Inuit microbiome compared to other hunter-gatherers and agrarians could also be due to progressive loss of microbial diversity over generations of low-fiber traditional food consumption, typical of a Western diet (Sonnenburg et al. 2016). Low diversity may also be due to short-term effects of diet: indeed, the dietary habit questionnaires used in this study failed to capture more subtle variations in food intake, that could have consequences on microbiome composition (David et al., 2013). Improved resolution in the dietary data used in the analyses presented here could help tease apart the reasons for the low diversity seen in Nunavut samples, and determine if they are the consequences of short or long-term changes.

Third, Inuit and Montrealers might both consume more meat than previously studied agrarians and hunter-gathers, possibly leading to a similar diversity and community structure in Inuit and Montreal microbiomes. It is known that shifts to extreme meat-based diets induce major changes in the gut microbiome (David et al. 2013; Torrey and Montu 1931). However, these studies used extreme animal-only diets, while the Inuit diet includes at least some fiber (Kuhnlein et al. 2004) and many of our participants consumed some market food. Furthermore, previous studies (David et al. 2013; Torrey and Montu 1931) followed a shift from a baseline to a meat-rich diet, while our Inuit participants have presumably been consuming a meat-rich diet throughout their whole lives. Therefore, some of the changes observed in previous studies could have been due to the drastic and rapid dietary changes, rather than the long-term effects of diet composition. Again, the type of the dietary information collected may mask part of the

differences between Nunavut and Montreal microbiomes at the community level. Indeed, the dietary information compiled here is composed of food frequency data averaged over a year, and therefore does not take into account diet over the past few days, which have been shown by others to be potentially reflected in the daily variations of the microbiome (David et al. 2013, David et al. 2014). However, although we may be missing fine scale variations due to the consumption of a traditional meal in the days prior to sampling, given the overall similarities between Nunavut and Montreal diets, we believe that the microbiomes remain similar at the community level.

Fourth, seasonal microbiome variation has been observed in other human populations (Davenport et al. 2014; Schnorr et al. 2015) and might explain the similarity between Montreal and Nunavut microbiomes. All sampling in this study was conducted in late July through early August, when most Inuit consume a mix of traditional and market food (Hopping et al. 2010), which might be relatively similar to the Montreal diet. Other times of year, when more traditional food is consumed, might yield more distinct microbiomes. Taxa associated with the Inuit diet could thus be long-term dietary biomarkers, persisting even in periods of Western food consumption.

Despite the overall similarity between Inuit and Montreal microbiomes, they differed in the relative abundance of certain OTUs and higher taxonomic units, possibly linked to long-term dietary differences. For example, *F. prausnitzii*, which has been linked with consumption of citrus fruit (David et al. 2014), was overrepresented in Montreal, where – unlike the Arctic – citrus is readily available year-round. The enrichment of the Lactobacillales family in Montreal was also expected, because dairy products are widely consumed in Montreal but not in Nunavut. Finally, *Prevotella* has been previously associated with fiber-rich diets (De Filippo et al. 2010; Schnorr et al. 2015) and was found as a biomarker of the Western diet within Nunavut. The association of *Prevotella* with the Western diet (in the entire cohort and specifically within Nunavut) is consistent with more fiber in the Western diet compared to the Inuit diet, even if the difference is slight compared to other agrarians and hunger-gatherers (De Filippo et al. 2010; Schnorr et al. 2015; Sharma et al. 2010). Not only is *Prevotella* relatively more abundant in Inuit consuming a Western diet, it is more diverse in the richness and evenness of oligotypes within the genus (Fig. 5). It is possible that lower strain diversity in the Inuit diet group is due to the

lower relative abundance of *Prevotella* in individuals consuming an Inuit diet (*e.g.* diversity exists, but strains are too rare to be detected). Some *Prevotella* OTUs (Fig. 4, Tables S1D & E) and oligotypes (Table S1H) are associated with Western diet and geography, and others with Inuit diet. Together, this suggests that populations consuming modestly different levels and types of fiber (*e.g.* diversity and abundance of fruits and vegetables) may differ in their relative abundance of different *Prevotella* strains. While *Prevotella* has been frequently associated with fiber-rich diets (De Filippo et al. 2010; Kovatcheva-Datchary et al. 2015; Schnorr et al. 2015), it has also been linked to inflammation in the gut (Dillon et al. 2015; Ley 2016). *Prevotella* strains vary from individual to individual (Zhu et al. 2015) and strains might have contrasting associations with health state and diet (*e.g.* some may correlate with fiber while others do not) (De Filippis et al. 2016). Moreover, it is likely that factors other than fiber also contribute to shaping *Prevotella* diversity, in our samples and more generally.

In the case of *Prevotella,* we were able to disentangle the effects of diet and geography. However, the small sample size (n=3) of Inuit consuming a Western diet prevented us from identifying differentially associated OTUs with DESeq2, and may have led to some false-positives among the 14 LEfSe biomarkers. Larger sample sizes of Inuit consuming both traditional and Western diets will be needed to replicate and confirm these biomarkers.

In other cases, diet could not be clearly disentangled from geography, genetics, and lifestyle. For example, *Akkermansia* contained strains associated with either Montreal/Western diet, or with Nunavut/Inuit diet (Fig. 6). Diet, geography, and lifestyle could all contribute to the distribution of these strains. The partitioning of these strains into two lineages also suggests an ancient divergence of "rare" and "abundant" lineages, followed by more recent diversification into Montreal/Nunavut strains within each lineage, perhaps associated with environmental or dietary pressures. It remains to be seen if "rare" and "abundant" *Akkermansia* strains are true monophyletic groups, if they are a general feature of other human microbiomes, and whether there are any ecological differences between them, or between Montreal/Nunavut strains.

In summary, the Inuit harbor a diversity of gut microbes that is not strikingly different from their urbanized, westernized counterparts. This may not reflect the ancestral Inuit microbiome: indeed, dietary transition and westernization, as well as increasing prevalence of

obesity may have reduced diversity and changed the composition of the Inuit microbiome over time. The modern Inuit microbiome resembles that of Southern Canadians and other Western populations. We did, however, pinpoint subtle differences in the composition of the Inuit and Western gut microbiomes, which may be due to contrasting diets. Like other native populations (Sankaranarayanan et al. 2015), the Inuit have a unique set of health risks, many of which could be modulated by the microbiome. Although the goal of our study was not to investigate health risks, we have presented a snapshot of an Inuit microbiome in transition, providing a foundation for future studies of how the microbiome changes over time, and how it interacts with diet and human genetics to affect health and disease.

Materials and methods

Participant enrollment and sample collection

We recruited 26 volunteers from the community of Resolute Bay, Nunavut (representing approximately 18% of the local adult population), a small hamlet where 95% of the population is Inuit (Fig. S1A and S1B) (Statistics Canada). Three individuals of European descent living in Resolute Bay were included in the study. We also recruited 33 residents of Montreal (Canada) mostly working or studying at a university, and all of European descent. Stool samples were collected from July-September 2014 from healthy participants who had not taken antibiotics in the previous month. Details on volunteer characteristics are presented in Table S1B. The community sampled in this study is the second-smallest in Nunavut (Statistics Canada). The size of the community, the shared family history and households of the participants as well as the small number of samples limits the degree to which these 26 volunteers represent all Inuit. Rather, this study provides a snapshot of a group of Inuit individuals, and our results should not be extrapolated to all Inuit. For the sake of clarity, the term “Inuit” is used in the text, but does not imply representation of all Inuit.

All volunteers gave written informed consent after the objectives and potential outcomes of the study were explained to them. Participants completed dietary habit questionnaires (Annex III), evaluating their typical diet over the course of a year (Table S1C). Dietary information was

compiled according to frequency of traditional Inuit food consumption, spanning a range from an entirely Western diet to a highly traditional Inuit diet. For all subsequent analyses, dietary information was broken down into two categories: Inuit (individuals who consumed traditional Inuit food at least twice a week) or Western diet (individuals who occasionally or never ate traditional Inuit food). The Western diet category included individuals from both Montreal and Nunavut who consumed traditional Inuit food infrequently or never. All work was approved by the Université de Montréal ethics review board for arts and sciences (CERFAS, certificate #2013-14-022-D). Permission for this work was granted by the Nunavut Research Institute (licenses #02 040 13N-A and #02 046 14N-A), by the Hunters & Trappers Association and Hamlet of Resolute Bay.

Participants wore sterile gloves while collecting stool samples into sterile specimen cups. In Nunavut, samples were kept outside (temperatures < 4 °C) for a maximum of 12 hours before being collected by a sampling team and frozen at -80 °C. In Montreal, samples were immediately frozen at -20 °C before being collected by a sampling team and frozen at -80 °C.

DNA extraction, library preparation and sequencing

DNA was extracted from stool samples using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.) (David et al. 2014). Library preparation was done using a two-step PCR method to amplify the V4 region of the 16S rRNA gene (Supplementary Materials and Methods). During the first step PCR, primers PE16S_V4_U515_F (5′ ACACG ACGCT CTTCC GATCT YRYRG TGCCA GCMGC CGCGG TAA-3′) and PE16S_V4_E786_R (5′-CGGCA TTCCT GCTGA ACCGC TCTTC CGATC TGGAC TACHV GGGTW TCTAA T 3′) were used to target and amplify the V4 region, as well as to add second-step priming sites (Caporaso et al. 2011; Preheim et al. 2013). Library size was confirmed at approximately 440 bp with a Qiaxcel Advanced System (QIAGEN). Libraries were quantified with a Qubit v.2.0 fluorometer (Life Technologies), pooled and denatured following the Illumina protocol. Paired end sequencing (2 x 250 bp) was performed using MiSeq reagent Kit V2 (Illumina) on a MiSeq (Illumina). All samples were sequenced in a single run, yielding a Q score greater than Q30 for 93.1% of reads, and a cluster density of 856 ± 12 K mm^{-2}.

OTU picking and data processing

We obtained 26 samples from our Nunavut participants, 19 of which were successfully sequenced (>5,000 raw reads per sample). Meanwhile, of the 33 Montreal samples collected, 26 were sequenced (>5,000 raw reads). The sequencing data was analyzed using QIIME (version 1.8.0) (Caporaso et al. 2010). Paired-end reads were concatenated using the join_paired_ends.py script, with default parameters. Libraries were demultiplexed with the split_libraries_fastq.py script according to barcode identification. Chimeric sequences were identified using the usearch61 method with the identify_chimeric_seqs.py script, and removed with the filter_fasta.py script. Sequencing produced a total of 6,345,335 reads and an average of 141,007 ± 54,899 reads per sample. Each sample was rarefied to 50,000 reads for subsequent analyses (unless otherwise indicated, *e.g.* for DESeq2 analyses). Open-reference operational taxonomic unit (OTU) picking was performed in QIIME (pick_open_references.py script) at a 97% identity level using Greengenes version 13_8, with a pre-filtering step to remove non-16S sequences (percent identity < 60%) (Rideout et al. 2014). OTUs with fewer than 10 observations across all samples were filtered from the OTU table (filter_otus_from_otu_table.py script). This left us with a final data set of 45 samples and 9,581 OTUs.

Data analyses

Alpha diversity was computed using phyloseq{} (McMurdie and Holmes 2013) in R (R Development Core Team) with several metrics: observed OTUs, Chao1 estimated OTUs, Shannon, Simpson and Fisher diversity indices. OTU diversity in our dataset was compared to 1,000 randomly selected American Gut project samples (ftp://ftp.microbio.me/AmericanGut/latest) (see Supplementary Methods for data filtering). For this comparison, we performed closed-reference OTU picking (pick_closed_reference_otus.py) at 97% identity, eliminated OTUs with less than 10 observations across all samples (filter_otus_from_otu_table.py) and rarefied samples to 10,000 reads (single_rarefaction.py).

Beta diversity analyses were performed on weighted and unweighted UniFrac and Bray-Curtis distances, as well as Jenson-Shannon divergence, then visualized with PCoA using ggplot2{} (Wickham 2009). Sample groups were compared by analysis of variance using a permutation test with pseudo-*F* ratios with the adonis() function in vegan{} (Oksanen et al. 2015). Clusters of samples were analyzed using the gap statistic, which estimates the number of clusters (groups) in a dataset (Tibshirani et al. 2001). We compared our data to 16S sequences from De Filippo et al. (EBI: project 'ERP000133'), Schnorr et al. (MG-RAST: project ID '7058') and Clemente et al. (EBI: projects 'ERA387449' and 'ERP008799') and publicly available sequences from the American Gut Project, filtered as described in Supplementary Methods (Clemente et al. 2015; De Filippo et al. 2010; Schnorr et al. 2015; Yatsunenko et al. 2012). To minimize methodological differences among studies, we performed closed-reference OTU picking, and OTUs with fewer than 10 observations across all samples were removed. Samples were then rarefied to 1,000 reads per sample, and compared using unweighted and weighted UniFrac.

We performed linear discriminant analyses (LDA) using LEfSe to identify microbial taxa (biomarkers, at all taxonomic levels, down to the genus level) that characterize the difference between groups of samples. The alpha value for Kruskal-Wallis and Wilcoxon tests was set at 0.05, and the logarithmic LDA score threshold was 2.0, and per-sample normalization of sum values was applied (LEfSe default parameters). These biomarkers are microbial taxa that differ in abundance between groups, as identified by a Wilcoxon rank-sum test. The effect size of each biomarker is then estimated by an LDA score (Segata et al. 2011). When LEfSe analyses were initially performed on our dataset, the domain Bacteria emerged as a biomarker for Montreal. We determined that this was due to unassigned reads being more associated with the Nunavut cohort. However, while the difference between populations was large enough to impact LEfSE analyses, unassigned reads accounted for a very small proportion of reads (average of 0.02% of reads per sample). We removed unassigned reads and Bacteria were no longer a biomarker. All other LEfSE results remained identical and are reported here.

To investigate differences at finer taxonomic levels (OTU level), we performed differential abundance analyses on unrarefied, filtered (minimum of 10 observations across all

samples) OTU tables using DESeq2 (Anders and Huber 2014; Love et al. 2014). Only taxa found as significant ($P < 0.05$ after multiple hypothesis testing) were reported.

To define strains within certain genera of interest, we used unsupervised oligotyping (also known as Minimum Entropy Decomposition, MED version 0.1-alpha; http://oligotyping.org/MED) (Eren et al. 2014a). By using the Shannon entropy, MED decomposes the dataset to find "MED nodes" that explain the maximum entropy. To filter noise, we removed MED nodes for which the most abundant unique sequence was represented by fewer than 100 reads (-M 100). We found eight MED nodes (strains) within *Akkermansia,* 48 within *Prevotella*, and 256 within *Bacteroides*. We calculated the Shannon diversity of these strains as described above. We excluded individuals (samples) with fewer than 100 reads within the genus of interest. For genera with relatively few MED nodes (*Akkermansia* and *Prevotella*), we were able to confirm the results with supervised oligotyping (http://oligotyping.org) (Eren et al. 2013; 2014b). Using oligotyping v1.4, We identified 45 *Akkermansia* oligotypes and 7 *Prevotella* oligotypes. We found that the minimal number of nucleotide positions explaining the diversity within these genera was respectively 36 and 14 high-entropy positions. Fig. S8 shows the distribution of entropy along the *Akkermansia* and *Prevotella* reads and positions. In order to minimize the impact of noise, we used parameters that removed any oligotypes with a frequency smaller than 100 modified (-M 100), and we eliminated oligotypes that appeared in less than three samples (-s 3). These filters removed 1.63% of *Prevotella* reads and 7.95 % of *Akkermansia* reads. We used LEfSe as described above to identify oligotypes (strains) associated with diet and/or geography (Table S1H). Based on the 11 high-entropy positions in the *Akkermansia* alignment, we constructed a Neighbour-Joining tree of the 7 oligotypes (strains).

Short-chain fatty-acid analysis

Short-chain fatty acids from stool samples were quantified by gas chromatography coupled with a flame ionization detector (GC-FID). For detailed method, see Supplementary Materials and Methods.

Data availability

Raw 16S rRNA gene sequences have been deposited in Qiita (http://qiita.microbio.me/) under Study ID 10439 and are available on GitHub (https://github.com/cgir/16S_inuitgut).

Acknowledgments

We would like to thank Debbie and Pilipoosie Iqaluk for their assistance in the field, the Hunters & Trappers Association and Hamlet of Resolute Bay for their support of this research, the residents of Resolute Bay for their participation and the Nunavut Research Institute for helpful comments. We are grateful to Lawrence David and Corinne F. Maurice for constructive comments on an early version of the manuscript. We also thank Yves Terrat, Geneviève Dubois, Inès Levade, Julie Marleau, Simone Perinet, and Sarah Preheim for help in the laboratory and with data analysis. We thank Sophie Breton, Alexandre Poulain and Rachel Carmody for their constructive comments.

Supplementary information

Supplementary materials and methods

Participant enrollment and sample collection

We recruited 26 volunteers from the community of Resolute Bay, Nunavut (representing approximately 18% of the local adult population), a small hamlet where 95% of the population is Inuit (Fig. S1A and S1B) (Statistics Canada). Three individuals of European descent living in Resolute Bay were included in the study. We also recruited 33 residents of Montreal (Canada) mostly working or studying at a university, and all of European descent. Stool samples were collected from July-September 2014 from healthy participants who had not taken antibiotics in the previous month. Details on volunteer characteristics are presented in Table S1B.

All volunteers gave written informed consent after the objectives and potential outcomes of the study were explained to them. Participants completed dietary habit questionnaires (Annex III), evaluating their typical diet over the course of a year (Table S1C). Dietary information was compiled according to frequency of traditional Inuit food consumption, spanning a range from an entirely Western diet to a highly traditional Inuit diet. For all subsequent analyses, dietary information was broken down into two categories: Inuit (individuals who consumed traditional Inuit food at least twice a week) or Western diet (individuals who occasionally or never ate traditional Inuit food). The Western diet category included individuals from both Montreal and Nunavut who consumed traditional Inuit food infrequently or never. All work was approved by the Université de Montréal ethics review board for arts and sciences (CERFAS, certificate #2013-14-022-D). Permission for this work was granted by the Nunavut Research Institute (licenses #02 040 13N-A and #02 046 14N-A), by the Hunters & Trappers Association and Hamlet of Resolute Bay.

Participants wore sterile gloves while collecting stool samples into sterile specimen cups. In Nunavut, samples were kept outside (temperatures < 4 °C) for a maximum of 12 hours before being collected by a sampling team and frozen at -80 °C. In Montreal, samples were immediately frozen at -20 °C before being collected by a sampling team and frozen at -80 °C.

DNA extraction, library preparation and sequencing

DNA was extracted from stool samples using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.) (David et al. 2013). Library preparation was done using a two-step PCR method to amplify the V4 region of the 16S rRNA gene. During the first step PCR, primers PE16S_V4_U515_F (5′ ACACG ACGCT CTTCC GATCT YRYRG TGCCA GCMGC CGCGG TAA-3′) and PE16S_V4_E786_R (5′-CGGCA TTCCT GCTGA ACCGC TCTTC CGATC TGGAC TACHV GGGTW TCTAA T 3′) were used to target and amplify the V4 region, as well as to add second-step priming sites (Caporaso et al. 2011; Preheim et al. 2013). The first step PCR reaction was performed as follows: 0.5 units of Phusion polymerase (New England BioLabs) with 1X High Fidelity buffer, 0.5 μL dNTPs, 2.5 μL of 3 μM PE16S_V4_U515_F and PE_16S_V4_E786_R primers and 5 ng of DNA were combined in a 25 μL reaction. PCR reactions were performed under the following conditions: initialization step at 98 °C for 30 seconds, denaturation at 98 °C for 25 seconds, annealing at 54°C for 40 seconds, extension at 72 °C for 30 seconds and final elongation at 72 °C for 2 minutes, over 20 cycles. Each sample was prepared in quadruplicate, with replicates pooled to a final volume of 100 μL and purified by Agencourt AMPure XP purification (Beckman Coulter), following the manufacturer's protocol.

During the second-step PCR amplification, 9-bp barcodes for sample identification and Illumina adapter sequences were added to step one products, using second step primers PE-III-PCR-F (5′-AATGA TACGG CGACC ACCGA GATCT ACACT CTTTC CCTAC ACGAC GCTCT TCCGA TCT 3′) and PE-III-PCR-001-096 (5′-CAAGC AGAAG ACGGC ATACG AGATN NNNNN NNNCG GTCTC GGCAT TCCTG CTGAA CCGCT CTTCC GATCT 3′), where Ns represent the unique barcode (Preheim et al. 2013). The second step PCR reaction was performed as follows: 5 units of Phusion polymerase with 1X High Fidelity buffer, 0.5 μL of dNTPs, 3.3 μL of 3 μM PE-PCR-III-F and PE-PCR-III-001-096 and 4 μL of step one product were combined in a 25 μL reaction. PCR reactions were performed under the following conditions: initialization step at 98 °C for 30 seconds, denaturation at 98 °C for 30 seconds, annealing at 83°C for 30 seconds, extension at 72 °C for 30 seconds and final elongation at 72 °C for 2 minutes, over 7 cycles. Each sample was prepared in quadruplicate, with replicates

pooled to a final volume of 100 µL and purified by Agencourt AMPure XP purification (Beckman Coulter), following the manufacturer's protocol.

Library size was confirmed at approximately 440 bp with a Qiaxcel Advanced System (QIAGEN). Libraries were quantified with a Qubit v.2.0 fluorometer (Life Technologies), pooled and denatured following the Illumina protocol. Paired end sequencing (2 x 250 bp) was performed using MiSeq reagent Kit V2 (Illumina) on the MiSeq (Illumina). All sequencing was done in a single run, with Q score was greater than Q30 for 93.1% of reads, and cluster density was of 856 ± 12 K mm^{-2}.

OTU picking and data processing

We obtained 26 samples from our Nunavut participants, 19 of which were successfully sequenced (>5,000 raw reads per sample). Meanwhile, of the 33 Montreal samples collected, 26 were sequenced (>5,000 raw reads). The sequencing data was analyzed using QIIME (version 1.8.0) (Caporaso et al. 2010). Paired-end reads were concatenated using the join_paired_ends.py script. Libraries were demultiplexed with the split_libraries_fastq.py script according to barcode identification. Chimeric sequences were identified using the usearch61 method with the identify_chimeric_seqs.py script, and removed with the filter_fasta.py script. Sequencing produced a total of 6,345,335 reads and an average of 141,007 ± 54,899 reads per sample. Each sample was rarefied to 50,000 reads for subsequent analyses (unless otherwise indicated, *e.g.* for DESeq2 analyses). Open-reference operational taxonomic unit (OTU) picking was performed in QIIME (pick_open_references.py script) at a 97% identity level, with a pre-filtering step to remove non-16S sequences (percent identity < 60%) (Rideout et al. 2014). OTUs with fewer than 10 observations across all samples were filtered from the OTU table (filter_otus_from_otu_table.py script). This left us with a final data set of 45 samples and 9,581 OTUs.

Data analyses

Alpha diversity was computed using phyloseq{} (McMurdie and Holmes 2013) in R with several metrics: observed OTUs, Chao1 estimated OTUs, Shannon, Simpson and Fisher diversity indices. The observed species metric counts the number of distinct OTUs, chao1 estimates richness, while Shannon's diversity index, Simpson's index and Fisher's alpha include measures of richness and community evenness. We compared our alpha diversity results to publicly available sequences from the American Gut project (ftp://ftp.microbio.me/AmericanGut/latest), randomly selecting 1,000 'healthy' samples from this dataset ('healthy' as described here, with the exception of a BMI range of 18.5-45, to match that of the Montreal/Nunavut samples from this present study : ftp://ftp.microbio.me/AmericanGut/paper_healthy_subset.txt. Briefly, we performed chimera filtering (identify_chimeric_seqs.py) in both datasets, and American Gut sequences were filtered for 'bloom' sequences attributable to transport by mail using SortMeRNA 2.0, as described here: http://americangut.org/removing-blooming-bacterial-sequences-from-american-gut-data/. To minimize methodological differences among studies, we then performed closed-reference OTU picking (pick_closed_reference_otus.py script) at 97% identity, and filtered out OTUs with less than 10 observations across all samples. Each sample was rarefied to 10,000 reads, and we compared observed OTUs across geography in both datasets.

Beta diversity analyses were performed on weighted and unweighted UniFrac and Bray-Curtis distances, as well as Jenson-Shannon divergence, then visualized with PCoA using the ggplot2 R package (Wickham 2009). Jensen-Shannon divergence (JSD) and Bray-Curtis measure similarity across two populations, while UniFrac does the same but includes information on the relatedness of OTUs by using phylogenetic distances (Lozupone and Knight 2005).

Sample groups were compared with the adonis() function (analysis of variance using a permutation test with pseudo-*F* ratios, vegan{}) (Oksanen et al. 2015). Clusters of samples were analyzed using the gap statistic, which estimates the number of clusters (groups) in a dataset (Tibshirani et al. 2001). We compared our data to 16S sequences from De Filippo et al. (EBI: project 'ERP000133'), Schnorr et al. (MG-RAST: project ID '7058') and Clemente et al. (EBI: projects 'ERA387449' and 'ERP008799'), and 200 randomly selected 'healthy' American Gut

Project samples, filtered as described above (ftp://ftp.microbio.me/AmericanGut/latest) (Clemente et al. 2015; De Filippo et al. 2010; Schnorr et al. 2015). We performed closed-reference OTU picking (pick_closed_reference_otus.py script) at 97% identity, and OTUs with less than 10 observations across all samples were filtered out. Reads were then rarefied to 1,000 reads per sample, and plotted using weighted and unweighted UniFrac.

We performed linear discriminant analyses (LDA) using LEfSe to identify microbial taxa (biomarkers, at all taxonomic levels, down to the genus level) that characterize the difference between groups of samples. These biomarkers are microbial taxa that differ in abundance between groups, as identified by a Wilcoxon rank-sum test. The effect size of each biomarker is then estimated by an LDA score (Segata et al. 2011). To investigate differences at finer taxonomic levels (OTU level), we performed differential abundance analyses on unrarefied, filtered (minimum of 10 observations across all samples) OTU tables using DESeq2 (Love et al. 2014). Only taxa found as significant ($P < 0.05$ after multiple hypothesis testing) were reported.

To define strains within certain genera of interest, we used unsupervised oligotyping (also known as Minimum Entropy Decomposition, MED version 0.1-alpha; http://oligotyping.org/MED) (Eren et al. 2014a). By using the Shannon entropy, MED decomposes the dataset to find "MED nodes" that explain the maximum entropy. To filter noise, we removed MED nodes for which the most abundant unique sequence was represented by fewer than 100 reads (-M 100). We found eight MED nodes (strains) within *Akkermansia*, 48 within *Prevotella*, and 256 within *Bacteroides*. We calculated the Shannon diversity of these strains as described above. We excluded individuals (samples) with fewer than 100 reads within the genus of interest. For genera with relatively few MED nodes (*Akkermansia* and *Prevotella*), we were able to confirm the results with supervised oligotyping (http://oligotyping.org) (Eren et al. 2013; 2014b). Using oligotyping v1.4, We identified 45 *Akkermansia* oligotypes and 7 *Prevotella* oligotypes. We found that the minimal number of nucleotide positions explaining the diversity within these genera was respectively 36 and 14 high-entropy positions. Supplementary Fig. S8 shows the distribution of entropy along the *Akkermansia* and *Prevotella* reads and positions. In order to minimize the impact of noise, we used parameters that removed any oligotypes with a frequency smaller than 100 modified (-M 100), and we eliminated oligotypes

that appeared in less than three samples (-s 3). These filters removed 1.63% of *Prevotella* reads and 7.95 % of *Akkermansia* reads. We used LEfSe as described above to identify oligotypes (strains) associated with diet and/or geography (Table S1H). Based on the 11 high-entropy positions in the *Akkermansia* alignment, we constructed a Neighbour-Joining tree of the 7 oligotypes (strains).

Short-chain fatty-acid (SCFA) analysis

Phosphoric acid was purchased from Fisher Scientific. Ethyl acetate anhydrous (99.8%) and all the 99% grade standards (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid and internal standard 4-methyl valeric acid) were purchased from Sigma-Aldrich.

Short chain fatty acids were extracted according to a protocol previously published by Garcia-Villalba et al. 2012. Right after collection, faeces were weighed and 1 mL of 0.5% phosphoric acid was added per 100 mg of material. The suspensions were frozen at -20°C until extraction. Once thawed, faecal suspensions were homogenized 2 min with a vortex mixer then centrifuged 10 min at 17949 g at 4°C. Supernatant was collected and an equal volume of ethyl acetate was added. To extract SCFAs, samples were once again homogenized 2 min with a vortex mixer and centrifuged 10 min at 17,949 g at 4°C. The organic phase was transferred to an autosampler vial and 4-methylvaleric acid added to obtain 500 µM of internal standard.

SCFAs were analysed with a GC-FID system (Shimadzu), constituted of a GC-2010 Plus gas chromatograph equipped with an AOC-20s auto-sampler, an AOC-20i auto-injector and a flame ionisation detector. The system was controlled by the GC solution software. A 1 µL sample was injected to the Nukol capillary GC column (30 m x 0.25 mm id, 0.25 µM film thickness, Supelco analytical) with a split ratio of 1:10 (v/v). The column flow was constant at 1.2 mL min^{-1} of hydrogen. Nitrogen (30 mL min^{-1}), hydrogen (40 mL min^{-1}) and air (400 mL min^{-1}) were used as auxiliary gases for flame ionisation detector. The injector was set on 230 °C and the detector on 250 °C. The oven temperature was initially programmed at 100 °C, then increased to 200 °C at 8 °C min^{-1} and maintained for 2 min at this temperature (total run time 14.5 min and 3 min equilibration).

Supplementary tables

All Supplementary Tables available from mSphere at:

http://msphere.asm.org/content/msph/2/1/e00297-16/DC1/embed/inline-supplementary-material-1.xlsx?download=true

TABLEAU SIA. Traditional food consumption in the Nunavut cohort. Examples of frequently consumed traditional foods are shown on the left. On the right, respondents are binned according to the frequency of their traditional food consumption, ranging from never, to infrequently (once a week or less), to frequently (at least twice a week), to every day.

TABLEAU SIB. Characteristics of study participants. The Inuit and Western cohorts had similar gender and age representation (t-test, $P > 0.05$). Inuit participants had higher BMI values (t-test, $P < 0.005$), and more varied ethnic representation. We collected samples from 26 Nunavut participants 19 of which were successfully sequenced (>5,000 raw reads per sample). 33 samples were collected in Montreal, 26 of which passed our quality filters. Sampling was restricted to individuals over 20 years of age, who had not used antibiotics in the month preceding sampling. 8 Nunavut participants shared households (4 households).

TABLEAU SIC. Dietary information collected during surveys on the field, showing how frequency was converted into bins (for Inuit and Western diets). Table also includes BMI, ethnicity and gender information.

TABLEAU SID. OTUs enriched in Nunavut or Montreal samples, identified with DESeq2. *P*-values were corrected for multiple comparisons using the False discovery rate (FDR). baseMean shows average normalized count values divided by size factor, lfcSE shows standard error estimate for log2FC. A negative Log2FC indicates enrichment in Montreal.

TABLEAU SIE. OTUs enriched in the Inuit or Western diet samples across all participants, identified with DESeq2. *P*-values were corrected for multiple comparisons using the False discovery rate (FDR). baseMean shows average normalized count values divided by size factor, lfcSE shows standard error estimate for log2FC. A negative Log2FC indicates enrichment associated with the Inuit diet.

TABLEAU SIF. OTUs enriched in BMIs inferior and greater than 25 across all participants, identified with DESeq2. *P*-values were corrected for multiple comparisons using the False discovery rate (FDR). baseMean shows average normalized count values divided by size factor, lfcSE shows standard error estimate for log2FC. A negative Log2FC indicates enrichment associated with BMIs < 25.

TABLEAU SIG. LEfSe analysis identified biomarkers of Nunavut (blue) and Montreal (yellow), of Western (green) and Inuit diets (purple), across the whole dataset, and biomarkers of the Western diet identified in Nunavut participants only (red). Biomarkers of high (dark purple) and low BMI (dark blue) are also reported. Taxa are ranked according to their estimated effect size (LDA score). Asterisks denote biomarkers of Nunavut also identified as biomarkers of the Inuit diet, crosses denote biomarkers of Nunavut, the Inuit diet and of BMI > 25.

TABLEAU SIH. LefSe analysis of *Akkermansia* and *Prevotella* oligotypes.

Supplementary figures

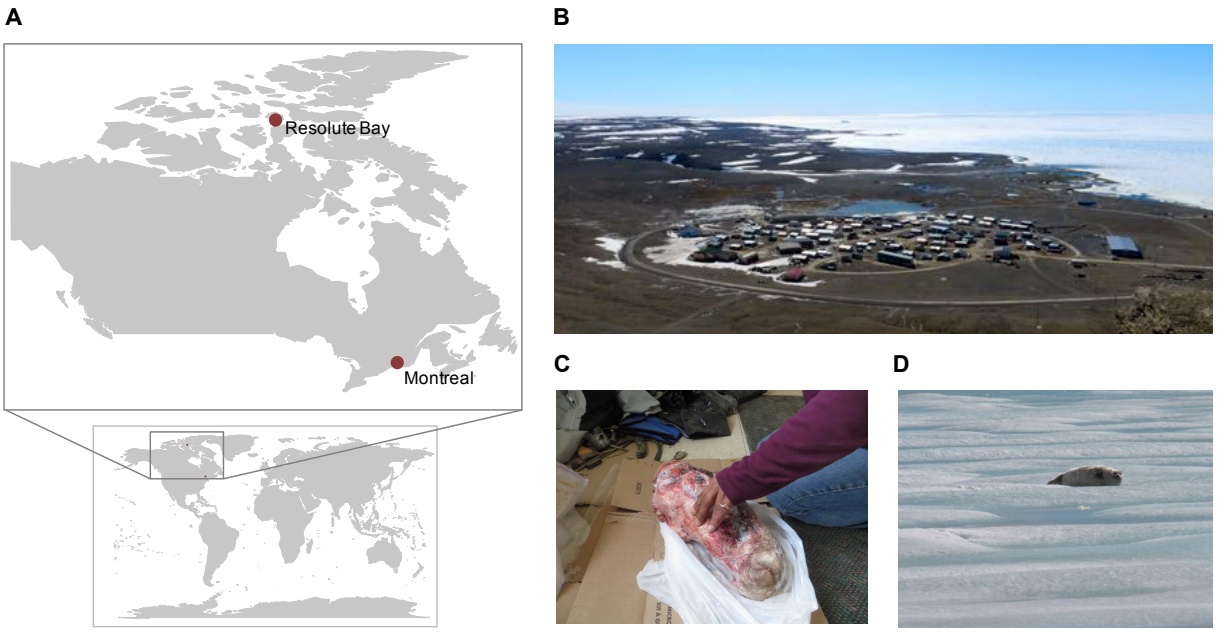

FIGURE S1. A and B. The Inuit community sampled is located in Nunavut, in the Canadian Arctic (74°41'51''N, 94°49'56''W), and has a population of 214 (2011 Canadian Census). The traditional Inuit diet is based on animals hunted in the Arctic environment, including various land and marine animals such as C. caribou and D. seal.

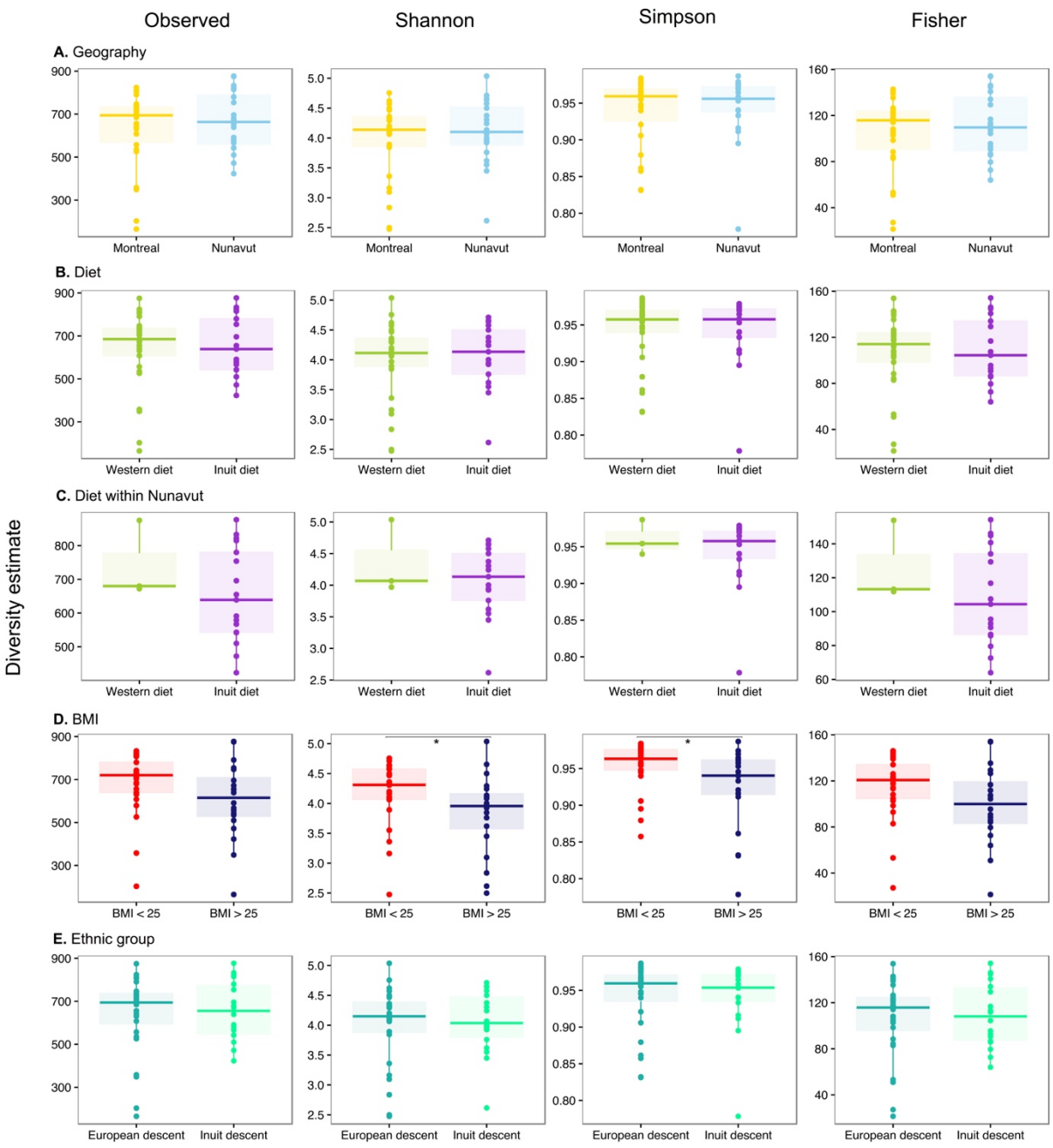

FIGURE S2. Comparison of alpha diversity metrics between A. populations, B. diets among all participants, C. diet only among Nunavut participants, D. BMI and E. ethnic groups. Only D. BMI had significant differences, with leaner BMIs being more diverse in Shannon and Simpson diversity metrics (Mann-Whitney test, $P < 0.05$). See Supplementary Materials and Methods for description of metrics.

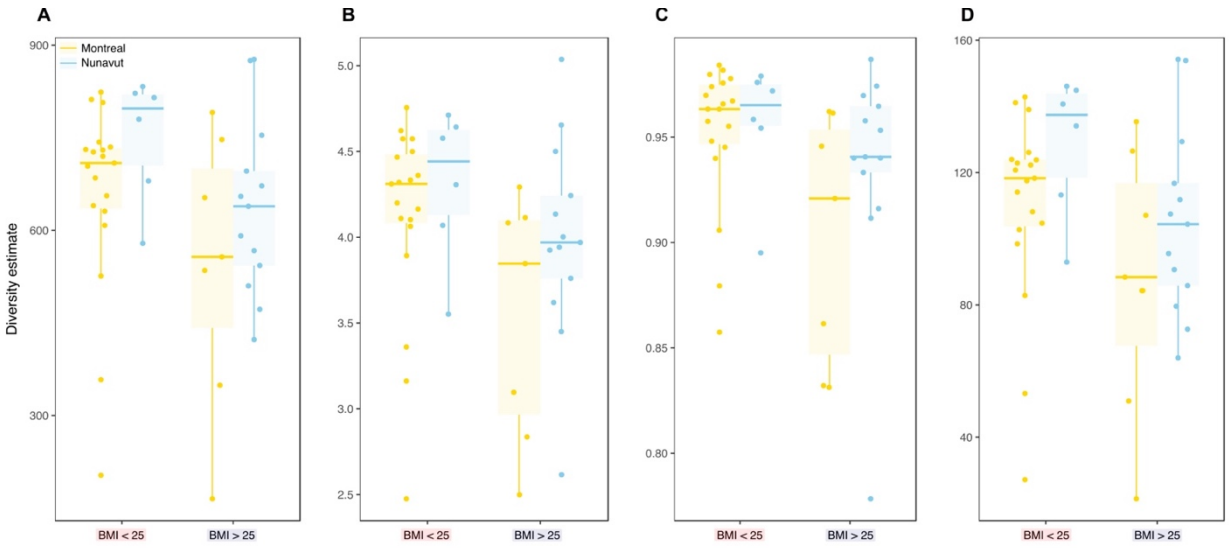

FIGURE S3. Comparison of alpha diversity between Montreal and Nunavut, binned by BMI, using A. observed OTUs, B. Shannon's diversity index, C. Simpson's index and D. Fisher's alpha. While there is a tendency for greater diversity in Nunavut for a given BMI, the difference is not statistically significant (Mann-Whitney test, $P > 0.05$). OTUs were counted using open-reference OTU picking (Methods).

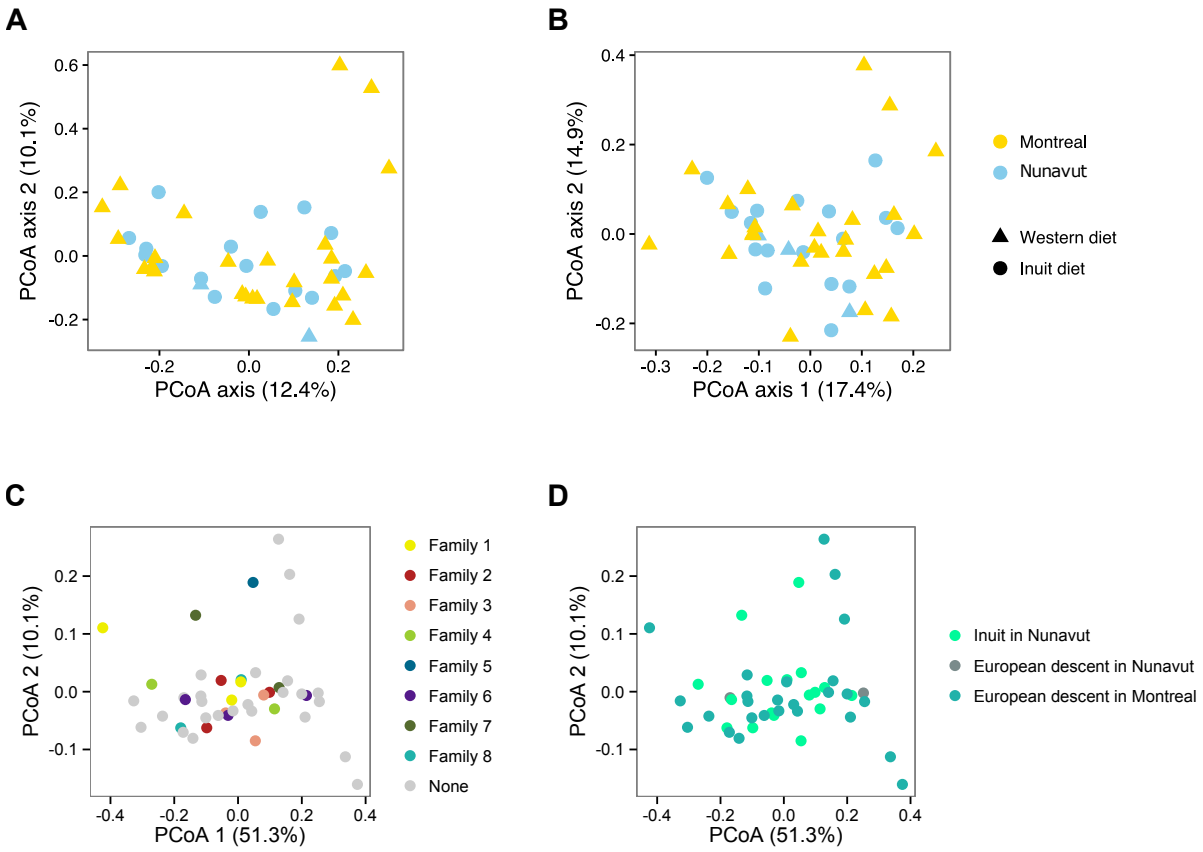

FIGURE S4. Beta diversity analyses using A. Bray-Curtis and B. Jenson-Shannon divergence reveal no clustering between populations or dietary types. See Supplementary Materials and Methods for description of metrics. UniFrac distances of the gut microbial community shows no clustering between C. members of the same household or family or D. ethnic groups included in this study.

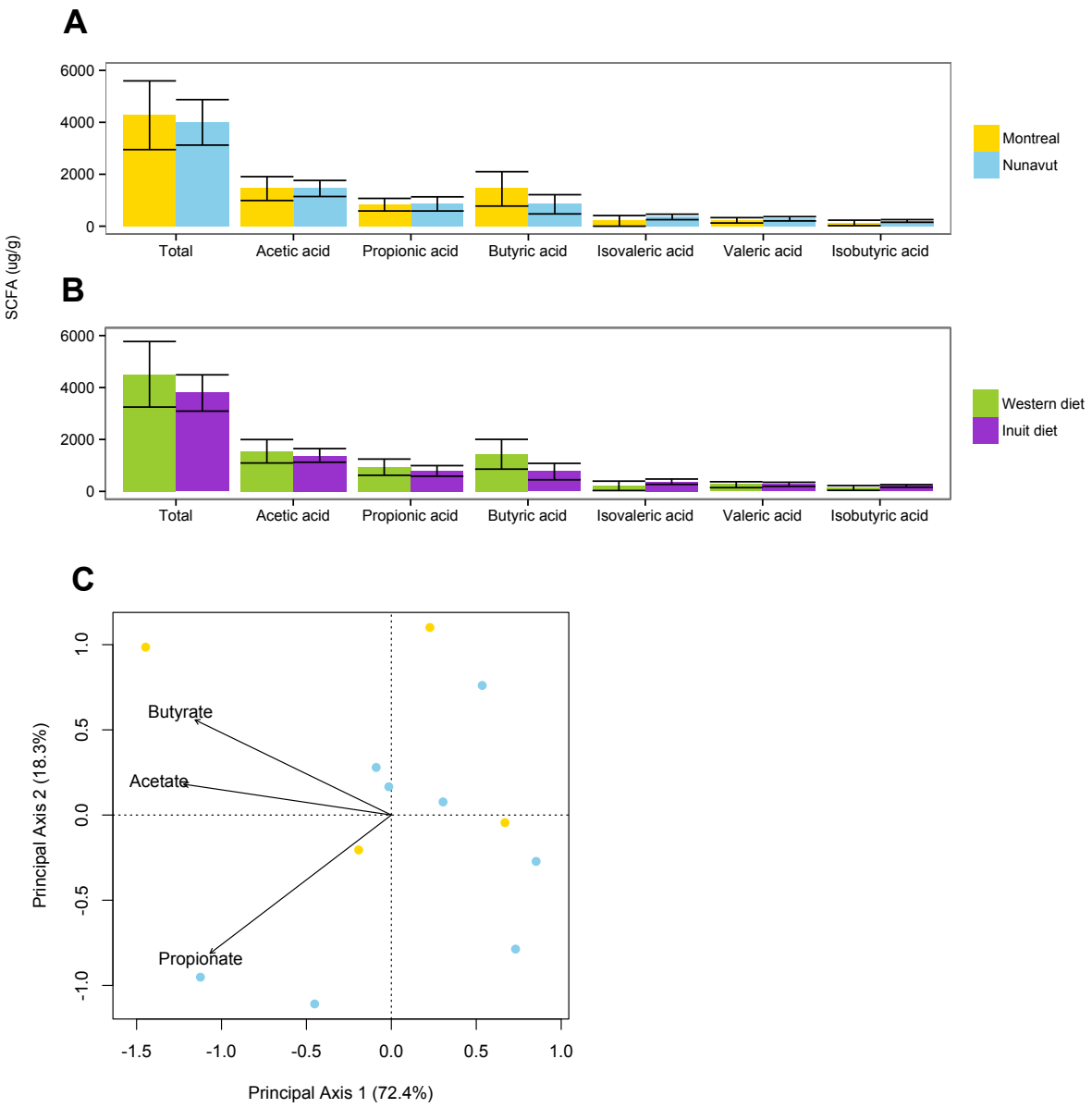

FIGURE S5. Short chain fatty acid (SCFA) content in stool samples according A. to geography and to B. diet. There were no significant differences between groups, C. and samples did not cluster together by geography based on similiarity in SCFA profiles for the three SCFAs analyzed (acetate, butyrate and propionate).

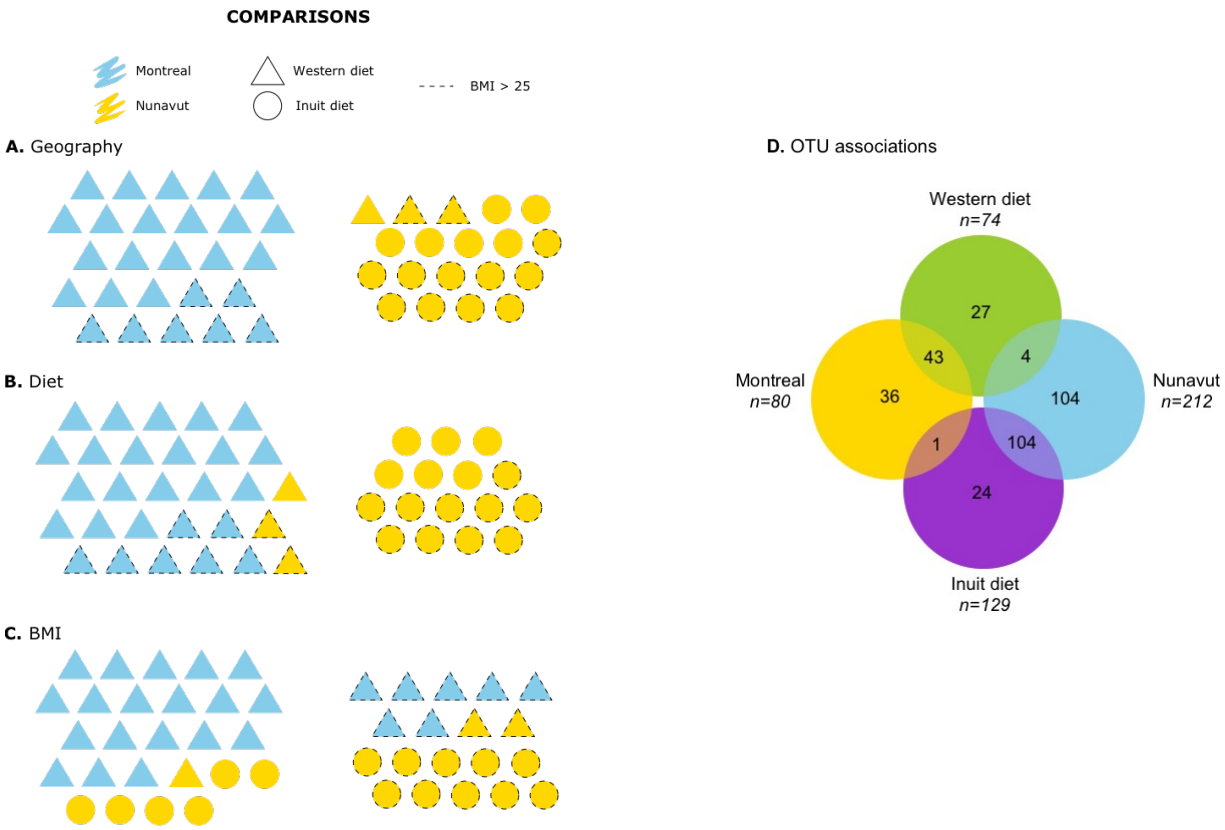

FIGURE S6. Diagram showing partitioning of samples and sample size when compared across A. geography, B. diet and C. BMI. D. Venn diagram of OTUs identified by DESeq2 associated with geography (Nunavut or Montreal) and diet (Inuit and Western diet). Numbers in Venn diagram show number of OTUs associated with each category, numbers in overlapping sections show number of OTUs associated with both categories, and *n* is the total number of OTUs associated with a category. Most of the OTUs identified by DESeq2 as associated with Montreal were also associated with the Western diet (53.8%) – however, 1 OTU (*Prevotella copri* OTU 326482) was associated with Montrealers and the Inuit diet. Half of the OTUs associated with Nunavut were also more abundant in consumers of the Inuit diet (49.1%), while 4 OTUs were associated with the Western diet. (*Eubacterium biforme* OTU 182483, unclassified member of the *Barnesiellaceae* family OTU 315846, unclassified member of the RF39 family OTU 569244, unclassified member of the YS2 family OTU 269386). Raw data for panel D. is presented in Tables S1D & E.

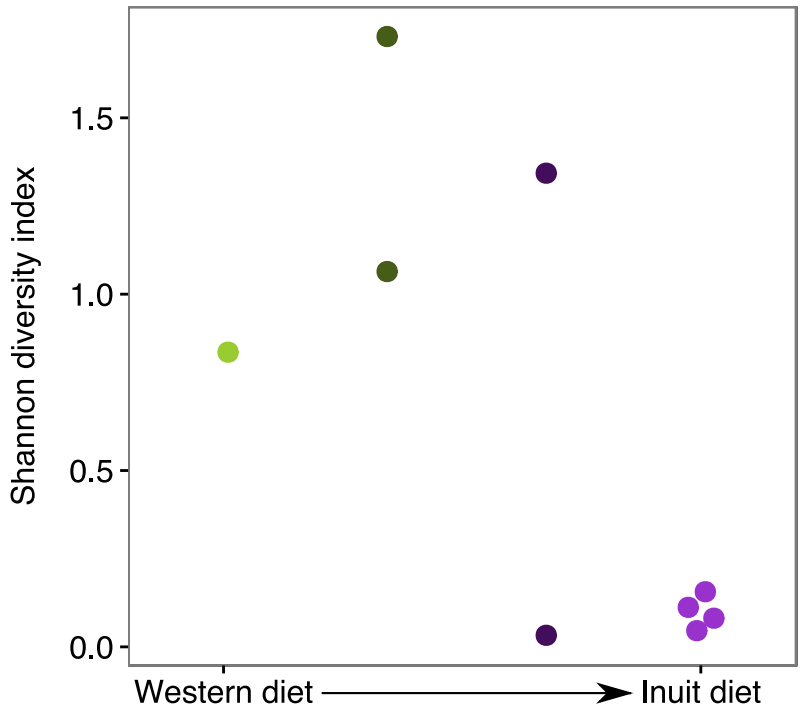

FIGURE S7. When diet is binned into 4 categories of increasing frequency of traditional food consumption (instead of 2 as in Figure 5), we observe the same trend of low *Prevotella* diversity being associated with an increasingly traditional diet. The extreme Inuit diet (right; n=4) is defined as individuals consuming traditional foods daily; the extreme Western diet (left; n=1) is composed of individuals who never consumed traditional foods; intermediates (each n=2) are composed respectively of individuals who rarely (less than weekly) or frequently (weekly) consume traditional foods (Tables S1A & C).

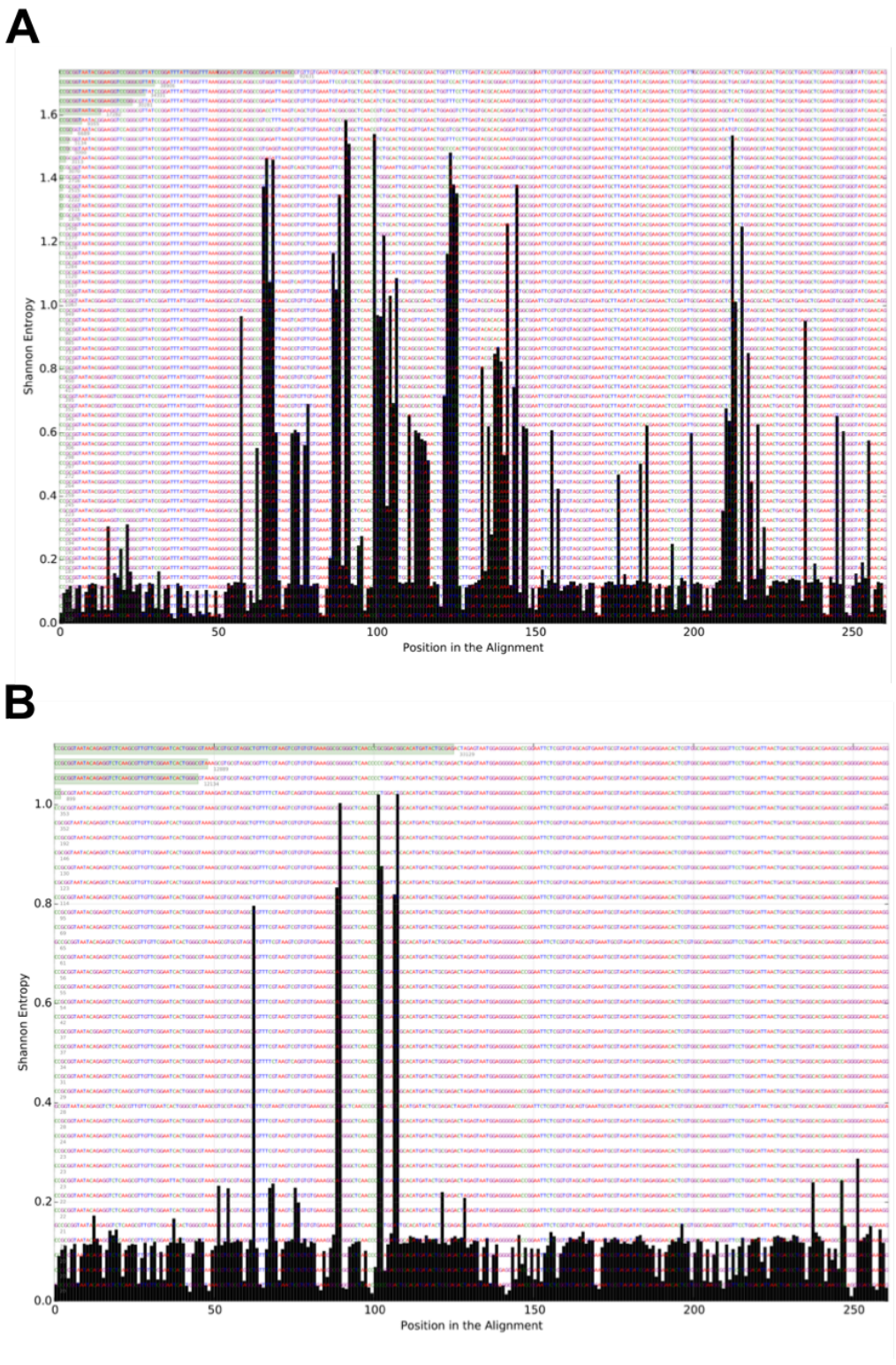

FIGURE S8. Shannon entropy at each position of the 16S alignment for A. *Prevotella* and B. *Akkermansia* oligotypes.

CHAPITRE 4. Profils fonctionnels métagénomiques et analyse de gènes de résistance et de méthylation du Hg dans le microbiome inuit

Metagenomic functional profiles and survey of mercury resistance and methylation genes in the Inuit gut microbiome

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In prep.

Abstract

Mercury (Hg) is a contaminant of major interest in the Arctic. In its organic form of methylmercury (MeHg), it can bioaccumulate through food webs into animals that are part of the traditional Inuit diet, such as fish and marine mammals. Consumers of this diet are thus exposed to greater concentrations than other individuals around the globe. Since environmental bacteria can readily transform Hg into the toxic MeHg form and back, it is critical to understand if these transformations also occur in the gut microbiome, as this may alter the contaminant's fate in the body and its toxicity to the host. Here, we sequenced metagenomes from stool samples of Inuit donors from Nunavut, Canada consuming traditional (n=5) or Western diets (n=5) and compared them to non-Inuit Southern North Americans with a Western diet (n=7). We found that geography and diet both drove differences in metagenomic profiles, and that mobile genetic elements (including plasmids, viruses) were important drivers of this variation. We also identified a *mer* operon (encoding Hg resistance) in one individual, including the gene *merA*, a reductase that can reduce Hg(II) into volatile Hg(0), which rapidly diffuses from cells. These genes were organized in an operon on a plasmid which also encoded antibiotic resistance. We found no evidence for the presence of *hgcAB*, the genes responsible for the production of toxic MeHg. We conclude that the traditional Inuit diet shapes the microbiome and its mobile gene pool. We also propose that Hg reduction may be possible in the gut, which could help better inform pharmacokinetic models that predict absorption of this toxic metal from food into the body.

Introduction

The human gut microbiome is a complex and diverse ecosystem, which contributes to host health in many ways, through nutrition, metabolism, immune development and behavior (Cryan and Dinan 2012; Nicholson et al. 2012; Turnbaugh et al. 2006). The gut microbiome is now recognized as a source of genetic and metabolic variation across human populations, with significant differences observed across human cohorts (De Filippo et al. 2010; Mueller et al. 2006; Yatsunenko et al. 2012). Diet is a major driver of community composition (David et al. 2013), and much attention has been paid to populations with traditional, non-Western diets. Indeed, subsistence strategies have been linked to variations in gut microbiome community structure and function (Clemente et al. 2015; De Filippo et al. 2010; Obregon-Tito et al. 2015; Schnorr et al. 2015; Yatsunenko et al. 2012). Many of these studies have focused on isolated or highly traditional peoples, with hunter-gatherer or agrarian practices. Recently, efforts have gone into describing the gut microbiome of North American Indigenous populations (Annex II, Dubois et al. 2017; Girard et al. 2017b; Sankaranarayanan et al. 2015), who are typically poorly represented in microbiome studies (Sankaranarayanan et al. 2015).

Hunting and gathering practices are deeply anchored in the culture of the Inuit of the Canadian Arctic, and their 'country food' diet is rich in animal fats and protein, and poor in fiber (Sharma 2010; Sharma et al. 2010). However, like many other Indigenous peoples around the globe, the Inuit diet is undergoing a transition towards a westernized state, which is associated with lower micronutrient intakes (Egeland et al. 2011; Kuhnlein et al. 2004; Mead et al. 2010; Sharma et al. 2010). Westernization can have consequences on human health and metabolism (Munch-Andersen et al. 2012), and may have also caused the Inuit microbiome community composition to converge with other North Americans (Annex II, Dubois et al. 2017; Girard et al. 2017b).

Country food in the Arctic is also a vector of exposure to contaminants, including heavy metals and organic pollutants (Donaldson et al. 2010; Van Oostdam et al. 2005). These contaminants, emitted at industrialized sites outside the Arctic, can be carried through long-range atmospheric or marine transport to the Arctic, where they may bioamplify through food webs, increasing in concentration with every trophic level (Donaldson et al. 2010). Mercury

(Hg) is a particular concern, as the Arctic is considered to be a sink for volatile anthropogenic Hg produced at lower latitudes (AMAP 2011; Ariya et al. 2004). Once deposited in Arctic aquatic ecosystems, Hg can be converted into the neurotoxin methylmercury (MeHg), which then accumulates through foodwebs, contaminating animals that are part of the country food diet (Donaldson et al. 2010; Lemire et al. 2014). While high oral exposure to another heavy metal has recently been shown to alter the gut microbiome of consumers of lead-contaminated water (Gao et al. 2017), little is known on the long-term effects of lifelong chronic metal exposure on the structure and function of the gut microbiome.

Pharmacokinetic models attempting to describe contamination patterns in humans have been found to be poor predictors of Hg in Northern Indigenous peoples (Canuel et al. 2006a). This could be due to human genetics or dietary practices altering the fate of Hg in the body (Girard et al. 2017a), but could also be caused by interactions with the gut microbiome. This has been previously observed for other metals, such as arsenic (Diaz-Bone and Van de Wiele 2010; Laird et al. 2009b). In the environment, Hg can transition between its volatile inorganic elemental form of Hg(0), the inorganic mercuric species Hg(II), and the toxic, organic methylated form of MeHg. Transformations from one chemical species to the other can all be mediated by microorganisms. Indeed, in the environment, Hg(II) is transformed regularly by bacteria, who can methylate it into MeHg (through the *hgcAB* gene product) (Compeau and Bartha 1985; Fleming et al. 2006; Pak and Bartha 1998). While *hgcAB* has not yet been identified in primate or human microbiomes (Gilmour et al. 2013; Martín-Doimeadios et al. 2017), it might be present in the Inuit microbiome, where microbes are potentially exposed to higher Hg concentrations. Environmental bacteria can also mediate the demethylation of MeHg into Hg(II), and the subsequent reduction of Hg(II) into volatile Hg(0) through the Hg resistance *mer* operon (Barkay et al. 2003). If these pathways are present in the gut microbiome, they may alter the cycling of Hg in the body, and impact toxicity to the host. Previous studies have shown that volatile Hg(0) can be produced from stool incubated with Hg (Rowland 1988), and that this production is halted after antibiotic treatment in mice (Nakamura et al. 2013). Furthermore, experiments on excretion rates in human have found that antibiotic treatments altered MeHg elimination (Caito et al. 2017). This suggests that interactions between Hg and the microbiome could alter the fate of this contaminant in country food consumers.

In this study, we explored how the Inuit microbiome is shaped by the traditional country food diet, and searched for genes involved in response to Hg exposure. We compared individuals from a remote Arctic community adhering to the traditional Inuit diet, to residents of the same community with a Western diet. We also included samples of Western diet consumers from an urban center. We used shotgun metagenomic sequencing from stool to compare the taxonomic composition and gene content of microbiomes between dietary and geographic groups. We show that while overall similar, the composition and gene functions of the Inuit microbiome are significantly affected by diet, and that Nunavut microbiomes are characterized by a greater proportion of mobile genetic elements. Then, we explored whether the Inuit microbiome has greater potential for Hg metabolism, as this might alter speciation of the metal between its inorganic, less bioavailable forms (Hg(0) and Hg(II)), and its toxic methylated species (MeHg). We did not identify any sequences of *hgcAB*, consistent with methylation of Hg into MeHg being unlikely to occur in the gut. However, we did identify a *mer* operon in one Inuit individual, suggesting the potential for other forms of Hg resistance in the gut. The *mer* genes were carried on a plasmid which also encoded antibiotic resistance. We thus show that the traditional country food diet can shape the composition and gene content of the Inuit microbiome, and that Hg resistance can be a feature of the Inuit microbiome (but is not necessarily linked to diet). Increased sample size would help better understand how widespread resistance is, and how it may impact Hg toxicology in humans.

Methods

Participant enrollment and sample collection

We recruited volunteers from the community of Resolute Bay, Nunavut in July 2014, as described in Girard et al. 2017b. We also recruited individuals living in Montreal, Canada, who were students or employees at a university. Two samples were chosen from the Montreal cohort for this metagenomic study, and 10 were selected from Nunavut. The 10 Nunavut samples were chosen to represent the most traditional consumers of the country food diet (NVT-TRAD, n = 5), and the most Westernized (NVT-WEST, n = 5) of the cohort described in Girard et al., to

maximize dietary contrast. Two donors from Nunavut shared a household. The small cohort sampled in this study cannot represent all Inuit. For the sake of clarity, the term "Inuit" here is used to refer to our small cohort, but should not be inferred as representing all Inuit worldwide. We also sequenced two metagenomes from Montrealers, and combined these with five previously sequenced metagenomes from the Human Microbiome Project, yielding a group of urbanized North Americans with a Western diet (MTL-HMP, *n*=7). The raw metagenomic sequences from the five randomly selected healthy HMP donors were downloaded from the HMP Portal (https://portal.hmpdacc.org/). These samples underwent the same trimming, assembly and annotation method as our own sequences (described below).

The objectives and outcomes of the study were explained to all Nunavut and Montreal participants, who then gave informed written consent. All participants were healthy and had not taken antibiotics in the previous month, and completed dietary habit questionnaires to assess their food intake (Girard et al. 2017b). This work was approved by the Université de Montréal internal ethics review board for arts and sciences (CERFAS; certificate no. 2013-14-022-D). Permission for this work was granted by the Nunavut Research Institute (Health Research Licenses no 02 040 13N-A and 02 046 14N-A), as well as by the Hunters and Trappers Association and Hamlet of Resolute Bay.

Participants wore gloves when collecting stool samples into sterile specimen containers. In Nunavut, samples were either kept at <4 °C for a maximum of 12 h before being collected by the sampling team and frozen at -80 °C. In Montreal, samples were immediately placed at -20 °C by participants, before being collected by the sampling team and kept at -80 °C until DNA extractions. Anonymized participant information is provided in Supplementary Table S1, and dietary binning was performed as described in Girard et al., 2017b.

DNA extraction, library preparation, and sequencing

DNA was extracted from stool samples with a PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc) (Girard et al. 2017b). DNA was purified with the PowerCleanPro DNA isolation kit (Mo Bio Laboratories, Inc) using the manufacturer's instructions, with an extra

RNAase A step and ethanol wash. DNA was quantified using a NanoDrop (Thermo Fisher), and smears were verified on a Qiaxcel Advanced system (Qiagen). Paired-end (2 x 300 bp) shotgun metagenomic libraries were prepared using a Nextera XT kit (Illumina). Libraries were quantified with a Qubit v.2.0 fluorometer (Life Technologies, Inc), denatured and sequenced using V3 Illumina chemistry (Illumina MiSeq), with 3 samples sequenced per run.

Quality filtering and trimming

All three sequencing runs yielded 79,013,572 reads (average per sample: 6,584,464 ± 1,936,661). Read quality was verified with the ShortRead{} (Morgan et al. 2009) package in R (R Development Core Team). Reads were trimmed with Trimmomatic (Bolger et al. 2014) to a minimal length of 75 bp and a minimal Phred score of 33. We analyzed these reads using two methods: 1) we performed taxonomic and functional annotation using the MG-RAST pipeline (Meyer et al. 2008) and searched these annotations for Hg metabolism genes, and 2) we assembled reads and annotated genes on the resulting assembly to compare sequence similarities between groups and search for Hg-related genes. Read counts before and after quality-filtering are presented in Supplementary Table S1.

Taxonomic and functional annotation

Trimmed and quality-filtered reads were uploaded to the MG-RAST server (Meyer et al. 2008) following dereplication (Gomez-Alvarez et al. 2009), filtering of host sequences (Langmead et al. 2009) and removal of low quality reads (minimal Phred score of 15). Taxonomic annotations were performed at 97% identity against the 16S Greengenes database (version 13_8) (DeSantis et al. 2006), and functional annotations were performed at 60% similarity against KEGG Orthologs (Kanehisa 2000) and SEED SubSystems databases (Overbeek et al. 2005).

We used the observed number of OTUs and Shannon's diversity index (measure of richness and community evenness) to assess alpha diversity, and Bray-Curtis distance (measure of similarity across two populations) for community-wide comparisons of beta diversity.

OTU differential abundance analyses were performed on filtered (minimum of 10 observations across all samples, using *filter_otus_from_otu_table.py* in QIIME 1.9.0 (Caporaso et al. 2010)) taxonomic data with DESeq2 (Anders and Huber 2014; Love et al. 2014). Only taxa significant after correction for multiple-hypothesis testing are reported.

Sequence cluster permutational analysis

Trimmed and quality-filtered paired-end reads were joined and assembled using the *de novo* assembler IDBA-UD (Peng et al. 2012), with minimal *k-mer* length set at 20, and maximum at 120. Contigs less than 2,500 bp were removed. This left us with a mean of 137,201 ± 47,884 contigs per sample. Gene annotation from the assembly was performed with Prokka (Seemann 2014), providing us with amino acid and nucleotide sequences of predicted genes.

Contigs were clustered using all-vs-all BLAST (Zhang et al. 2000), resulting in a sequence similarity network. Network analyses were performed with EGN (Halary et al. 2013), and links between contigs were established based on 80% sequence identity, covering at least 80% of the shortest sequence in the alignment. Clusters in the network were labelled according to the types of samples it contained (combinations of MTL-HMP, NVT-WEST, and NVT-TRAD), resulting in 6 possible types of clusters: MTL-HMP only, NVT-WEST only, NVT-TRAD only, MTL-HMP+NVT-WEST, MTL-HMP+NVT-TRAD, NVT-WEST+NVT-TRAD, or all three types. Permutations (n=100,000) were performed by re-shuffling the contig labels (preserving only the number of contigs/nodes in a cluster), and the frequency of each cluster type was re-calculated (Supplementary Figure S1). This analysis allowed us to assess how the observed frequency of dietary clusters compared to their frequencies expected in the network by chance.

We also investigated the contribution of mobile genetic elements (plasmids, viruses, prophages) to the dietary cluster types. The 20 most common backbone gene families in viruses and plasmids (involved in replication and transport) were used to find these mobile elements in our contigs (Garcillán-Barcia et al. 2011) with BLAST (e-value < $1e^{-5}$) (Supplementary Table S2). The ratio of plasmid and virus contigs to total contigs was calculated within each cluster type, and overall.

Mercury metabolism genes, assembly and annotation

To investigate the diversity of genes related to Hg in the dataset, genes associated with resistance, transport or metabolism of metals were first identified in SEED annotations from MG-RAST, using the following keywords: *mercury*, *mercuric*, *Hg*, *arsenic*, *As, zinc, copper, cobalt, lead, cadmium, chrome, chromate, chromium, metal, resistance, tolerance.* The abundance profiles of these genes were then filtered for low-abundance genes (with less than 10 hits across all samples), and used to produce heatmaps.

Hg associated genes were also identified in the Prokka-annotated assembly. Briefly, a keyword search was performed with NCBI's E-Utilities (esearch and efetch), with the names of all the gene families involved in the mer operon. Networks were created with EGN and visualized to visually curate clusters that may contain mis-annotated sequences. This dataset of reference sequences was then expanded by performing a BLAST alignment against NCBI's non-redundant (NR) database (e-value cut-off $10e^{-40}$). The expanded dataset was then clustered at 90% using CD-HIT-EST (Fu et al. 2012) and aligned with Mafft (Katoh and Standley 2013). The final dataset of reference sequences, which includes *mer* genes from Acidobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Terrabacteria are listed in Supplementary Table S5A.

Hidden-Markov models in HMMER (Finn et al. 2011) were used to search the Prokka-annotated assembly against the NCBI references. Sequences in the Prokka assembly that were hits to these *mer* genes were extracted from their contigs, and trees were computed with FastTree (Price et al. 2009). The 5 HMP samples that were assembled and annotated with Prokka as described above were also searched with HMMER for *mer* genes and added to trees. Bowtie 2 was used to map reads onto *mer* gene hits (Langmead and Salzberg 2012).

Statistical analyses

Taxonomic and functional profiles (KEGG and SEED annotations) were analyzed in STAMP (Parks et al. 2014). Differentially abundant features were identified with Welch's t-test with Benjamini-Hochberg False Discovery Rate (FDR) correction for multiple hypothesis

testing, and ANOVA with Games-Howell post-hoc test and Storey's FDR. Taxonomic and functional profiles were visualized with the ggplot2{} package (Wickham 2009) in R (R Development Core Team). Alpha diversity metrics were compared with the Mann-Whitney test. Heatmaps were produced with the Heatplus{} package (Ploner 2015), and distances for dendrograms were computed with vegdist() in vegan{} (Oksanen et al. 2015). Analyses of variances were performed on Bray-Curtis distance matrices using the adonis() function in vegan. Principal component analyses (PCA) were performed with vegan and plotted with ggplot2 and ggfortify{} (Tang et al. 2016), and constrained correspondence analyses (CCA) were performed with the cca() function. CCAs were tested by a permutation ANOVA-like test using the anova.cca() function in vegan. Environmental factors (diet, geography) were fitted onto CCA ordinations with the envfit() function in vegan{}.

Results

Taxonomic and functional composition

Our previous studies based on 16S amplicon sequencing of stool samples have shown that the Inuit microbiome is surprisingly similar to the Western microbiome (Annex II, Dubois et al. 2017; Girard et al. 2017b). Here, we show that metagenomic sequencing of a subset of 'extreme' dietary types described in Girard et al. (2017b) supports these observations. Greengenes taxonomic clustering at 97%, based on shotgun sequencing, shows a (non-significant) trend for Nunavut and country-food diet samples to be slight less diverse, for both observed OTUs and Shannon's diversity index (Figure 1A&B).

Grouping samples by diet (Western vs Traditional) explained 17.5% of the observed variance in microbiome community structure (adonis $P < 0.05$). Individuals adhering to the traditional country food diet were more scattered along the PC2 axis, when data was ordinated using Bray-Curtis as a distance metric (Figure 1E). When samples were compared in CCA space, diet was also a significant explanatory variable ($P < 0.05$) (Figure 1F).

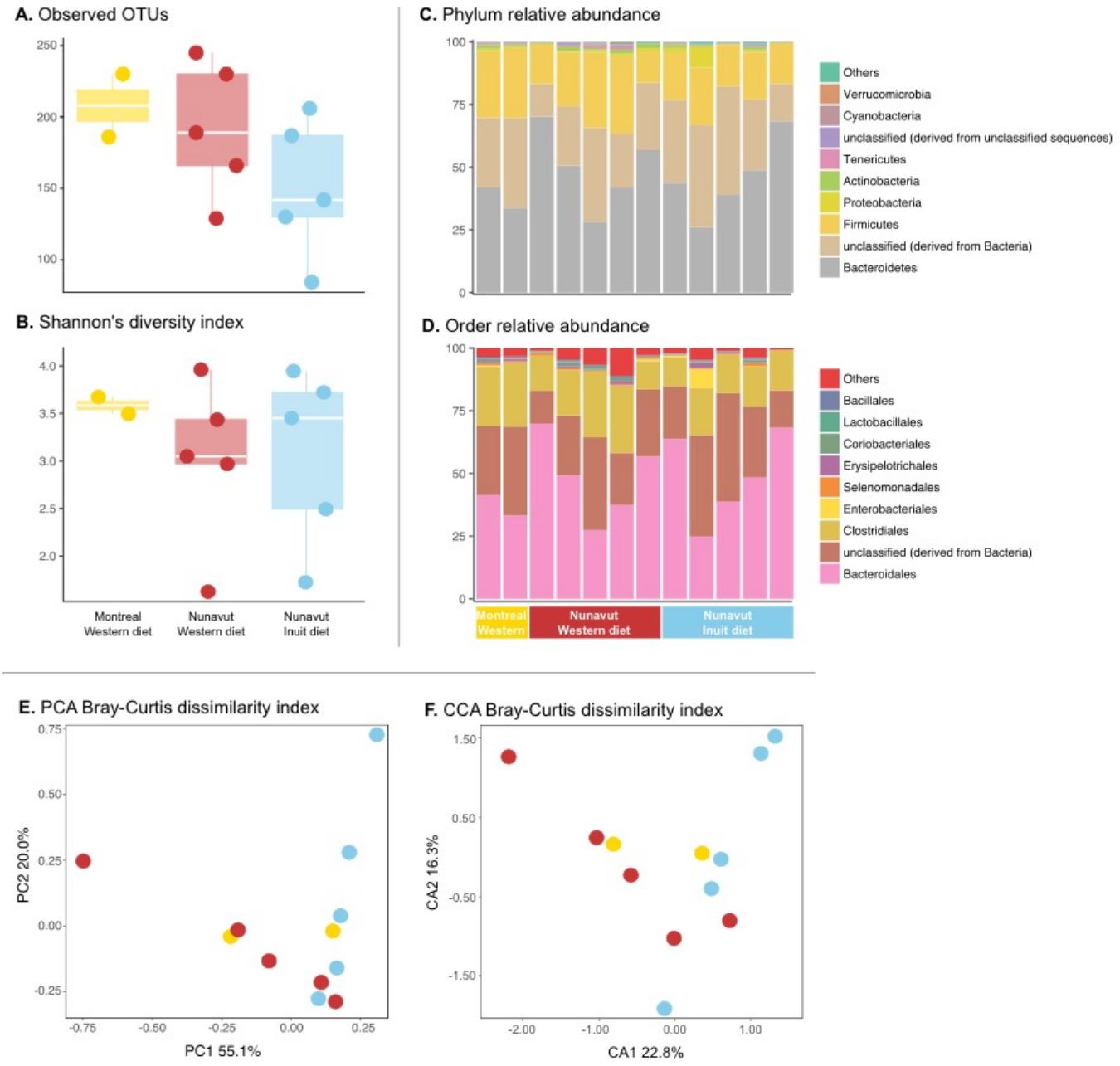

FIGURE 1. Community composition and alpha and beta diversity are broadly similar for all samples. (A & B) Alpha diversity, shown through observed OTUs and Shannon's diversity index, shows that diversity is similar across Montrealers, and individuals from Nunavut adhering to a Western or traditional diet. However, there is a slight (non-significant) trend for traditional individuals in Nunavut to be slightly less diversified. (C & D) Phylum and order-level abundance show that the composition of the microbiomes is similar across all groups. (E & F) Diet was a significant explanatory variable when considering beta-diversity, calculated with Bray-Curtis dissimilarity index in PCA ($P < 0.05$) and CCA space ($P < 0.05$).

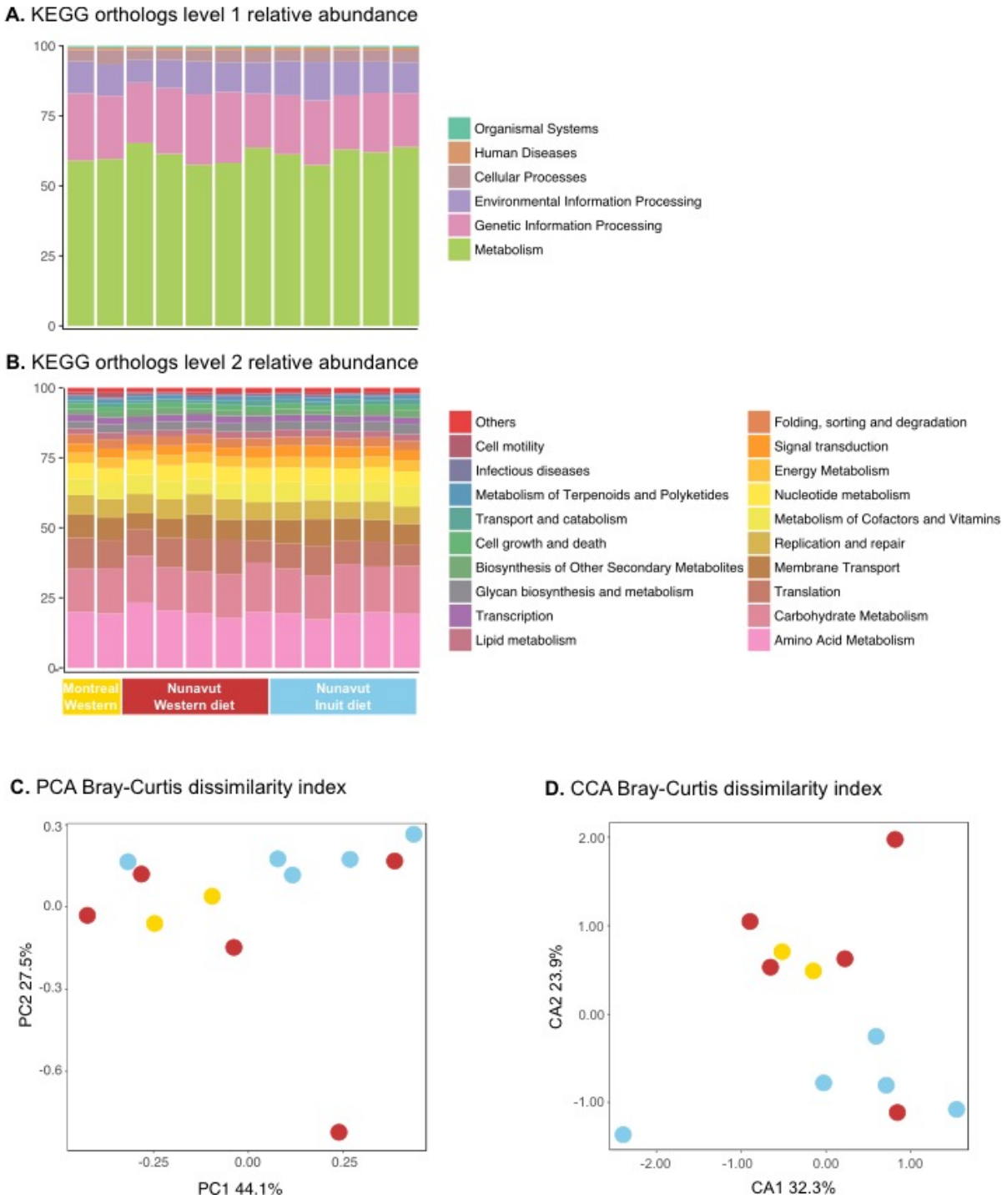

FIGURE 2. Functional profiles are broadly similar for all samples. (A & B) Pathways identified through KEGG Orthologs show that all three cohorts are broadly similar in their functional profiles. (C & D) Diet was a significant explanatory variable for distances calculated with Bray-Curtis dissimilarity index in CCA space ($P < 0.05$).

Differential abundance comparing samples by diet revealed that *Bacillus coagulans* and *Symbiobacterium thermophilum* were also associated with the Western diet, including samples from Montreal and Nunavut ($P < 0.05$). Meanwhile, *Bacteroides* sp., *Prevotella albensis* and *Bacteroides finegoldii* were all significantly more abundant in consumers of the traditional country food diet of Nunavut ($P < 0.05$) (Supplementary Table S3). No higher taxonomic groups emerged as significant features when comparing samples across dietary types (Western vs Traditional), or when crossing geography and diet (Montreal-Western vs Nunavut-Western vs Nunavut Traditional).

Functional profiles annotated through the KEGG Ortholog database show that samples are similar across geography and dietary types (Figure 2A&B). We verified if any pathways were differentially abundant across diets, and specifically searched for pathways identified by David et al. as being associated with either a plant-based or protein-based diet (David et al. 2013): however, none emerged as significant ($P > 0.05$). However, in CCA ordination space, diet explained 26.4% of the variance observed in the abundance of KEGG Ortholog annotations called through MG-RAST (envfit $P < 0.05$).

Sequence-similarity clustering and the mobilome

Contigs from assembled metagenomes were clustered by sequence similarity (Methods). We then examined the dietary types represented in each cluster. Observed frequencies were compared to randomized permutations, to determine how they differed from random expectations (Supplementary Figure S1). We hypothesized that individuals within the same dietary category or from the same geographic regions would have more contigs that cluster together, suggesting they are more similar. This allowed us to compare samples at a finer level, outside of taxonomic or functional assignments. Results from permutations show that clusters of contigs containing reads from all dietary groups were as abundant as expected from randomization. However, clusters containing only Montreal and HMP data (MTL-HMP) were more abundant than predicted, as was the case for clusters containing only Nunavut samples from individuals with a traditional diet (NVT-TRAD) (Figure 3A). This suggests that individuals with a Western diet from Montreal and from the HMP have more similar contigs,

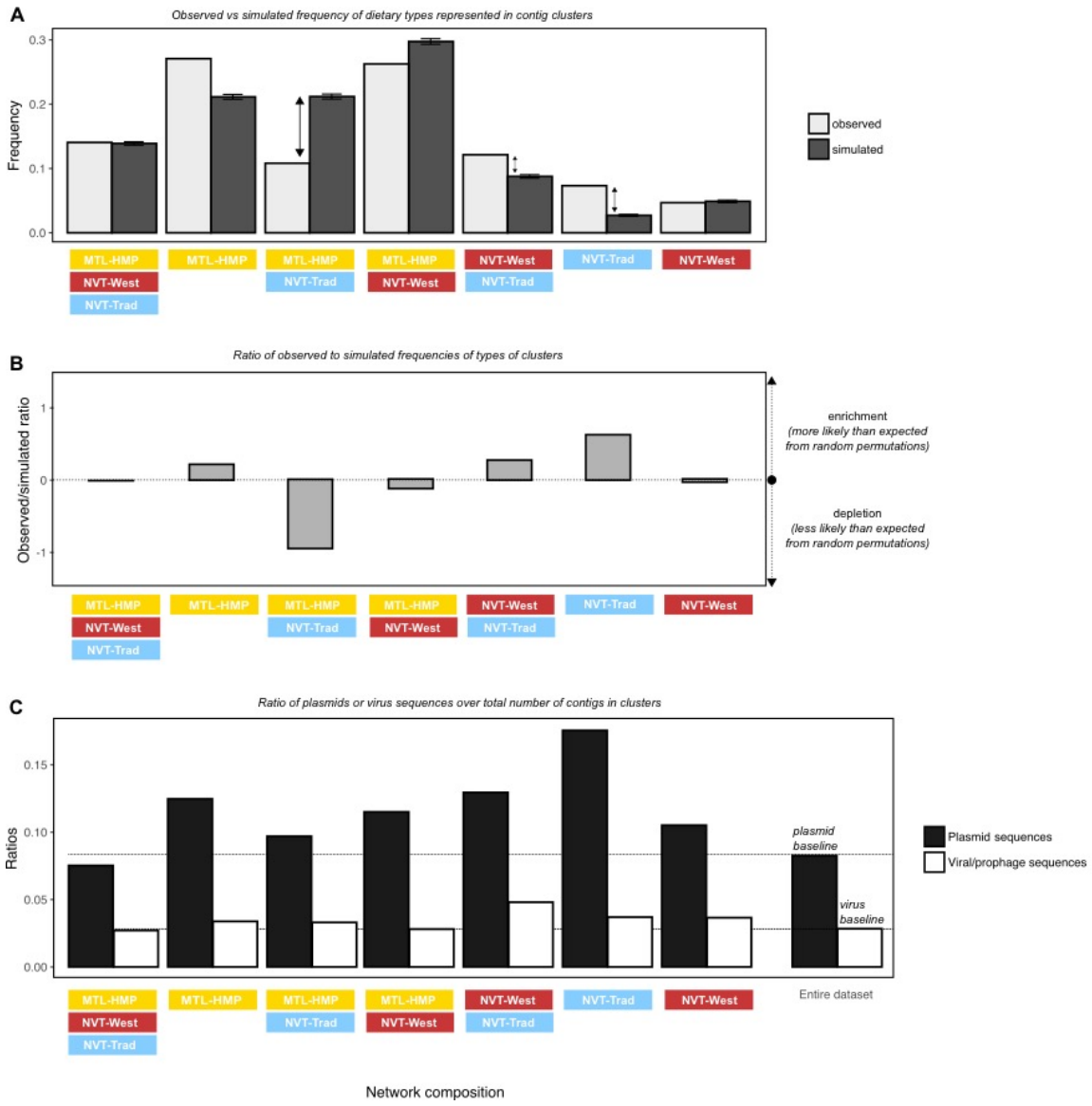

FIGURE 3. Similarity-based clustering of contigs into 6 possible cluster types (MTL-HMP, NVT-WEST, NVT-TRAD, MTL-HMP+NVT-WEST, MTL-HMP+NVT-TRAD, NVT-WEST+NVT-TRAD, all 3 diets) from observed and simulated (100,000 permutations) contig clusters. (A) Observed vs expected frequencies of dietary groups from clustered contigs. (B) Observed ratios for each dietary group, showing that there were less groups made up of MTL-HMP and NVT-TRAD in the observed dataset, but overrepresentation of NVT-TRAD alone and Nunavut (NVT-TRAD+NVT-WEST) clusters. (C) Ratios of mobile genetic elements compared to all contigs for plasmids and viruses/prophages, in each of the dietary clusters presented in Panel A. Baselines are marked at the overall ratio in all contigs. All clusters (except for the one containing all sample groups) were enriched in plasmids. The effect was not as strong in viruses, where Nunavut samples tended to be slightly more enriched.

and tend to group together, away from Nunavut samples. Clusters containing both western samples (MTL-HMP) and Nunavut samples from the traditional diet (NVT-Inuit was lower than that was expected from simulations (Figure 3B). This shows that contigs from the MTL-HMP group and the NVT-TRAD were unlikely to be clustered together, and were therefore less similar to each other than expected.

We also found that mobile genetic elements (including plasmids and viruses) were significant contributors to the groupings observed in Figure 3A. When compared to our baseline (ratio of plasmids/viruses to the total number of contigs in the dataset), we found that clusters composed of only one dietary type were enriched in both plasmids and viruses (Supplementary Figure 3C), and explained part of the groupings seen in Figure3A&B. Clusters containing Nunavut samples from both dietary groups (traditional Inuit and Western diets) were also more enriched in mobile genetic elements. Clusters containing all three dietary types on the other hand were not more enriched than the total dataset (Supplementary Figure 3C). The plasmid enrichment was greatest in NVT-TRAD-only clusters, and were mostly composed of genes annotated as hypothetical proteins (Supplementary Table S6). This suggests that the 'uniqueness' of clusters composed only of NVT-TRAD contigs (Figure 3A) is driven at least in part (17%) to plasmid sequences (Figure 3C).

Mercury methylation

No similar sequences were found in any samples for the mercury methylation genes *hgcA* or *hgcB*, on contigs or on Prokka-predicted proteins. We did not identify any mercury methylation sequences in the HMP dataset either. As *hgcA* and *hgcB* have not yet been identified in the human gut microbiome (Rothenberg et al. 2016), any genes present may be highly divergent.

Mercury resistance

SEED Subsystems annotations suggested the presence of several genes involved in metal transport and metabolism. This includes heavy metal translocation proteins, that allow for

transport of cadmium, cobalt, mercury, lead and zinc (Figure 4A). None of these were not significantly differentially abundant across dietary types (Welch's t-test, $P > 0.05$). This absence of significant association was confirmed using annotated genes from metagenomic assemblies instead of SEED annotations. We conclude that none of the metal metabolism genes tested were differentially abundant across dietary groups. In contrast with our expectation that mercury resistance genes would be associated with the traditional Inuit diet, we noted the presence of multiple *mer* operon genes in most samples regardless of diet, including the gene encoding the mercuric reductase MerA (Figure 4). One Nunavut participant consuming a Western diet (R22), contained hits to most genes involved in the *mer* operon (Figure 5, Supplementary Table S4), notably *merA, merE* (a gene encoding an unknown function) (Barkay et al. 2003) as well as *merD*, and *merR* (regulatory proteins) (Barkay et al. 2003). To further explore these results, we considered the abundance profiles of only mercury-related genes from the SEED annotation, and saw that four out of five traditional individuals group together because of a relative enrichment of reads mapping to the *mer* operon (Figure 4B).

Since sequences of *mer* genes can be genetically diverse (Silver and Phung 1996). possibly leading to false positive and negative identification, we performed an in-depth analysis of reference sequences for the different *mer* gene families, independently from MG-RAST annotations. Using HMMER, we searched our annotated contigs for *mer* genes listed in our reference dataset containing *mer* genes from 5 phyla collected from NCBI (Supplementary Table S5A. We were only able to confirm the presence of *merA*, *merR*, *merD* and *merE* in one sample, R22 (Figure 5). All genes were present on the same contig (#1951) of R22, suggesting that the microbiome of this individual has the potential to reduce Hg into its gaseous form (via MerA) under the control of regulatory genes *merR* and *merD* (with *merE* being of unknown function)**.** While sequences similar to *mer* genes were identified in samples from all dietary categories as well as in the HMP dataset, phylogenies of these genes suggest that most of these are distant paralogs of *mer* genes. This can be seen by the clades formed by these sequences in Supplementary Figure S2, annotated as various proteins including a dihydrolipoyl dehydrogenase, a soluble pyridine nucleotide transhydrogenase, a glutathione reductase and a Coenzyme A disulfide reductase. Only the R22-1951 sequence is annotated as *merA*, and forms a monophyletic group with other *merA* reference sequences (Supplementary Figure S2). We

obtained similar results for *merD* (Supplementary Figure 2), while *merR* and *merE* only yielded one hit in R22 (Supplementary Figures 3 & 4).

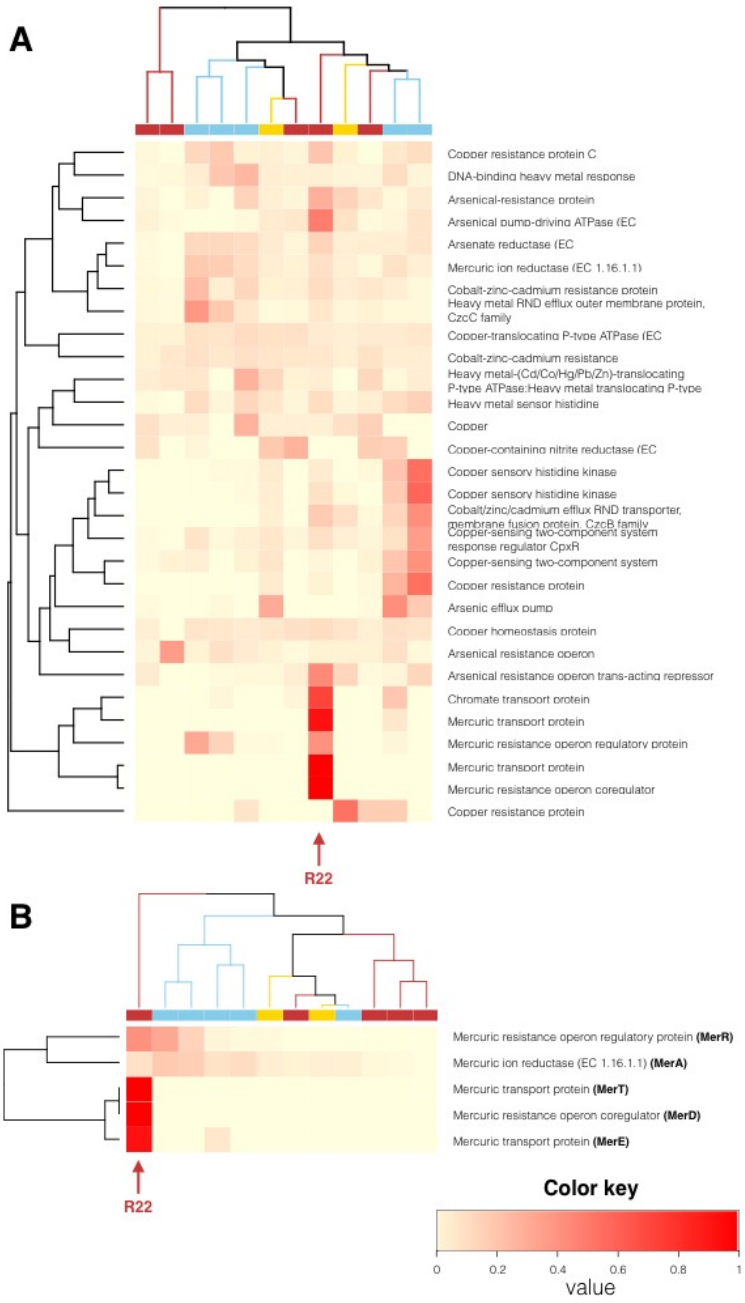

FIGURE 4. Heatmaps showing relative abundance of (A) Metal metabolism pathways from SEED annotations (annotated with MG-RAST) and (B) mercury-specific genes, after row-wise normalization. Dendrograms were computed with Bray-Curtis dissimilarity index. Yellow shows Montrealers with a Western diet, red represents Nunavut individuals with a Western diet, and blue shows Nunavut individuals adhering to a traditional Inuit diet.

Reads from other samples were mapped onto the #1951 contig, and we found no alignments. This allowed us to confirm that the *mer* genes were only present in R22.

We then verified if the *mer* operon genes we identified were scattered amongst different genomic regions, or grouped together on a single contig, which would suggest that a single cell could encode full Hg-resistance. We found that contig #1951 from R22 contained four *mer* genes, as well as a transposon resolve gene, a TEM beta-lactamase, and three genes of unknown function. A BLAST search in the NCBI NR database for the full #1951 contig points to this sequence belonging to an anaerobic or facultative anaerobic Gram-negative bacteria, like *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Escherichia coli* or *Salmonella enterica* (based on the taxonomic annotation of the top 10 hits in BLAST).

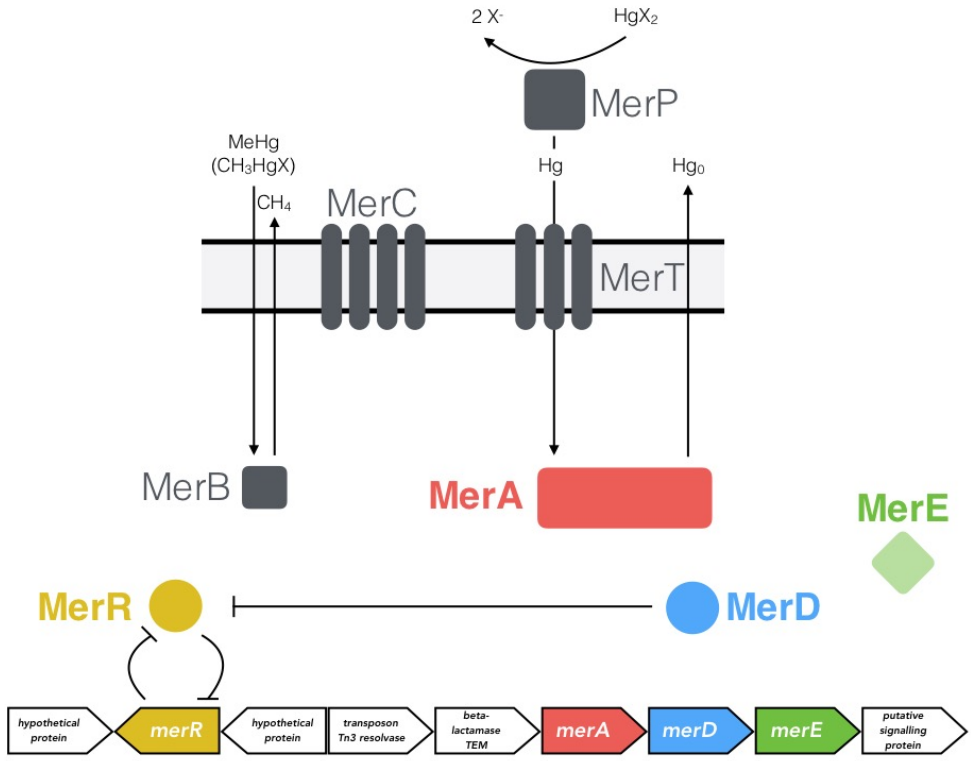

FIGURE 5. *mer* operon encoded pathways, adapted from Barkay et al. 2003. Proteins shown in grey were not identified in our samples. The genes encoding MerA, MerR, MerE and MerD (in colour) were found on the same contig #1951 in R22. The structure of the R22-1951 contig is presented, showing the presence of hypothetical proteins, transposon genes and an antibiotic resistance gene. In the figure, MerE is transparent, because it is unknown if the gene actually encodes a protein or not (Barkay et al., 2003).

A BLAST search using only the *mer* operon from contig #1951 (which includes *merA*, *merR*, *merD* and *merE* and the antibiotic resistance gene) showed it was a close match (99% identities over the length of the operon, 6,669 bp) to a plasmid isolated from a wastewater treatment plant (pEFC36a, accession number JX486126) (Rahube et al. 2014), where *mer*-encoded resistance was also found. The pEFC36a plasmid also included other genes not found on the 1951 operon (*merC*, *merT* and *merP*).

Discussion

Previous studies of the gut microbiome have shown major differences across populations and dietary practices. While the Inuit live in a unique environment and rely heavily on country food (*ie*. animals harvested from the tundra or the ocean) (Kuhnlein et al. 2004), their transition to a Westernized diet and increasing consumption of market foods is making their microbiomes more similar to those of individuals living at urbanized latitudes (Annex II, Dubois et al. 2017; Girard et al. 2017b). Our findings from the metagenomic survey confirms previous observations based on 16S only (Girard et al. 2017b) that the Inuit microbiome's community composition is very similar to Montrealers (Figure 1). However, in this sub-cohort, we did find that diet explained 17.5% of beta diversity taxonomic composition, similarly to what Dubois et al. observed in a 16S survey of the same population (Annex II, Dubois et al. 2017). This may be due to the fact that for this study, we chose the most 'extreme' practitioners of both diets, ie. the strongest adherents to the traditional Inuit diet, and the least traditional Inuit participants of the study. This shows that while the Inuit may be undergoing a dietary transition towards a more westernized state, the signal for diet remains when the most extreme individuals are compared.

The dietary transition and significant overlap in the diets of participants (Girard et al. 2017b) also explains the homogeneity of gene abundances from KEGG Ortholog annotations (Figure 2). While metabolic profiles have been found to be stable among healthy individuals in spite of variations in taxonomic profiles (The Human Microbiome Project Consortium 2012), studies comparing extreme diets have revealed differentially abundant metabolism pathways (David et al. 2013). A comparison of extreme plant-based and animal-based diets has shown

that these dietary types have distinct enrichment patterns for amino acid and glucose metabolism (David et al. 2013). This supports observations made in carnivorous and herbivorous microbiomes, where amino acid metabolism has opposite abundance patterns (Muegge et al. 2011). In this study, we found no significant enrichment in the pathways identified in David et al. 2013, either when comparing across geography or diet. However, this may not be surprising, in light of the dietary transition underway in many Indigenous communities around the North, and especially in the Arctic (Kuhnlein et al. 2004). We did however find that diet significantly explained variation in the pathways present (Figure 2), suggesting that animal protein and fat-enriched diets can impact the microbiome's metabolism but in subtle ways, such that no individual pathways or genes emerge as differentially abundant. While the dietary questionnaires used in this study do not capture short-term changes in diet, which could impact the microbiome (David et al., 2013), the 17.5% of taxonomic variation explained by diet is very close to what was observed by Dubois et al. from 16S analyses in the same population (17%) (Annex II, Dubois et al. 2017) using 48 hour dietary recalls. This suggests that while variation in this study may be masked by low resolution dietary information, is appears robust with more detailed studies performed in the same community.

We observed further geographic and dietary signals when we performed the contig clustering analysis, which allowed us to explore associations that may not be captured by taxonomic or functional annotations. Indeed, clusters containing Montrealers and HMP samples alongside either Nunavut dietary group were underrepresented, suggesting geographic effects (Figure 3). However, the effect was much greater when considering the traditional Inuit sampled (NVT-TRAD), showing that the Inuit diet leads to a different microbiome. The overrepresentation of clusters containing only traditional Inuit samples further shows that this group may have a specialized assemblage of genes (45% of which are annotated as hypothetical proteins, Supplementary Table S6) that was not observed in individual KEGG pathways, but echoes the multivariate analyses presented above. This contig similarity analysis is therefore an independent confirmation of the MG-RAST analyses, that does not rely on databases of gene annotations, but on unbiased sequence similarity.

Our results also show that the cluster types could at least be partially explained by mobile genetic elements, such as plasmids and viruses (Supplementary Figure S1). Indeed, while approximately 8% of all sequences were identified as plasmids in the general dataset, clusters based on dietary types show an enrichment in plasmids. Here we observed both a geographic signal (as seen by the cluster containing Nunavut samples from both diets, NVT-TRAD+NVT-WEST) as well as dietary (with clusters containing only one dietary type each having their own signal).

Contigs from the NVT-TRAD group for example, which includes clusters of similar contigs that are all from consumers of the Inuit traditional diet, were more likely to cluster together based on sequence similarity (Figure 3A), and 17% of the contigs in these clusters were plasmids (Figure 3C). This suggests an adaptation to dietary practices from this portion of the mobilome. For viruses, the greatest enrichment was found in clusters containing Nunavut samples from both dietary types, pointing towards a geographic driver of adaptation. The absence of enrichment in the cluster type containing all samples (both Nunavut cohorts, Montrealers and HMP samples) further supports these hypothetical dietary and geographic drivers of mobilome presence.

The over-representation of these mobile genetic elements suggests that these plasmids and viruses may be important in microbiome adaptation, as they have been found in general microbial populations (Smets and Barkay 2005). These results show that Montreal and Nunavut microbiomes are more different than would be expected from 16S marker gene analyses and from KEGG pathway annotation. While the sample size of this study limits the implications that we can draw from these results, it does suggest that mobile genetic elements can be drivers of microbiome structure. In the Fiji Community Microbiome Project, differences in annotated genes from the mobilome reflected variations in plant-degradation genes in the rest of the microbiomes of different populations (Brito et al. 2016). The authors also found that the mobilome could be used to tease apart fine variations between neighboring populations, who otherwise had very similar microbiomes (Brito et al. 2016). While most functions encoded by the mobile gene pool of Inuit diet consumers is unknown (Supplementary Table S7), they may respond to local adaptations associated with the consumption of country food. To further understanding this, it will be necessary to explore the function of these hypothetical proteins.

While annotation pipelines often use the best-hit to a database, Prokka uses a hierarchical method which also includes searching in domain-specific databases (Seemann 2014). Nonetheless, large numbers of proteins of unknown function (labelled as hypothetical proteins) remain, which is an issue of growing concern (Mills et al. 2015). A way to further explore the functions of these proteins could be by using tertiary-structure or local active site sequence similarities (Mills et al. 2015), followed by validation in the laboratory.

As the country food diet of the Inuit is a vector of Hg, we were interested in investigating Hg-metabolism genes in the microbiomes of the Nunavut cohorts. Metals have been shown to be able to perturb microbial communities, as has been established in soil science (Frossard et al. 2017; Hemme et al. 2010). Less work has been done on human-associated microbiomes, although a recent study has demonstrated that lead-contaminated drinking water could significantly alter the composition and metabolism of the gut microbiome (Gao et al. 2017). In our study, as there were few differences between the functional and taxonomic profiles of our participants (Figure 1 and 2) it is unlikely that Hg is differentially impacting the Inuit microbiomes presented here. In any case, the absence of quantified Hg intake for each participant prevents us from drawing conclusions on this.

The microbiome has been shown to be able to transform xenobiotics, including contaminants (Claus et al. 2016; Koppel et al. 2017). *In vitro* simulations of the human gut have noted that gut microbiota can transform arsenic into its more toxic, bioavailable form (Laird et al. 2007; 2009b). This is a question of interest for Hg, as its transformations between its inorganic forms into its toxic, highly bioavailable species (MeHg) can be catalyzed by bacteria.

First, we found no evidence of the presence of the *hgcAB* gene in our cohort, nor in the 5 healthy HMP samples that were included in the *merA* analysis. This is important in terms of Hg toxicity in the gut, as the methylated form of MeHg is much more bioavailable than the inorganic species. The *hgcAB* gene, first isolated from the known methylators *Geobacter sulfurreducens* PCA (Parks et al. 2013), has since been identified in a wide range of environments (including the fish microbiome), in Archaea and Bacteria (Gilmour et al. 2013). An *hgcAB* ortholog has also been identified in a methanogen isolated from the human gut (Dridi

et al. 2012; Gilmour et al. 2013). However, there is limited evidence for *in vivo* Hg methylation by the gut microbiome. In a study of 17 pregnant women tracking hair, stool and cord-blood Hg levels as well as shotgun sequencing of the gut microbiome, Rothenburg et al. found no matches for *hgcA* or *hgcB* (Rothenberg et al. 2016). Furthmore, in a survey of 1,250 human microbiomes from HMP, Podar et al. found no hits for *hgcAB*, concluding that MeHg production in the human gut is unlikely (Podar et al. 2015). Our results from a Hg-exposed population support these observations, with no *hgcAB* detected in our samples or those selected from the HMP. However, other unknown genes may be involved in the process of MeHg production (Parks et al. 2013), and further work on methylation in microbiome may reveal more on the cycle of Hg, both in humans and in other actors of the foodwebs where Hg bioaccumulates (Martín-Doimeadios et al. 2017). It is also important to note that our sample size is relatively small – including more samples with greater sequencing depth will be important to confirm that *hgcAB* is indeed absent from the gut microbiome.

Second, we focused on *mer* genes found on the *mer* Hg resistance operon, which is often present on mobile elements such as plasmids. A study performed on monkeys showed that Hg-containing dental amalgams acted as a selective agent for Hg-resistant bacteria in the oral and gut microbiome (Summers et al. 1993). This suggests that oral exposure to Hg, such as that of consumers of country food (Van Oostdam et al. 2005) may also allow for the selection of *mer* resistance genes in the Inuit. The mercury reductase (EC1.16.1.1) was identified in US-sampled microbiomes a large-scale study comparing samples from different populations (Yatsunenko et al. 2012), and *merA* was detected in low concentrations in pregnant women (Rothenberg et al. 2016). Further evidence from stool sample incubations and mice models suggests that some members of the gut microbiome may alter Hg cycling in the body (Nakamura et al. 2013; Rowland 1988). However, there has been little further research on Hg resistance in the gut microbiomes of human populations likely to be exposed to the contaminant.

While we identified *mer* genes from SEED analyses in several samples, we were only able to confirm hits for *merA* (encoding the mercuric reductase), *merR*, *merD* (regulatory genes) and *merE* (unknown function) in R22 with the more stringent HMMER method. We only considered genes identified by HMMER analyses, which included a wider diversity of reference sequences than what is included in typical annotation databases, and is more conservative than

the SEED annotations. For example, the SEED database entry for the mercuric ion reductase encoded by *merA* (EC1.16.1.1) is based on 13 UniProtKB/Swiss-Prot sequences, while our NCBI NR curated database contained 878 sequences covering 5 phyla. Based on both permissive SEED annotations (Figure 4) and more conservative HMM-based annotations (Figure 5), the *merB* gene was completely absent, leading us to conclude that the microbiomes sampled do not have the ability to mediate the demethylation of MeHg via the *mer* operon. We therefore only focused on the reduction of Hg by the *merA* gene product, the mercuric reductase.

The presence of *merA*, *merR*, *merD* and *merE* in R22 suggests that in this individual, there is potential for Hg reduction by the gut microbiome. The absence of transport proteins however does not mean that Hg reduction cannot occur. Indeed, Hg can be transported into bacterial cells in many ways, including by the diffusion of lipid-soluble Hg complexes at low concentrations (Morel et al. 1998). It is also possible that transport proteins are present, but were missed due to low sequencing coverage. The bacteria carrying the *mer* operon in R22 may therefore still be able to passively import Hg and reduce it to volatile Hg(0).

The *mer* operon has been found to have high sequence diversity across Bacteria (Barkay et al. 2003; Barkay and Wagner Döbler 2005). This is reflected in the wide diversity shown in the reference sequences in Supplementary Figure S2. While sequences from all our Nunavut and Montreal samples as well as all HMP samples tested contained hits to *merA* identified by HMMER, all of these, apart from those carried by the #1951 contig, were paralogs of other function. Therefore, true *merA* orthologs do not appear to be a common feature of the human gut microbiome. Only sequences from Bacteria were included in this analysis. It may be important to include archaeal representatives, since the *mer* operon has been found in Archaea (Barkay and Wagner Döbler 2005; Wang et al. 2008). However, very few complete *merA* archaeal sequences are available on NCBI (2, against the 328 found for Bacteria), and alignments show that these sequences greatly resemble the bacterial ones included in this analysis. Furthermore, HMMER is very sensitive, and is designed to capture distant homologs (Finn et al. 2011): while it would be interesting to include Archaea here, the impact on our results would likely be minimal.

The *mer* operon is known to be very mobile across genomes (Hall et al. 2015), and we found that the *mer* genes in R22 were organized on a operon likely carried by a plasmid (Figure

5). Indeed, the contig carrying the operon contains a Tn*3* transposon resolvase. This class of transposon includes Tn*21*, a subfamily of transposable elements which frequently carry *mer* genes (Pal et al. 2017). The presence of *mer* gene on a mobile genetic element supports observations by other authors stating that mobile genes can be of importance in understanding adaptations of the gut microbiome across human populations (Brito et al. 2016).

The contig carrying the *mer* operon in R22 also carried a TEM beta-lactamase, an antibiotic resistance gene. Thus, it is not clear whether this contig was maintained due to selective pressures for Hg resistance, antibiotic resistance, or both. Metal resistance genes are often carried on mobile elements alongside antibiotic resistance genes, and Hg resistance has been frequently found to be co-selected with antibiotic resistances in aquatic environments (Di Cesare et al. 2016) but also in the microbiome of a species of fish (Lloyd et al. 2016). In the case of R22, as this individual did not adhere closely to the traditional Inuit diet (and thus not in the most potentially Hg-exposed group), selection of this operon may have more likely occurred following a course of antibiotics (prior to the one month exclusion criteria of our sampling). There is a major knowledge gap in our understanding of antibiotics use and resistance in Northern indigenous populations (Public Health Agency of Canada 2016): while data pooled from all three Northern Canadian territories show that antibiotic use is similar to that of more southerly provinces, it has been decreasing over the past decade (Public Health Agency of Canada 2016), and it remains difficult to assess actual intake in remote northern communities (Public Health Agency of Canada 2016). In any case, *mer* genes in the gut microbiome may be selected for Hg resistances, or may be carried over by selection of other features, like antibiotic resistance.

As we did not find any *merB* in our cohort, it appears that MeHg demethylation into Hg does not occur in the microbiomes surveyed. However, demethylation by the *mer* operon is only one of several pathways that are thought to be involved in the conversion of MeHg into inorganic Hg. Furthermore, *mer*-mediated demethylation is a reductive processes (Barkay and Wagner Döbler 2005), thought to occur in oxic conditions with high concentrations of Hg (Oremland et al. 1991). The human gastrointestinal tract may be more conducive to other less well-known demethylation pathways. Oxidative demethylation for example, which is favored at lower Hg concentrations and anoxic conditions (Schaefer et al. 2002), has been found in the environment

to be performed by methanogens and sulfate-reducing bacteria (SRB) (Barkay and Wagner Döbler 2005). Multiple SRB taxa have been previously identified in human feces including *Desulfovibrio desulfuricans*, *Desulfovibrio fairfieldensis* and *Desulfovibrio piger* (Loubinoux et al. 2002), all of which were also found in our samples. Similarly, our metagenomes contained multiple archaeal methanogens like *Methanobrevibacter smithii*, *Methanobrevibacter gottschalkii* and *Methanosphaera stadtmanae*. However, oxidative demethylation remains poorly understood, and no pathways or genes have been characterized to date. For now, the presence of this pathway of MeHg degradation in the gut is speculative, but possible in light of the redox and Hg concentration conditions found in the human gastrointestinal tract.

The role of the mobilome in structuring the Inuit microbiome could also contribute to our understanding of the metabolism of Hg and of other metals in the gut. Indeed, the plasmids in our survey frequently carried hypothetical proteins and metal (other than Hg) or antibiotic resistance genes, and have previously been found to be vectors of local adaptation to variations in diet (Brito et al., 2016). Better understanding the functions of the numerous hypothetical proteins could reveal domains associated with Hg binding, import or export from cells (outside of functions encoded by the *mer* operon) for example. Studying the microbiome of exposed populations from the point of view of mobile genetic elements may thus help further our understanding of metal resistance and cycling in the gut.

With the dietary transition currently underway in Northern populations, it is important to understand how changing dietary practices may alter the microbiome. Here we show that while the Inuit have a similar functional profile as Westerners, it is shaped by the traditional country food diet. This diet may also be a vector of Hg-exposure, making it a contaminant of major interest in the Arctic. However, Hg resistance does not appear to be a common feature in the gut microbiome, and its presence in one of our samples could indicate that it is preferentially selected throughout antibiotic exposure. In the Hg-exposed population surveyed here, we found no evidence for the presence of the methylation gene *hgcAB*, nor for the demethylator *merB* (which was not found in the HMP dataset either). However, we did find that *merA*, the mercuric reductase, is present in one individual from Nunavut. This suggests that the human gut microbiome may have the ability to reduce Hg(II) into volatile Hg(0), which is relatively inert

and lipid soluble. Hg(0) can then diffuse through cell membranes passively. If these genes are transcriptionally active, they may promote the reduction of Hg(II) burden in the gut. Indeed, experiments performed on mice have shown that eliminating the gut microbiome is associated with a reduced excretion of Hg in the feces (Nakamura et al. 2013). The presence of the *mer* operon thus has the potential to alter the cycling of Hg in the body, and the mobility of this element (especially when encoded on transposons) suggests it may move around easily in the microbiome.

Current pharmacokinetic models attempting to describe Hg-burden in humans are typically poor predictors in Indigenous populations, including in the Inuit (Canuel et al. 2006b). This could be due to dietary practices (Girard et al. 2017a), but may also be caused by the presence or absence of *mer* genes. While the fact that this gene is limited to only one individual suggests this may not have widespread effects on Hg pharmacokinetics in the North, our small sample size limits our ability to interpret what this might mean for human populations in the Arctic. If the gene is present in 20% of individuals as it was in our study (and transcribed), there may be large variations across human populations. Broader sampling efforts will be necessary to confirm this. Even if there are currently no associations between the abundance of these genes and diet, they may still be informative for assessing Hg risk. More knowledge on how Hg cycles in the gut may thus ultimately improve our understanding of this contaminant's fate in the body.

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Data availability

Reads will become available on the MG-RAST server under a stable Study ID before publication.

Supplementary Tables

Supplementary tables S1-S7 are available at:

https://github.com/cgir/metagenomics_inuit/blob/master/SuppTablesS1-S7.xlsx

TABLEAU SI. Metadata, read counts before and after quality-filtering and number of assembled contigs for the 12 samples used in this study.

TABLEAU SII. Top 20 (A) plasmid and (B) virus backbone genes used for identification of mobile elements in our dataset. Backbone genes were all involved in replication or transport of these mobile genetic elements.

TABLEAU SIII. Differentially abundant OTUs across diet. Taxa were identified using DESeq2, and *P* values were corrected for multiple comparisons using FDR. baseMean shows the average normalized count values divided by size factor; lfcSE shows the error estimate for the log2FC value. Negative log2FC values show enrichment in the traditional Inuit diet. Only results significant after multiple-hypothesis testing are presented here.

TABLEAU SIV. Prokka annotations and gene lengths of the R22 #1951 contig, where the *mer* operon was identified.

TABLEAU SV. List of reference sequences from NCBI and hits from this study and HMP samples analyzed by HMMER for presence of *merA* (used for phylogeny in Figure S2). Colors reflect diet of participants from this study (yellow for Montreal Western, red for Nunavut Western and blue for Nunavut traditional). The sequence for *merA* in R22 #1951 (R22-FOLIJDAJ_46054) is identified by asterisks.

TABLEAU SVI. Genes present in the NVT-TRAD-only clusters of contigs. 45% of genes are annotated as hypothetical.

TABLEAU SVII. Plasmid genes in the NVT-TRAD-only clusters of contigs.

Supplementary Figures

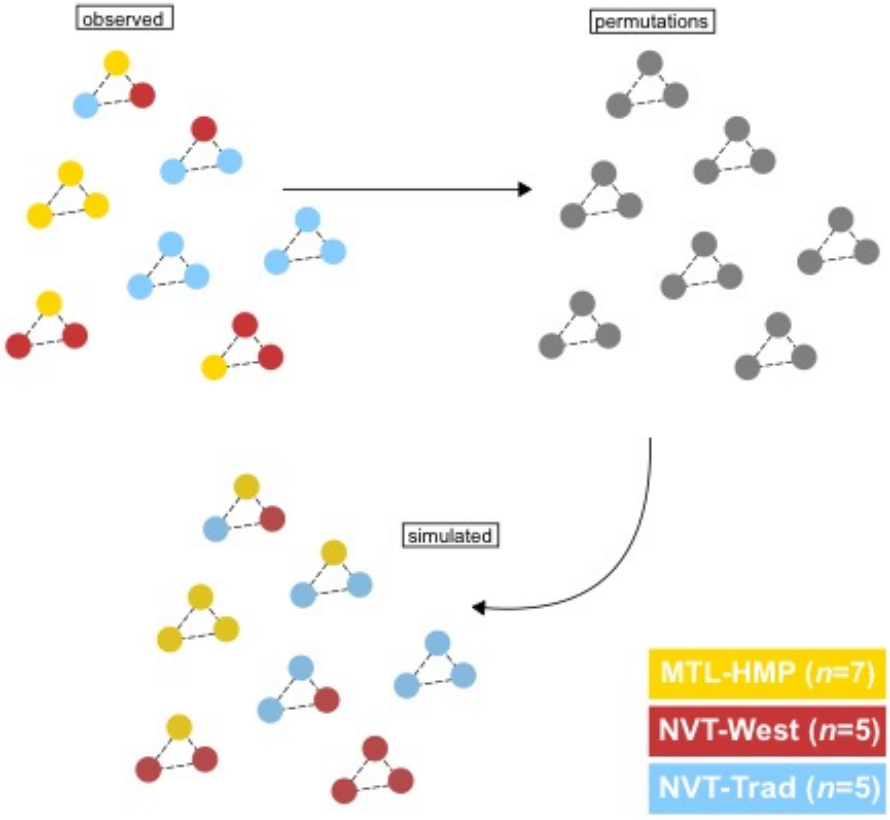

FIGURE S1. Contigs from this study and 5 HMP samples were clustered by similarity, and frequency of clusters was computed for the 6 groups (MTL-HMP, NVT-WEST, NVT-TRAD, MTL-HMP+NVT-WEST, MTL-HMP+NVT-TRAD, NVT-WEST+NVT-TRAD, all 3 diets). 100,000 permutations were performed, and frequencies were computed on simulated data.

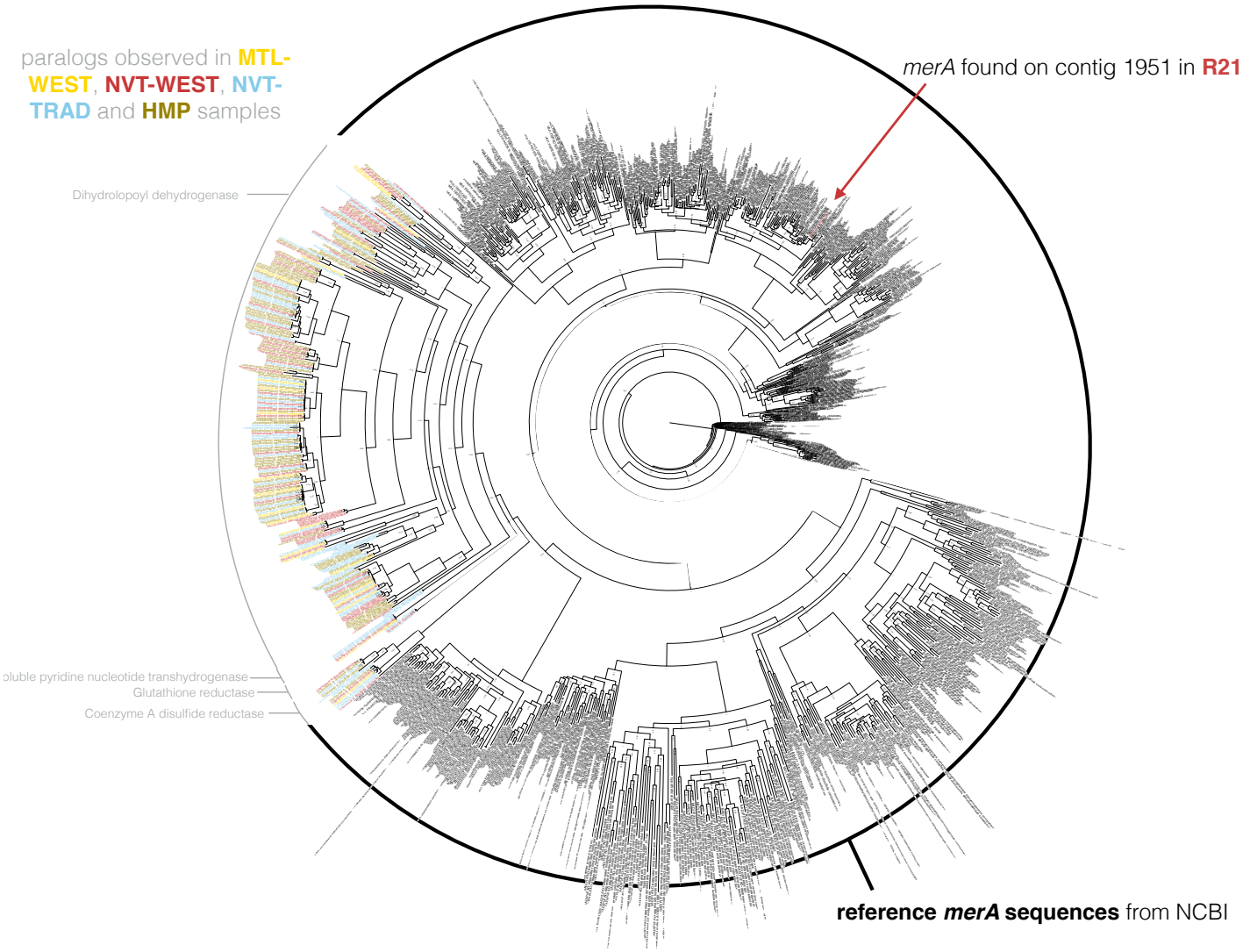

FIGURE S2. Phylogenetic tree of *merA* hits in this study and in 5 HMP samples, along with reference sequences collected from NCBI. Yellow shows Montrealers with a Western diet, red represents Nunavut individuals with a Western diet, blue shows Nunavut individuals adhering to a traditional Inuit diet and brown shows HMP samples. Most hits were paralogs, grouping outside of the mercuric reductase clade. Only one contig from R22 (#1951) contained a true *merA* gene, that grouped with reference sequences.

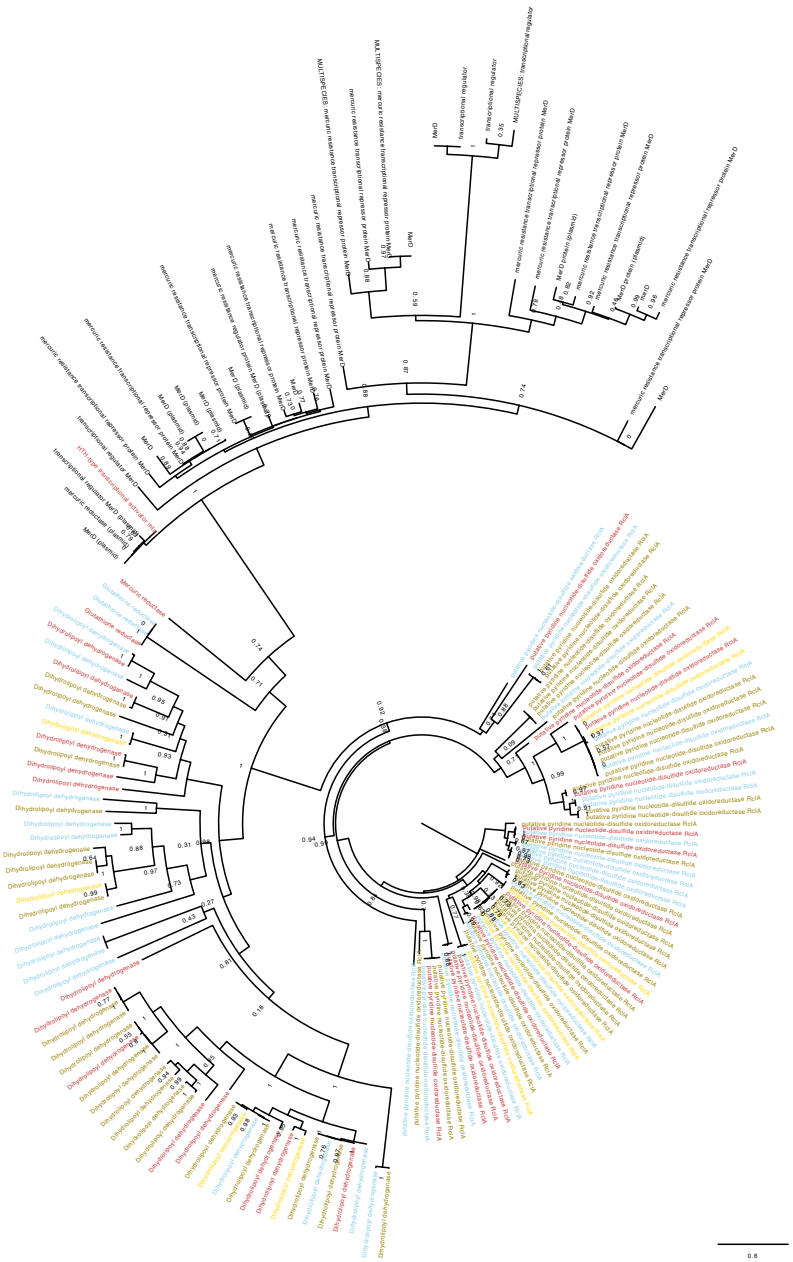

FIGURE S3. Phylogenetic tree of *merD* hits in this study and in 5 HMP samples, along with reference sequences collected from NCBI. Yellow shows Montrealers with a Western diet, red represents Nunavut individuals with a Western diet, blue shows Nunavut individuals adhering to a traditional Inuit diet and brown shows HMP samples. Only one contig (#1951 from R22) contained a sequence that grouped in a monophyletic clade with known *merD* reference sequences, all other sequences were paralogs.

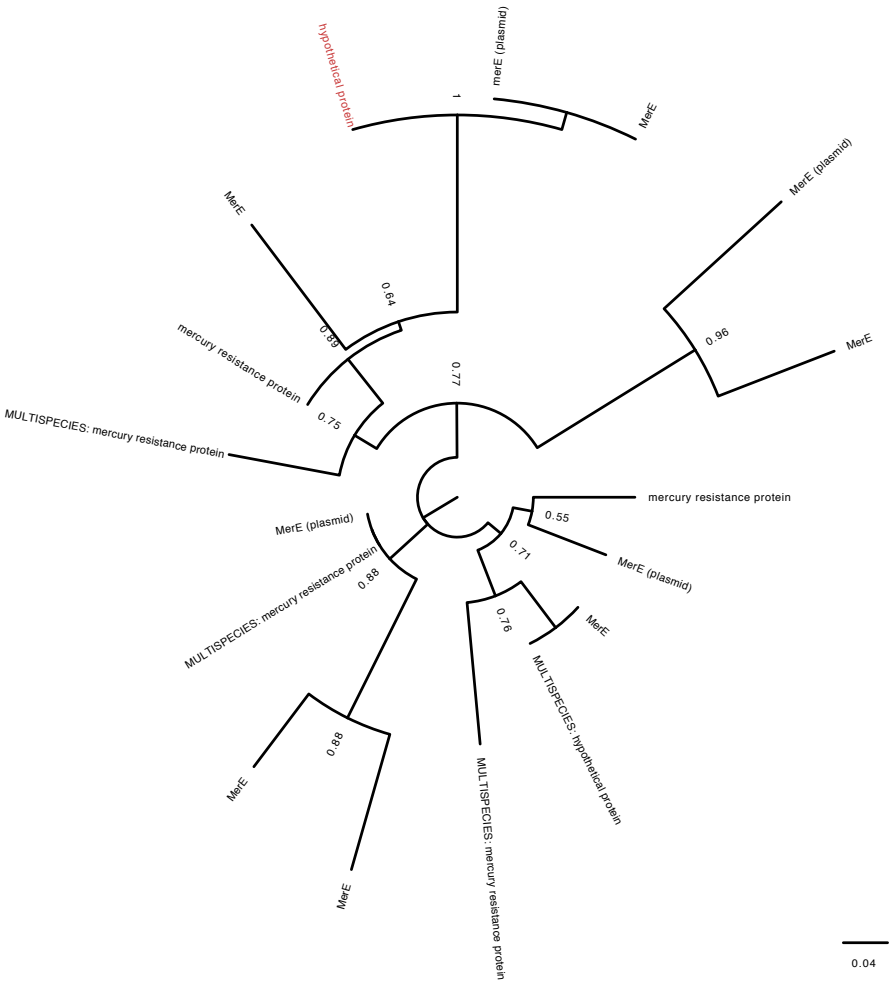

FIGURE S4. Phylogenetic tree of *merE* hits in this study and in 5 HMP samples, along with reference sequences collected from NCBI. Only one contig from R22 (#1951) contained a sequence that grouped with references, shown in red.

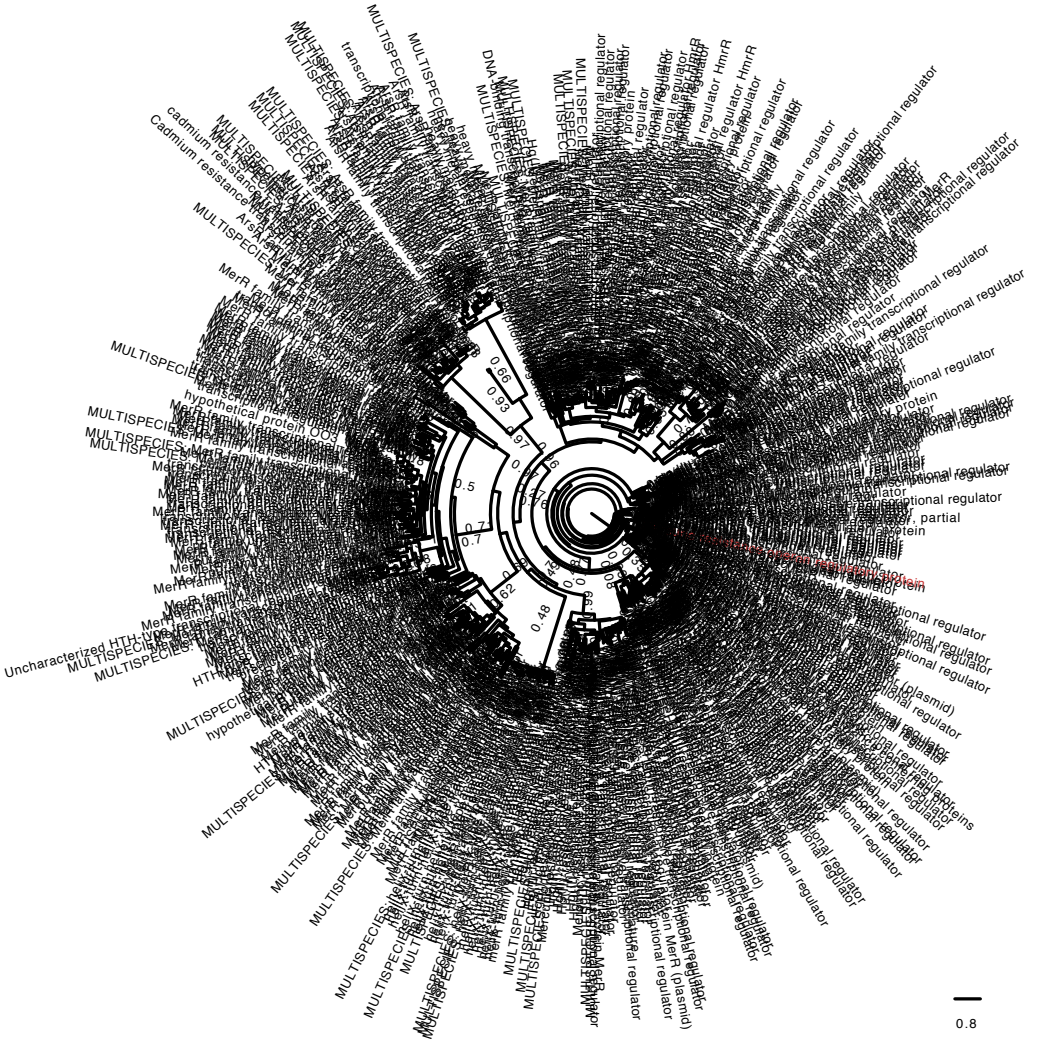

FIGURE S5. Phylogenetic tree of *merR* hits in this study and in 5 HMP samples, along with reference sequences collected from NCBI. Only one contig from R22 (#1951) contained a sequence that grouped with references, shown in red.

Conclusions

L'Arctique en changement

Les projections prédisant les futures concentrations de Hg dans les écosystèmes arctiques varient selon les modèles utilisés. Si les activités économiques et industrielles mondiales se poursuivent au même rythme, les émissions de Hg anthropique continueront d'augmenter, menant à une plus grande déposition en Arctique (Figure 1A). Même en prenant en compte un scénario où des mesures limitant les émissions de Hg sont implantées (comme l'adoption de la Convention de Minamata), on s'attend à voir les diminutions de taux de déposition de Hg les plus faibles dans l'Arctique (Figure 1B). De plus, si les émissions atmosphériques et la déposition du Hg ralentissent dans les années à venir, la réduction des concentrations en Hg dans d'autres compartiments environnementaux (comme les réseaux trophiques lacustres ou les animaux consommés par les Inuits) risque de se faire plus lentement (AMAP 2011). Ainsi, même si la Convention de Minamata aura un effet mesurable, le Hg restera un enjeu majeur en Arctique pour les années à venir.

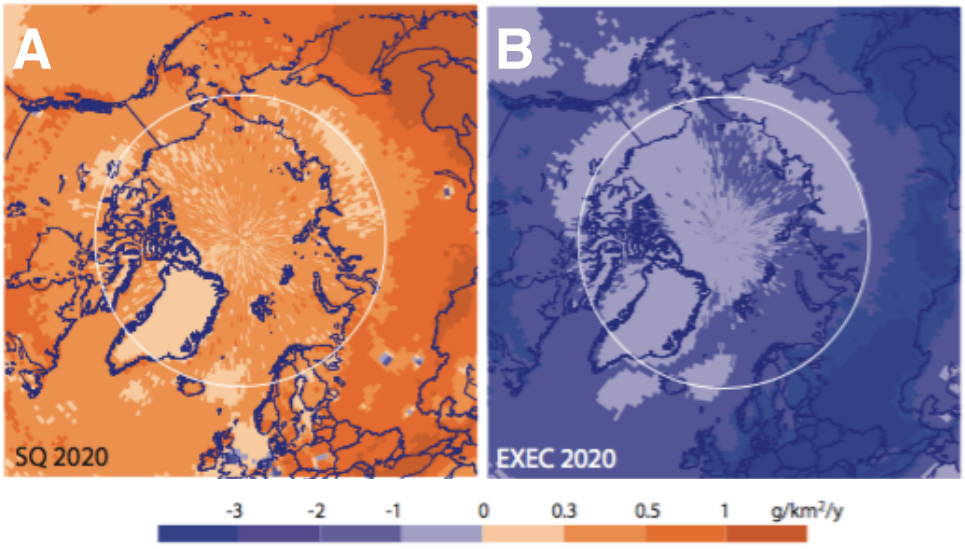

FIGURE 1. Modèles prédictifs de la variation des valeurs absolues de Hg déposé entre 2005 et 2020, selon les scénarios (A) de maintien des émissions actuelles ou (B) de diminution. Adapté de AMAP 2011.

Par ailleurs, les changements climatiques risquent également d'avoir des impacts sur le cycle du Hg dans le Nord. Les premières conséquences du réchauffement auxquelles on pense ont souvent trait à l'océan Arctique, comme la fonte de la banquise et la perte de glace multi-annuelle (Stroeve et al. 2007). Toutefois, les changements climatiques affecteront aussi les écosystèmes aquatiques d'eau douce : l'augmentation des pluies favorisera le ruissellement du Hg vers les eaux de surface (Prowse et al. 2006), et la dégradation du pergélisol pourrait augmenter la relâche de Hg qui y est stocké (Klaminder et al. 2008). Ces mêmes variables auront également des conséquences sur la productivité des écosystèmes nordiques, en augmentant les apports de matière organique (Klaminder et al. 2008). Le Hg ayant une affinité très forte pour les groupes soufrés de la matière organique, ces changements affecteront la spéciation, la mobilité et la biodisponibilité de ce métal (Driscoll et al. 1995; Ravichandran 2004). De plus, la présence de matière organique pourrait augmenter l'atténuation lumineuse dans les eaux douces, limitant la pénétration de radiation UV nécessaire à la photodéméthylation (Lehnherr and St Louis 2009).

Le réchauffement pourrait aussi favoriser la méthylation du Hg (Paranjape and Hall 2017). D'abord, les écosystèmes aquatiques et les milieux humides seront libres de glace plus longtemps, augmentant la période de temps pendant laquelle le MeHg peut être produit (AMAP 2011). Étant donné que l'activité bactérienne est étroitement liée à la température, on pourrait s'attendre à observer une augmentation tant de la déméthylation (par *merB*) que de la méthylation (par *hgcAB*), et assumer que les bilans actuels nets de production et de dégradation actuels du MeHg ne changeront pas. Toutefois, des observations par Canário et al. suggèrent que la méthylation pourrait être plus susceptible d'être accélérée à des températures croissantes (Canário et al. 2007).

Ainsi, les changements climatiques risquent d'avoir des conséquences importantes en Arctique (ACIA 2005), et même si la Convention de Minamata encouragera une diminution des émissions de Hg, le Hg et le MeHg continueront d'être un problème environnemental dans le Nord. Il importe donc de mieux comprendre le cycle de ces contaminants, en portant une attention particulière aux variables impliquées qui pourraient être affectées par le réchauffement de l'Arctique.

Hg dans les mares de fonte : vers une recherche *in situ* approfondie

Les mares de fonte sont parmi les écosystèmes aquatiques les plus abondants dans l'Est de l'Arctique canadien, et l'**Annexe I** démontre que la contamination en MeHg de ces mares est étroitement liée à la matière organique et aux nutriments. Malgré la petite taille de ces mares, leur haute connectivité pourrait faire d'elles des sources de MeHg pour leurs environnements. Par ailleurs, l'augmentation des apports en matière organique avec les changements climatiques suggère que ces sites pourraient demeurer des points chauds de la contamination en MeHg dans le Nord. Il devient alors critique de comprendre le sort de ce MeHg, particulièrement les mécanismes pouvant permettre sa dégradation. Le **Chapitre 1** présente l'importance de la matière organique et de la pénétration lumineuse dans la photodéméthylation du MeHg, deux variables risquant d'être affectées par les changements climatiques. Cette étude démontre également que la photodéméthylation n'est pas ubiquitaire dans le Nord, et que même lorsqu'elle est présente, elle n'a pas nécessairement d'effet net sur le bilan de MeHg de l'écosystème (Girard et al. 2016).

Plusieurs questions demeurent ouvertes suite à cette étude : il sera nécessaire de confirmer le rôle de la matière organique en réalisant des expériences à ajouts dosés. Ces expériences pourraient être réalisées en tenant compte des prédictions d'augmentation de la matière organique dans le Nord avec les changements climatiques, et pourraient donner un aperçu du futur potentiel de photodéméthylation des mares thermokarstiques dans le futur. Ces ajouts dosés devraient également être appliqués aux eaux oligotrophes du lac Char, où il n'y avait pas de photodéméthylation mesurable sur le long terme, pour nous permettre de confirmer que l'absence de matière organique est responsable de nos observations. Enfin, une gamme de concentrations de matière organique nous permettrait de confirmer la relation à seuil hypothétique présentée dans le **Chapitre 1**, entre la matière organique et les taux de photodéméthylation. Dans toutes ces expériences, il sera essentiel de faire un suivi de la qualité de la matière organique pendant son irradiation solaire (via l'absorbance spécifique d'ultraviolets, par exemple), afin d'avoir un meilleur portrait des réactions photochimiques en cours, et pour mieux comprendre comment des sources allochtones ou autochtones de carbone pourraient affecter la photodéméthylation.

Si nos résultats suggèrent que le rôle de la matière organique dans la photodéméthylation est capital, celui des thiols est moins clair. Alors que certains auteurs ont montré que les thiols favorisent la photodéméthylation dans des eaux simulées (Zhang and Hsu-Kim 2010), nos résultats en eaux naturelles ont montré que les thiols n'ont pas d'effet, ou limitent la photodéméthylation, et ne nous permettaient pas d'expliquer les différences dans les taux de photodéméthylation à nos sites. Davantage d'expériences en eaux naturelles portant sur la complexation du MeHg aux thiols et sur la dégradation simultanée des thiols par la radiation solaire sont nécessaires. Ces expériences pourraient nous informer sur les conséquences de ces petites molécules soufrées sur la photochimie du MeHg, et devraient prendre en compte les conséquences intramoléculaires de l'irradiation de la matière organique. La médiation de la photodéméthylation par la matière organique pourrait également s'opérer de manière indirecte, via la production d'espèces réactives d'oxygène. Si le rôle des espèces réactives dans la photodéméthylation a été identifié dans un lac oligotrophe (Hammerschmidt and Fitzgerald 2010) ainsi que dans des expériences de laboratoire (Zhang and Hsu-Kim 2010), il faudrait le confirmer formellement dans les sites étudiés au **Chapitre 1**. L'ajout de producteurs d'oxygène singulet ou de radical hydroxyl dans l'eau du lac Char par exemple, où le signal de photodéméthylation était quasi-absent, nous permettrait de déterminer si ces molécules à elles seules peuvent induire la photodéméthylation.

Les expériences en bouteilles *in vitro* sont utiles pour étudier des questions mécanistiques. Toutefois, elles courent le risque de masquer des processus à l'échelle de l'écosystème, comme les interactions entre les sédiments, la colonne d'eau et l'atmosphère. Les mares thermokarstiques, avec leur petite taille et le bon brassage de leur colonne d'eau, sont un milieu idéal pour mener des expériences de manipulation d'écosystème, et pourraient être un excellent modèle pour étudier la photodéméthylation dans son contexte environnemental. Un design expérimental amélioré pour ces manipulations *in situ* par rapport au **Chapitre 1** (notamment au niveau de la couverture de la surface de l'eau) pourrait inclure des expériences sur des ajouts de matière organique qui sont représentatifs des augmentations que le Nord subira avec le réchauffement climatique. Ces expériences devraient impérativement s'accompagner de mesures de la méthylation et de la déméthylation du Hg, afin de comprendre les dynamiques de

production et de dégradation du MeHg, et pour bien comprendre le rôle réel de la photodéméthylation dans cet équilibre.

Enfin, les hauts niveaux de MeHg observés dans les mares arctiques, combiné à leur haute connectivité hydrologique (du moins pendant la période de fonte des neiges) (Woo and Guan 2006) (Figure 2), suggèrent qu'ils pourraient être des sources de MeHg pour les autres milieux aquatiques du Nord. Ceci pourrait poser problème pour les populations humaines, si les le MeHg des mares thermokarstiques est exporté vers des lacs où le poisson est pêché, ou vers l'océan. Toutefois, les liens hydrologiques entre ces mares et les écosystèmes aquatiques environnants sont peu connus, particulièrement dans le contexte de la dégradation actuelle du pergélisol et de l'augmentation des températures moyennes arctiques.

FIGURE 2. Mares de fonte de pergélisol de la vallée Qarlikturvik sur l'île Bylot, Nunavut. Les mares de fonte sont connectées entre elles, et sont hydrologiquement reliées aux autres environnements aquatiques environnants (milieux lacustres, riverains et marins), pour lesquels elles pourraient être une source de MeHg.

Il serait donc intéressant de suivre les mouvements de l'eau entre les mares thermokarstiques, afin de déterminer si le MeHg peut être exporté d'une mare à l'autre. Ces mesures pourraient être particulièrement intéressantes à réaliser au printemps polaire, où la fonte de la neige cause des échanges hydrologiques importants entre les mares (Woo and Guan 2006). Ensuite, pour déterminer si le MeHg peut être exporté des mares à d'autres écosystèmes dans le paysage, il serait possible de tracer des molécules auxquelles le contaminant est fréquemment lié, comme la matière organique. En utilisant des approches de spectroscopie à fluorescence et d'analyse par facteurs parallèles (comme PARAFAC) (Stedmon et al. 2003), on pourrait vérifier si la matière organique dans les lacs, rivières et régions marines côtières possède une signature indiquant qu'elle provient de mares thermokarstiques du milieu terrestre (Walker et al. 2009). Ceci pourrait être un indicateur de la connectivité des mares thermokarstiques au reste du système hydrologique nordique, et nous aiderait à déterminer si ces mares sont réellement une source de MeHg pour leurs environs. De plus, ceci nous permettrait de mieux explorer le rôle de la matière organique dans la photodéméthylation, particulièrement dans un contexte où les apports de matière organique terrestre vers les milieux aquatiques risquent d'augmenter avec les changements climatiques (Klaminder et al. 2008).

Réchauffement et bioaccumulation vers les niveaux trophiques supérieurs

Le réchauffement de l'Arctique affectera également les réseaux trophiques, et la manière dont le MeHg s'y accumulera. Les températures élevées et apports en nutriments et en matière organique risquent d'augmenter la productivité des écosystèmes aquatiques, ce qui aura des répercussions (positives ou négatives) sur la bioaccumulation vers les niveaux trophiques supérieurs (AMAP 2011). Dans de nombreuses espèces de haut niveau trophique en Arctique, des études temporelles démontrent que les concentrations de MeHg sont à la hausse (Figure 3). S'il existe des patrons géographiques mal compris ainsi que des espèces où les tendances sont à la baisse, ces données démontrent que le MeHg risque de rester un enjeu de santé environnemental mais aussi pour la santé humaine, chez les consommateurs de nourriture traditionnelle.

Les interactions entre les changements climatiques et les contaminants comme le Hg sont donc complexes et difficiles à prédire (Lennert 2016), et le réchauffement a déjà des conséquences sur les pratiques traditionnelles et l'accessibilité aux proies des Inuits (Wenzel 2009). Il importe donc de comprendre comment le Hg et le MeHg passent des réseaux trophiques vers l'humain, et comment ce contaminant est absorbé par le corps après son ingestion.

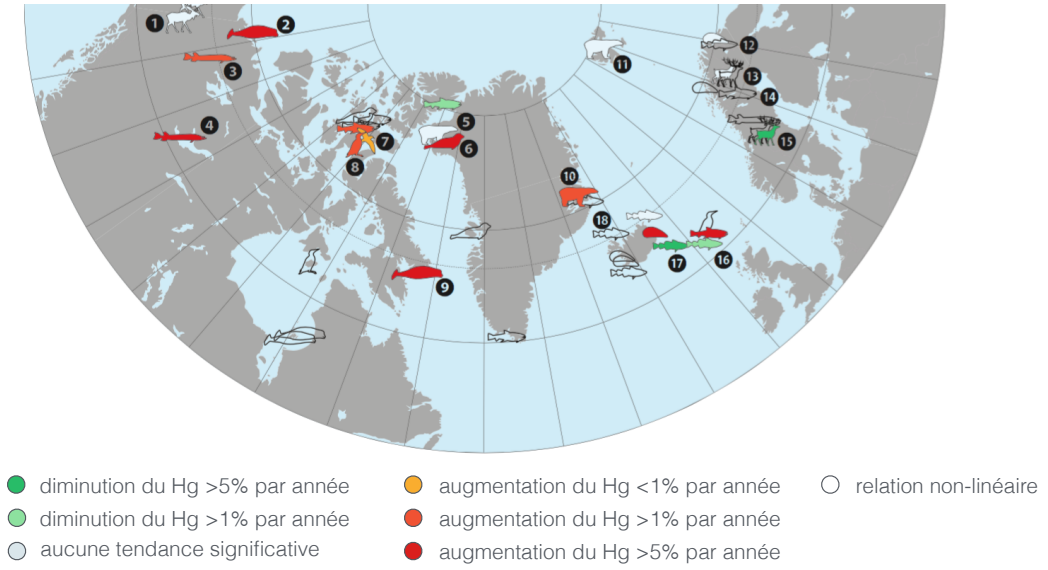

FIGURE 3. Tendances temporelles d'accumulation de Hg dans la faune arctique, montrant des variations géographiques importantes. La majorité des espèces du Nord canadien voient leurs concentrations augmenter. Adapté de AMAP 2011.

Le sort du Hg et du MeHg dans le corps

Pratiques alimentaires et bioaccessibilité : vers une validation *in vivo*

Étant donné les augmentations potentielles du Hg et MeHg dans la faune arctique canadienne et les risques posés pour les populations Inuits ayant une alimentation traditionnelle, il importe de bien comprendre le sort de ces contaminants une fois ingérés dans le corps humain. Le **Chapitre 2** démontre que plusieurs pratiques alimentaires couramment employées dans le Nord peuvent altérer la bioaccessibilité *in vitro* du MeHg, affectant potentiellement sa capacité à être absorbée. La cuisson réduit la bioaccessibilité de manière significative, alors que la

congélation n'a pas d'impact. Le thé et le café ont aussi limité la bioaccessibilité du MeHg. Le rôle des polyphénols dans l'effet du thé sur la bioaccessibilité a été confirmé pour la première fois, et nous avons noté un effet cumulatif de ces pratiques sur la solubilisation du MeHg. Ces résultats prennent beaucoup d'importance lorsqu'on considère que les recommandations gouvernementales actuelles visant à limiter l'exposition au MeHg assument que 100% du contaminant est absorbé : il semblerait donc qu'on surestime le risque (Girard et al. 2017a).

Cette étude ouvre la porte à plusieurs questions de recherche qui doivent être explorées, avant d'utiliser ces résultats pour modifier les lignes directrices actuelles. D'abord, le mécanisme d'action de la cuisson doit être élucidé : si plusieurs études ont supposé que les changements de conformation protéiques induits par les températures élevées sont en cause, ceci n'a jamais été démontré. Une première avenue de recherche serait donc de tester différentes températures de cuisson le long d'un gradient, particulièrement autour des 100 °C, point à partir duquel l'oxydation des protéines peut causer l'agrégation de la matrice qui limiterait la dégradation enzymatique (Bax et al. 2012). Il sera alors critique de faire des analyses de spéciation chimique du Hg lié aux protéines, notamment par ICP-MS (Sanz-Medel et al. 2003), afin de déterminer si l'effet de la cuisson est effectivement dû à la rétention du MeHg dans la matrice protéique partiellement digérée.

De la même manière, il faudra également approfondir la question des polyphénols, dont l'effet limitant sur la bioaccessibilité serait dû à leurs propriétés chélatrices, mais qui n'a jamais été testé dans le contexte de la bioaccessibilité des métaux. Les thés verts et noirs sont communément consommés dans les communautés inuites, comme en témoigne notre cohorte du **Chapitre 3** et des données de la littérature (Kuhnlein et al. 2004), et pourraient donc être une source de polyphénols pouvant limiter la bioaccessibilité chez les populations nordiques. Toutefois, nous avons également constaté que seuls certains composés polyphénoliques ont un effet sur la bioaccessibilité. Il serait important d'évaluer la composition en polyphénols de plantes et baies qui font partie de la diète traditionnelle inuite, afin de vérifier s'ils pourraient être intégrés à des stratégies de diminution de la bioaccessibilité. Par exemple, si le thé du Labrador (*Rhododendron groenlandicum*) ne semble pas contenir de rutine (Rapinski et al. 2014), l'un des polyphénols les plus efficaces identifiés dans le **Chapitre 3**, il comporte de l'épicatéchine, qui a également eu un impact sur la bioaccessibilité. Ainsi, un inventaire plus

complet des polyphénols présents dans la diète traditionnelle inuite et ainsi qu'un portrait des mécanismes de leurs interactions avec le Hg seraient d'intérêt.

Enfin, le **Chapitre 2** s'est limité à tester la bioaccessibilité du MeHg dans le poisson. Pourtant, la composition de la chair de poisson varie d'espèce en espèce (par exemple au niveau de la représentation des différents types de muscles) (Sänger and Stoiber 2001), ce qui pourrait affecter la bioaccessibilité du MeHg. De plus, la diète traditionnelle des Inuits comprend d'autres espèces animales à risque pour la contamination au MeHg, telles que les mammifères marins. Laird et al. ont mené une étude sur un grand nombre d'échantillons de nourriture traditionnelle nordique (incluant différentes espèces de baleines et de phoques, en plus du poisson et du caribou), et ont déterminé que la bioaccessibilité du MeHg varie entre les espèces testées (Laird et al. 2009a). Il serait intéressant d'explorer les raisons pour lesquelles des matrices de viande différentes, que ce soit entre espèces de poisson ou entre poissons et mammifères, exhibent des profils de bioaccessibilité variables. Des analyses de protéomique pourraient fournir des informations plus détaillées sur la composition de la viande, et les coupler à des analyses de spéciation du MeHg pourrait nous aider à comprendre la manière dont le contaminant est solubilisé des protéines pendant la digestion.

Par ailleurs, ces expériences sur la cuisson, les polyphénols et l'effet de matrices devraient être accompagnées d'expériences avec des cellules épithéliales intestinales (comme le modèle Caco-2), afin de pouvoir comprendre quelle fraction du MeHg bioaccessible se retrouve biodisponible. Ceci nous permettrait d'explorer davantage la remise en question de l'absorption totale du MeHg, postulée par des études des années 60-70 (Aberg et al. 1969; Miettinen et al. 1971) et acceptée par les guides de recommandation alimentaires. S'il s'avère que les lignes directrices surestiment le risque, y intégrer les effets des pratiques alimentaires pour diminuer la bioaccessibilité du MeHg pourrait encourager la consommation de nourriture traditionnelle, qui comporte de nombreux bienfaits pour la santé (Kuhnlein et al. 2006).

Avant de modifier les guides de consommations pour être plus représentatifs du risque réel, il sera critique de valider les résultats obtenus en *in vitro* avec des études *in vivo*. Des expériences dans le porc, qui peut être un bon modèle du tractus digestif humain (Moreda-Piñeiro et al. 2011) pourraient être employées pour confirmer nos observations en laboratoire. Des porcs pourraient être nourris de repas à concentration connue en MeHg, cuits ou

accompagnés de polyphénols. Des prises de sang pourraient être utilisées comme méthode peu invasive pour suivre l'absorption du MeHg. Cette approche permettrait également d'explorer des questions impossibles à traiter dans les simulations *in vitro* : par exemple, elle pourrait tester l'hypothèse de la stimulation de la re-circulation entérohépatique par le thé, qui remettrait du MeHg stocké dans le foie en circulation dans le corps (Canuel et al. 2006b). Ainsi, si le thé a un impact prometteur sur la bioaccessibilité *in vitro*, des tests *in vivo* serviraient à mieux comprendre les conséquences de ce traitement sur le corps.

Les modèles pharmacocinétiques actuels semblent mal prédire la contamination dans les populations autochtones, et il semblerait que d'autres facteurs (génétiques, environnementaux, habitudes de vie) pourraient affecter le sort du MeHg de différentes manières selon la population (Canuel et al. 2006a; Chapman and Chan 2000). Il est peu probable qu'à elles seules, les pratiques alimentaires puissent expliquer cette différence, et une bonne manière d'intégrer d'autres facteurs serait au moyen d'études épidémiologiques. Si plusieurs études ont déjà dénoté des corrélations étroites entre la consommation de poisson et la charge en Hg (Abdelouahab et al. 2008; Cole et al. 2004; Kosatsky et al. 2000; Legrand et al. 2005; Mahaffey and Mergler 1998), ces études estiment l'apport en Hg depuis des questionnaires alimentaires. Ainsi, la dose exacte à laquelle les consommateurs sont exposés est inconnue (Abdelouahab et al. 2008; Sunderland 2007). S'il sera difficile de mener une telle étude en mesurant le Hg et le MeHg dans chaque poisson ou mammifère consommé par les participants, intégrer des cohortes de différentes populations ainsi que les modes de préparation qu'ils emploient (cuisson, co-ingestion) pourrait nous permettre d'améliorer les modèles pharmacocinétiques du Hg chez l'humain.

Microbiome intestinal et Hg : interactions directes et indirectes

Le microbiome intestinal pourrait également avoir une incidence sur la bioaccessibilité du Hg. En effet, des études ont montré que le microbiome peut contribuer à limiter l'absorption de cadmium et de plomb (Breton et al. 2013), voire altérer la spéciation de l'arsenic en sa forme plus biodisponible (Laird et al. 2007; 2009b). La prise en charge du Hg par le microbiome inuit

pourrait donc être une autre variable expliquant le manque de précision des modèles pharmacocinétiques. Les gènes bactériens responsables de transformations du Hg dans l'environnement pourraient être présents dans le microbiome humain, et altérer le sort du Hg dans le corps. Ces gènes incluent ceux de l'opéron de résistance *mer* (comme *merB*, encodant une lyase organomercuriale qui dégrade le MeHg, et *mer A*, qui encode une réductase qui réduit le Hg(II) en Hg(0)), ou le groupe de gènes *hgcAB*, qui catalyse la méthylation du Hg en MeHg.

Rothenberg et al. 2016 ont précédemment vérifié si le microbiome humain avait le potentiel de déméthyler le MeHg via MerB. En constatant l'absence de *merB*, les auteurs ont conclu qu'il était peu probable que cette voie métabolique soit présente chez l'humain (Rothenberg et al. 2016). Toutefois, la cohorte sélectionnée par ces auteurs était restreinte à des femmes enceintes. Étant en général très bien sensibilisées à la question du Hg alimentaire, il est possible que ce groupe ne soit pas le meilleur pour étudier la résistance au Hg. Puisque que la contamination en MeHg exerce une pression sélective positive sur l'opéron *mer* (Barkay et al. 2003), les Inuits, avec leur taux d'exposition plus élevés, pourraient être un meilleur groupe pour tenter de détecter ces gènes. Par contre, dans le **Chapitre 4**, nous montrons que nous n'avons pas identifié de gènes dont les produits pourraient déméthyler le MeHg dans le microbiome inuit. Ces gènes étaient également absents de la cohorte occidentale échantillonnée dans le Projet du microbiome humain (HMP). L'étude de Rothenberg et al. rapportait également l'absence de *hgcAB* dans les selles exposées au Hg (Rothenberg et al. 2016), appuyant les conclusions de d'autres études ayant criblé de grands nombres de microbiomes humains pour ce gène (Gilmour et al. 2013; Martín-Doimeadios et al. 2017). L'absence de *hgcAB* dans tous les échantillons analysés dans le **Chapitre 4** supporte donc ces observations. Toutefois, il est possible que l'absence de *hgcAB* et de *merB* soient de faux-négatifs : en effet, une couverture trop basse dans le séquençage de ces gènes, ou une divergence évolutive trop importante par rapport aux gènes déjà catalogués pourraient nous faire manquer d'autres séquences encodant des protéines capables de déméthyler ou méthyler le MeHg. De plus, il faudra également explorer d'autres avenues (pour l'instant peu caractérisées) pouvant mener à la dégradation du MeHg, comme la déméthylation oxdative, qui pourrait être plus probable dans l'environnement anoxique du tractus digestif que la déméthylation via *merB* (Schaefer et al. 2002). Cela étant dit,

les résultats rapportés dans le **Chapitre 4** suggèrent que le microbiome inuit n'a pas le potentiel d'altérer la bioaccessibilité du MeHg.

Par contre, la présence de *merA* et d'autres gènes impliqués dans l'opéron de résistance *mer*, présentée dans le **Chapitre 4**, pourrait avoir des conséquences sur la bioaccessibilité du Hg dans le corps. Il est vrai que la biodisponibilité du Hg(II) dans le tractus digestif est moindre que celle du MeHg. Toutefois, elle est plus grande que celle du Hg(0), qui serait négligeable dans l'intestin (Gochfeld 2003). Ainsi, l'activité de *merA* pourrait permettre la réduction du Hg(II) ingéré avec la nourriture en Hg(0) peu absorbable. La majorité de l'exposition au Hg chez l'humain se fait par la consommation de muscle de poisson, sous forme de MeHg (Harris 2003), ce qui pourrait limiter l'intérêt biologique de la réduction du Hg(II) dans le corps. Toutefois, la diète inuite inclut la consommation d'autres organes animaux comme le foie et les reins, qui eux contiennent davantage de Hg inorganique (AMAP 2011). La réduction du Hg(II) pourrait donc avoir des conséquences toxicologiques sur les gens porteurs de ce groupe de gènes.

Afin de déterminer l'importance biologique de *merA* chez les Inuits, et de déterminer si sa présence peut affecter la bioaccessibilité, il faudra d'abord réaliser un échantillonnage beaucoup plus étendu. Si le gène est détecté chez d'autres individus, il importera de valider que le gène est réellement actif dans le microbiome. Ceci pourrait être réalisé par séquençage transcriptomique, accompagné d'expériences d'incubations de selles avec du Hg(II) et de mesures de Hg volatilisé par CVAFS. Étant donné que l'ingestion n'est pas la voie d'exposition principale du Hg(0), elle est peu étudiée par rapport à l'inhalation : il serait donc important de réaliser des expériences préliminaires de biodisponibilité du Hg(0) dans le tractus digestif. Ceci pourrait d'abord être réalisé avec des lignées cellulaires épithéliales comme Caco-2, avant de progresser vers des modèles *in vivo* tels que décrit précédemment, nous permettant de mieux comprendre comment la bioaccessibilité (et la biodisponibilité) du Hg peuvent être modulées dans le corps humain. Si nous n'avons détecté l'opéron que chez un seul individu, la petite taille de notre échantillonnage ne nous permet pas d'écarter la possibilité que les gènes *mer* soient présents chez d'autres individus, et qu'ils pourraient affecter le sort du Hg dans le corps. D'ailleurs, ce gène a aussi été détecté dans le groupe de femmes enceintes rapporté par Rothenberg et al. 2016. Les gènes de résistance au Hg ne pourront donc probablement pas

expliquer à eux seuls pourquoi les modèles pharmacocinétiques d'absorption du Hg s'appliquent mal aux Inuits. Par contre, avec les pratiques alimentaires, ils pourraient nous permettre d'élucider une partie-clé de cette question.

Le microbiome pourrait également altérer la bioaccessibilité du Hg et du MeHg de manière indirecte. Le maintien d'un mucus intestinal peut limiter l'absorption du MeHg et du Hg(II) (Vázquez et al. 2013), et la santé mucosale est typiquement associée au genre *Akkermansia* (Belzer and de Vos 2012). Rothenberg et al. ont d'ailleurs identifié une corrélation positive entre l'abondance d'*Akkermansia* chez les femmes enceintes échantillonnées et la concentration de MeHg dans leurs selles, émettant l'hypothèse que le microbiome pouvait indirectement affecter l'absorption du Hg, en stimulant une bonne santé mucosale. Dans le **Chapitre 3**, nous avons observé qu'*Akkermansia* était un marqueur taxonomique du microbiome inuit, et qu'il existe des souches *A. muciniphila* fortement associées au microbiome inuit et à la diète traditionnelle. Ces différentes souches d'*Akkermansia* pourraient avoir une incidence sur la qualité du mucus intestinal, et éventuellement affecter l'absorption du Hg chez les Inuits. Cette association pourrait être explorée en isolant et en cultivant les différentes souches d'*Akkermansia* uniques à la cohorte du Nunavut et aux consommateurs de diète traditionnelle, et en les utilisant pour coloniser des substrats de mucus artificiels. En combinant ces mucus à des modèles d'absorption Caco-2, on pourrait déterminer si les différentes souches affectent la bioaccessibilité du MeHg.

Un autre mécanisme par lequel le microbiome pourrait indirectement affecter la bioaccessibilité serait par le métabolisme des polyphénols, identifiés dans le **Chapitre 2** comme étant des chélateurs potentiels du MeHg et limitant la bioaccessibilité. En effet, les interactions entre le microbiome et les polyphénols sont de mieux en mieux documentées (Ozdal et al. 2016), et une étude a montré que les microorganismes du microbiome peuvent générer des composés comme l'acide gallique à partir de précurseurs de théaflavine (Chen et al. 2012). Dans le **Chapitre 2**, l'acide gallique a été identifié comme l'un des polyphénols les plus efficaces dans la réduction de la bioaccessibilité du MeHg. Ainsi, des variations dans les taxons bactériens capables de métaboliser les polyphénols pourraient avoir des conséquences indirectes sur la bioaccessibilité du MeHg. Les interactions mécanistiques entre la composition taxonomique du microbiome, le métabolisme microbien des polyphénols et la bioaccessibilité du MeHg

pourraient être examinés davantage à l'aide du SHIME, un modèle de simulation *in vitro* du microbiome intestinal (van Duynhoven et al. 2011).

Il semble donc possible que le microbiome puisse moduler la bioaccessibilité du MeHg, et le microbiome inuit pourrait contribuer à expliquer pourquoi les modèles pharmacocinétiques s'appliquent mal à cette population. Les applications de ces connaissances semblent à priori moins directes que celles se rattachant aux pratiques alimentaires. En effet, si on peut imaginer un avenir où on pourrait développer des probiotiques portant des gènes de résistance au Hg ou favorisant une meilleure santé mucosale, cet avenir reste lointain. Toutefois, en attendant, ces connaissances nous ouvrent les yeux sur une partie méconnue du cycle du Hg.

Communication du risque du Hg, un défi continu

L'exploration de ces questions de bioaccessibilité pourrait nous permettre de mieux comprendre le risque auquel les Inuits font face dans le cas du Hg dans leur nourriture, et pourrait valoriser la diète traditionnelle. Ceci pourrait également servir aux populations côtières et aux adeptes de la pêche sportive, qui elles aussi sont exposées à des concentrations appréciables de Hg (Cisneros-Montemayor et al. 2016).

Toutefois, la communication du risque posé par les contaminants alimentaires est un défi de taille dans le Nord. S'il existe parfois un manque de consensus au sein de la communauté scientifique sur le risque d'un contaminant donné, l'interprétation par le grand public peut aussi être très variable (Donaldson et al. 2010; Sjöberg 2002). Une mauvaise communication du risque peut avoir des effets néfastes en causant de la confusion, de la peur ou des changements indésirables aux pratiques alimentaires, qui peuvent avoir des conséquences sur la santé (Furgal et al. 2005; Krümmel and Gilman 2016). Ainsi, la communication du risque posé par les contaminants ne doit pas se baser uniquement sur les dangers représentés par le Hg : on tentera d'inclure des alternatives culturellement acceptables dans le contexte de cette diète traditionnelle, comme le reflète cette recommandation du *Inuit Health Survey* réalisé en 2007-2008 :

> *Country foods provide many essential nutrients that can lower the risk of chronic diseases. Most Inuit adults in Nunavut need not be concerned by contaminant-related effects from country food consumption. Generally, the benefits of eating country foods outweigh the risks from contaminant exposure. Inuit women of child-bearing age who may become pregnant, are planning to get pregnant, or are pregnant should avoid eating ringed seal liver due to its high mercury content. Instead, ringed seal meat is a great and healthy alternative […]* (Chan 2012)

La recherche sur les conséquences des pratiques alimentaires sur la bioaccessibilité et la biodisponibilité des contaminants offre donc des avenues de recherche intéressantes pour la communication du risque. Des changements simples et peu invasifs, opérés par les individus dans l'apprêtement de leurs repas, pourraient devenir une manière de protéger les consommateurs des risques potentiels de la diète traditionnelle tout en respectant les pratiques culturelles. L'effet putatif des polyphénols sur la bioaccessibilité du MeHg par exemple pourrait être facilement intégré à la communication du risque, vu la consommation fréquente de thé par les Inuits dans le Nord. Ces techniques de préparation alimentaires pourraient ainsi représenter une manière peu invasive de protéger du Hg, tout en valorisant la consommation de la diète traditionnelle inuite, excellente pour la santé.

Évidemment, intégrer les concepts de bioaccessibilité dans les recommandations et efforts de communication de risque dans le Nord serait prématuré : il est nécessaire de procéder à des validations *in vivo* et épidémiologiques, afin de bien comprendre l'effet réel de ces pratiques sur la biodisponibilité du Hg. De plus, une bonne communication de risque doit obligatoirement être élaborée en collaboration étroite avec des intervenants de l'Arctique : ces résultats devraient donc être minutieusement étudiés avec des instances sociales, gouvernementales et du monde de la santé du Nord, afin de confirmer d'abord qu'ils pourraient être incorporés à des analyses de risque, et comment les communiquer de manière optimale.

Le microbiome inuit

Diète traditionnelle, microbiome et santé

La communication de résultats scientifiques est un défi dans le monde de la toxicologie, mais également dans le domaine du microbiome. Beaucoup d'études se sont penchées sur le microbiome de populations traditionnelles, et font le rapprochement entre le microbiome de ces groupes agraires, non industrialisés et le microbiome « ancestral » des humains (Obregon-Tito et al. 2015; Schnorr et al. 2015). Il est vrai que de comprendre l'évolution du microbiome pourrait nous aider à mieux comprendre les effets de l'urbanisation et de l'occidentalisation sur la santé humaine. Toutefois, affirmer que le microbiome ancestral est associé à une meilleure santé est sans doute exagéré. Par ailleurs, il est peu probable qu'il n'existe qu'un seul microbiome dit « ancestral » (Sonnenburg and Sonnenburg 2014), et la dispersion humaine vers des emplacements géographiques, des diètes et des modes de vie différents a contribué une radiation évolutive rapide du microbiome (Moeller et al. 2014).

Ceci est supporté par le **Chapitre 3**, où nous avons présenté la première description du microbiome des Inuits, une population ayant une diète dite traditionnelle unique existant depuis des milliers d'années (Raghavan et al. 2014). Toutefois, la diète traditionnelle inuite, comme celle de beaucoup de populations autochtones dans le monde, est en transition vers un état plus « occidentalisé », où la nourriture de supermarché occupe une place grandissante (Crittenden and Schnorr 2017; Kuhnlein et al. 2004; Sharma et al. 2010). Notre étude du microbiome inuit présente le premier portrait d'un microbiome traditionnel qui n'est pas plus diversifié que le microbiome occidental. Nous proposons que la faible diversité de fibres disponibles pour la consommation dans le Nord ainsi que la transition alimentaire vécue par les Inuits pourraient expliquer ce phénomène, mais il est possible que d'autres variables, comme la latitude (Dikongué and Ségurel 2017), puisse contribuer à cette diversité similaire (Girard et al. 2017b).

Si notre étude survient trop tard dans la transition alimentaire en cours pour faire figure d'un point de comparaison basal, elle appuie l'hypothèse qu'il existe sans doute de nombreux états ancestraux du microbiome, vu les différences entre le microbiome inuit et celui de populations agraires traditionnelles. Nos résultats suggèrent également que malgré la similarité du microbiome inuit au microbiome occidental, le microbiome inuit conserve des particularités

associées à la diète riche en protéines, pauvre en fibres des communautés nordiques (Hopping et al. 2010; Kuhnlein et al. 1996), tel qu'illustré par le profil métagénomique fonctionnel partiellement expliqué par la diète dans le **Chapitre 4** et dans l'**Annexe II** (Dubois et al. 2017). Ceci pourrait notamment se traduire par un profile d'éléments génétiques unique, mais de fonction inconnue pour l'instant (**Chapitre 4**). Dans l'**Annexe II**, nous présentons un suivi temporel de la même cohorte étudiée au **Chapitre 3**, et concluons que la plus grande variabilité dans le microbiome inuit pourrait être due à des pratiques alimentaires plus opportunistes, associées à la transition alimentaire nordique et au coût élevé des aliments de marchés dans le Nord (Dubois et al. 2017).

Plusieurs questions sur le microbiome inuit demeurent à explorer. Si les études utilisant le gène 16S de l'ARNr sont utiles pour explorer la complexité du microbiome intestinal d'une population, une étude plus ciblée sur le génome entier de taxons d'intérêt augmenterait la résolution des résultats et pourrait permettre de tirer des conclusions plus probantes. Dans le **Chapitre 3**, nous avons démontré une faible diversité de souches au sein du genre *Prevotella*, impliqué dans la dégradation des fibres. L'isolement des quelques souches présentes dans le microbiome inuit et leur culture en laboratoire en présence de différentes sources de fibres pourrait donner un meilleur aperçu du potentiel métabolique de ces souches, et des interactions entre le microbiome intestinal inuit et les fibres alimentaires (Chung et al. 2016; De Filippis et al. 2016). Étant donné que beaucoup de souches bactériennes ne sont pas cultivables en laboratoire, le séquençage du génome de ces souches et la reconstruction de leur génome serait un complément nécessaire à cette approche.

De manière plus anecdotique, l'effet de certains aliments de la diète traditionnelle inuite sur le microbiome pourrait également être évalués. L'impact d'aliments fermentés sur le microbiome par exemple est bien documenté, mais uniquement au niveau de composés d'origine végétale (Bianchi et al. 2014). Toutefois, la diète traditionnelle inuite a la particularité d'inclure de la viande fermentée (Speth 2017) comme l'*igunaq* (viande de morse fermentée), qui pourrait représenter un apport de bactéries anaérobiques unique issues de la putréfaction vers le microbiome intestinal. Une courte série temporelle incluant les semaines précédant et suivant la consommation d'*igunaq* chez une petite cohorte d'individus pourrait nous en apprendre davantage sur l'effet que pourraient avoir ces bactéries ingérées avec la diète sur le microbiome

intestinal résident, et pourrait contribuer à expliquer une partie de la variabilité observée dans l'**Annexe II** (Dubois et al. 2017).

Les liens entre le microbiome et la santé de l'hôte sont de plus en plus explorés, et la communauté scientifique réfléchit davantage aux approches thérapeutiques ciblant le microbiome intestinal. Évidemment, une cohorte de la taille de celle présentée au **Chapitre 3** ne permet pas de tirer de conclusions assez fortes pour justifier des interventions médicales ciblant le microbiome, et les conclusions de cette étude ne peux être appliquées à tous les Inuits. Des efforts d'échantillonnage à plus grande échelle doivent donc être mis de l'avant, ne se limitant pas à une seule communauté, mais incluant des Inuits de partout dans le Nord. Une meilleure représentation des Inuits, ainsi que des métadonnées de plus grande résolution pourraient alors servir à répondre à des questions spécifiques aux Inuits. Un enjeu d'intérêt qui pourrait être exploré dans un tel projet serait *Helicobacter pylori*, responsable du développement de gastrites chroniques, d'ulcères peptiques et de cancers. Si aucun membre de la cohorte du **Chapitre 3** ne présentait des séquences d'*H. pylori*, les populations autochtones nordiques sont particulièrement touchées par cet agent infectieux (Goodman et al. 2008). Une approche similaire pourrait être adoptée par rapport au genre *Akkermansia*, associé à l'obésité (Belzer and de Vos 2012; Everard et al. 2013). Dans le **Chapitre 3**, nous avons détecté des souches d'*Akkermansia* spécifiquement associées aux Inuits et à la diète traditionnelle mais dont les fonctions demeurent inconnues : *Akkermansia* pourrait donc potentiellement être utilisée pour explorer davantage les questions reliées à l'obésité, un phénomène prévalent dans le Nord (Kuhnlein et al. 2004). Enfin, suite à la communication des résultats du **Chapitre 3** à la cohorte échantillonnée, d'autres intérêts de recherche ont émergé : la question des parasites a été soulevée plusieurs fois par la communauté, vu les risques de contraction associés à la consommation de nourriture traditionnelle (Goyette et al. 2014). Ceci représente un exemple de la manière dont de futurs projets sur le microbiome devraient être développés à partir d'échanges avec les Inuits pour cibler leurs intérêts de recherche.

La recherche sur le microbiome se tournant davantage vers des avenues thérapeutiques, il est critique que les communautés autochtones soient mieux représentées dans ces études (Sankaranarayanan et al. 2015), comme dans d'autres domaines de la recherche biomédicale (Stephens et al. 2006). Une base de données regroupant des données sur le microbiome d'Inuits

de partout au Canada donnerait une opportunité à cette population d'être mieux représentés dans le monde de la recherche sur le microbiome, et d'éventuellement bénéficier des découvertes et traitements qui en découleront. Par contre, il est crucial que cette base de données soit planifiée et développée en étroite collaboration avec les gens du Nord. Les études génétiques et de biologie moléculaire chez les populations autochtones se sont trop souvent déroulées sans la consultation des communautés concernées, ou avec un manque de tact envers les enjeux socioculturels (Arbour and Cook 2006). De plus, la question de la propriété de l'information découlant de ce type de projet est un enjeu éthique important dans les communautés autochtones (Arbour and Cook 2006). Toute recherche sur le microbiome doit donc approcher la question d'une perspective bilatérale, en incorporant activement les intérêts et inquiétudes des Inuits et de la communauté scientifique.

Perspectives

La Convention de Minamata, adoptée en 2013, regroupe 128 pays signataires, et a pour objectif de protéger la santé humaine et l'environnement d'émissions anthropiques et de relâche de Hg (United Nations Environment Programme 2013). Le succès de la convention dépendra évidemment du respect de ses articles par les pays qui la ratifient. Toutefois, la Convention n'atteindra ses objectifs que si elle intègre aussi de nouvelles aires de recherche sur le Hg (Gustin et al. 2016). En effet, les articles et buts de la Convention sont basés sur des modèles prédictifs, dont la précision repose sur nos connaissances sur le cycle du Hg, et comme le remarquent Gustin et al. (2016) notre compréhension des émissions de Hg et de son cycle biogéochimique demeure incomplète. Pour assurer son succès, il est donc capital que la Convention de Minamata tienne compte de nouvelles percées portant sur le Hg. Ceci pourrait inclure la photodéméthylation, qui pourrait altérer les bilans nets de MeHg, particulièrement dans le contexte des apports en nutriments aux écosystèmes aquatiques dus aux changements climatiques.

De la même manière, nous suggérons que cette approche doit être appliquée à la recherche en toxicologie. Les modèles pharmacocinétiques doivent être révisés au vu de la littérature croissante suggérant que nos connaissances sur le sort du Hg dans le corps sont

partielles. Si la bioaccessibilité demeure une mesure issue d'observations *in vitro* peu réalistes, trop d'études indépendantes s'entendent pour dire que la solubilisation du MeHg est imcomplète pour que nous l'ignorions. Les études sur les taux d'absorption datant des années 70 doivent être révisées, et le rôle des pratiques alimentaires et du microbiome doivent être explorés davantage dans des modèles *in vivo*. Il est essentiel d'intégrer ces nouveaux champs de connaissance afin de raffiner nos évaluations du risque posé par le Hg.

Le déploiement de nouveaux projets hydro-électriques dans le Nord du Canada, dont les territoires alors inondés deviennent facilement des sources de MeHg (St Louis et al. 2004), suscite de nombreuses inquiétudes dans les communautés nordiques, comme en témoigne le développement actuel du projet de Muskrat Falls (Labrador). Selon des prédictions, ce barrage qui inondera approximativement 41 km^2 de territoire causera une hausse significative du MeHg dans les eaux douces touchées, et induira une augmentation d'un facteur 1.3 – 10 du MeHg dans la faune chassée par les Inuits de la région, doublant l'exposition chez les Inuits (Calder et al. 2016).

La contamination de la nourriture traditionnelle demeure donc un enjeu dans le Nord, particulièrement dans le contexte socioéconomique actuel. En effet, l'insécurité alimentaire touche disproportionnellement les communautés Inuits comparativement aux populations du Sud du Canada, touchant plus de 60% des demeures de l'Arctique (Huet et al. 2017). Les pratiques de chasse et la consommation de nourriture traditionnelle sont associées à une meilleure sécurité alimentaire (King and Furgal 2014), mais elles représentent un vecteur d'exposition important au Hg. Les concentrations de MeHg dans les animaux qui font partie de la diète traditionnelle seront probablement affectées par les changements climatiques (AMAP 2011), contribuant à l'incertitude sur le risque posé par ce contaminant. « Est-ce sécuritaire de manger de la nourriture traditionnelle ? » est donc une question fréquente dans les communautés nordiques (Bordeleau et al. 2016; King and Furgal 2014).

La réponse à cette question n'est pas simple, et se trouve à l'intersection de nombreux champs d'étude, comme la toxicologie, la nutrition et les effets des changements climatiques, mais également du savoir traditionnel inuit. La participation active des instances autochtones

dans l'élaboration et la réalisation de recherche environnementale nordique est essentielle : cette approche bilatérale contribuera à la décolonisation du Nord en permettant l'autonomie et l'autodétermination des communautés inuites (Black and McBean 2016). De plus, l'inclusion de savoir traditionnel autochtone a des conséquences positives sur la recherche scientifique (Mantyka-Pringle et al. 2017), et les entrevues utilisées pour récolter ces informations (telles que celles utilisées dans les **Chapitres 3 & 4**) facilitent le développement d'une relation de confiance entre les chercheurs et les communautés du Nord. Ce lien de confiance est par ailleurs essentiel à la communication subséquente des résultats, qui doit être adaptée à la communauté et réalisée dans le respect de la communauté (Furgal et al. 2005; Jack et al. 2010).

L'avenir de la recherche sur le Hg et le MeHg dans le Nord devra donc adopter cette approche bilatérale et être fondée sur une communication du risque réfléchie et développée en partenariat avec les gens du Nord, afin de protéger la santé humaine et environnementale de cette région en changement.

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Annexe I. Les niveaux de méthylmercure élevés de mares arctiques et subarctiques sont reliés aux nutriments dans l'Arctique canadien en réchauffement

High methylmercury in Arctic and Subarctic ponds is related to nutrient levels in the warming Eastern Canadian Arctic

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Contribution as second author: field campaign organizing, sample collection, contributing to chemical and statistical analyses and data interpretation.

Annexe II. Le microbiome intestinal inuit est dynamique dans le temps mais ne présente pas de variation saisonnière

The Inuit gut microbiome is dynamic over time but lacks seasonality

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Contribution as second author: project development, sampling and recruitment of volunteers, contributing to laboratory and statistical analyses.

Annexe III. Exemple de questionnaire d'habitudes alimentaires administré à Resolute Bay

DIETARY HABIT QUESTIONNAIRE (ENGLISH)

Project title

Bioaccessibility of organometallic contaminants in food of Canadian populations

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Information on participant

Participant number:

Participant age:

Height:

Weight:

Dietary habits

This section concerns your dietary habits regarding food that can potentially be contaminated, as well as that of food that can modify contaminant bioaccessibility. These questions concern your consumption in **the past year**.

1. Fish

In the past year, have you eaten **fish** collected by your or other members of the community?

Yes ☐ No ☐

If you answered yes, complete the following table. Check any box that applies.

Fish species	Cooking method						How often do you eat it?				
	Frozen	Raw	Grilled	Boiled	Dried	Fermented	Never	A few times per year	A few times per month	A few times per week	Everyday
Arctic char											
Other:											

2. Birds

In the past year, have you eaten **birds** hunted by your or other members of the community?

Yes ☐ No ☐

If you answered yes, complete the following table. Check any box that applies.

Bird species	Cooking method						How often do you eat it?				
	Frozen	Raw	Frozen	Raw	Frozen	Raw	Never	A few times per year	A few times per month	A few times per week	Everyday
Canada goose											
Snow goose											
Duck											
Ptarmigan											
Other:											

3. Mammals

In the past year, have you eaten **mammals** hunted by your or other members of the community?

Yes ☐ No ☐

If you answered yes, complete the following table. Check any box that applies.

Mammal species	Cooking method						How often do you eat it?				
	Frozen	Frozen	Frozen	Frozen	Frozen	Frozen	Never	A few times per year	A few times per month	A few times per week	Everyday
Polar bear											
Muskox											
Caribou											
Arctic hare											
Bearded seal											
Harp seal											
Ringed seal											
Walrus											
Beluga											
Narwhal											
Bowhead whale											
Other:											

4. Beverages

Do you drink **tea or coffee**?

Yes ☐ No ☐

If you answered yes, complete the following table. Check any box that applies.

Beverage	Do you drink it during meals?		How often do you drink it?				
	Yea	No	Never	A few times per year	A few times per month	A few times per week	Everyday
Green tea							
Black tea							
Coffee							
Other:							