Université de Montréal

Dynamique des communautés bactériennes et effet du glyphosate lors du compostage de biomasse lignocellulosique

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Thèse présenté(e) en vue de l'obtention du grade de *Philosophiae Doctor* (Ph.D.)

en Sciences biologiques

Août 2021

Université de Montréal

Département de Sciences biologiques, Faculté des Arts et Sciences

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Cette thèse intitulé(e)

Dynamique des communautés bactériennes et effet du glyphosate lors du compostage de biomasse lignocellulosique

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Résumé

Le compostage est un procédé anthropique basé sur le processus naturel de décomposition de la biomasse qui exploite l'activité enzymatique des microorganismes sous le contrôle de plusieurs facteurs environnementaux. Les résidus lignocellulosiques de par leur composition et leur faible pourcentage d'humidité sont particulièrement adaptés au compostage dans lequel ils jouent le rôle d'élément structurant. Bien que majoritairement d'origine végétale, la matière organique dirigée vers les sites de compostages est très diversifiée, tout comme les types de contaminants qu'elle peut incidemment contenir et dont l'impact sur les processus de biodégradation, et de surcroit leur rémanence dans l'environnement, reste largement à investiguer.

L'objectif de cette thèse vise ainsi à faire état de l'effet de la composition de la biomasse lignocellulosique et de la présence d'un contaminant fréquent tel que le glyphosate sur le compostage. Pour ce faire, le suivi de la transformation de la matière organique végétale et de la dégradation du glyphosate, l'évolution des paramètres physicochimiques et la dynamique de recrutement des populations bactériennes ont été effectués tout au long du processus.

Deux expériences menées sur le terrain visaient dans un premier temps à mesurer l'effet de l'âge d'une plante ligneuse, dans ce cas-ci le saule arbustif (*Salix*), et d'une période d'entreposage hivernal sur la transformation de la biomasse, et dans un deuxième temps à étudier les dynamiques de succession bactériennes impliquées dans le cycle du carbone et de l'azote lors du compostage de résidus végétaux. Les résultats obtenus ont révélé une différence dans la composition de la biomasse des tiges âgées de 2 ans et de 3 ans. Alors que les premiers contenaient plus de composés extractibles, les seconds étaient plus riches en sucres structuraux. Ces différences expliquent une hausse des températures plus forte et plus rapide dans le tas de copeaux de tiges plus jeunes. La diminution des composés extractibles, la conservation des sucres structuraux et l'augmentation de la proportion de lignine démontrent l'importance de la source de carbone soluble pour l'initiation de la

décomposition du bois et la récalcitrance des éléments lignocellulosiques durant l'entreposage hivernal. La seconde expérience a mis en évidence une très grande diversité de bactéries responsables de la décomposition de la cellulose, des hémicelluloses et de la lignine durant la phase thermophile du compostage. Cette phase qui était le théâtre d'une activité intense comptait moins d'espèces, mais ces dernières étaient très abondantes, une tendance qui s'est inversée avec la maturation de la matière organique. La dynamique observée traduit une redondance fonctionnelle des communautés qui semblent évoluer selon la température, le taux d'oxygène et la nature du substrat disponible.

Une troisième expérience menée en milieu contrôlé a ensuite démontré l'impact négligeable du glyphosate sur l'activité microbienne et l'évolution des paramètres physicochimiques lors du compostage. Le glyphosate était presque ou entièrement dégradé à l'issue du compostage et la présence du principal produit de dégradation, l'acide aminométhylphosphonique (AMPA) n'a d'ailleurs même pas pu être quantifiée durant l'expérience. L'impact du glyphosate sur les communautés bactériennes était également négligeable. Seules quelques bactéries étaient différentiellement abondantes entre les deux traitements, la grande majorité étant moins abondante dans le traitement contenant du glyphosate. La richesse en espèces aux différents temps d'échantillonnage était la même entre le traitement témoin et le traitement contenant du glyphosate « pur » et l'analyse de la bêta-diversité n'a relevé aucune différence significative entre les communautés présentes dans le traitement témoin et le traitement glyphosate.

Cette thèse a ainsi fait valoir l'importance de la nature initiale de la matière organique sur l'activité microbienne, le recrutement et la dynamique des communautés durant le compostage, tandis que la présence du contaminant glyphosate s'est présenté comme un facteur beaucoup moins déterminant sur les processus de décomposition et l'abondance des espèces bactériennes. Ces informations devraient non seulement permettre d'optimiser le traitement de la matière organique par compostage, mais aussi de mieux évaluer les risques potentiels associés au compostage de biomasse contaminé.

Mots-clés : Compostage, lignocellulose, saule, glyphosate, bactérie, métagénomique

Abstract

Composting is an anthropic process based on the natural decay of biomass that exploits the enzymatic activity of microorganisms under the control of several environmental factors. Due to their composition and low moisture content, lignocellulosic residues are particularly suitable for composting and serve as a structuring element, which confers them an important role in the process. Although mostly of plant origin, the organic matter (OM) directed towards composting sites is highly diversified, as are the types of contaminants it can contain. The impact of these contaminants, such as glyphosate, on the biodegradation process and their persistence in the environment remain to be investigated.

The objective of this thesis is thus to report on the effect of the composition of the lignocellulosic biomass and the presence of glyphosate on the evolution of the physicochemical parameters and the recruitment of bacteria during composting, while ensuring the follow-up of the transformation of the vegetable organic matter and the degradation of glyphosate during the process.

Two field studies were conducted to measure the effect of stem age and winter storage on the transformation of wood chips, and to study the dynamics of bacterial succession involved in the carbon and nitrogen cycle during the composting of plant residues. The results obtained revealed a difference in the composition of 2-year-old and 3-year-old stems from shrub willow (*Salix* sp.), with the younger ones containing more extractable compounds and the more mature ones richer in structural sugars. These differences were reflected in a higher and faster temperature rise in the younger chip pile. A decrease in extractives, retention of structural sugars, and an increase in the proportion of lignin demonstrate the importance of the soluble carbon source for the initiation of wood decomposition and recalcitrance of lignocellulosic elements. The second experiment revealed a very high diversity of bacteria responsible for the decomposition of cellulose, hemicelluloses and lignin during the thermophilic phase of composting. This phase, during which intense activity took place, had fewer species, but they were very abundant, a trend

that reversed as the organic matter matured. The observed dynamics reflect a functional redundancy of the communities, which seems to evolve according to the temperature, oxygen level and nature of the available substrate.

A third experiment conducted in a controlled environment demonstrated the negligible impact of glyphosate on microbial activity and the evolution of physicochemical parameters during composting. Glyphosate was almost or completely degraded after composting, while the main product of degradation, aminoethylphosphonic acid (AMPA), was not detected. The impact of glyphosate on bacterial communities was also negligible, while species richness at different sampling times was the same when comparing the control treatment and the treatment containing "pure" glyphosate. The beta-diversity analysis found no significant difference between the communities present in the control and glyphosate treatments, while a few bacteria were differentially abundant between the two treatments, the vast majority being less abundant in the glyphosate treatment.

This thesis has thus highlighted the importance of the initial nature of the organic matter on microbial activity as well as on the recruitment and dynamics of bacterial communities during composting, while the presence of glyphosate was shown to be a weak determinant of decomposition processes and species abundance. This information should help to optimize the treatment of organic matter by composting and to better assess the potential risks associated with composting contaminated biomass.

Keywords: Composting, lignocellulose, willow, glyphosate, bacteria, metagenomic

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Liste des sigles et abréviations

(Les caractères italiques indiquent les termes en anglais)

AG :	Grade Analytique / *Analytical Grade*
AMPA :	Acide aminométhylphosphonique / *Aminoethylphosphonic acid*
AOB :	Bactérie Oxydantes de l'Ammonium / Ammonia-Oxidizing Bacteria
BRF :	Bois raméal fragmenté
C:	Carbone / *Carbon*
CAP :	Canonical Analysis of Principal Coordinates
CH_4:	Méthane / *Methane*
CO :	Monoxyde d'azote / Carbon monoxide
CO_2:	Dioxyde de carbone / *Carbon dioxyde*
EPSP :	5-énolpyruvyl-shikimate-3-phosphate
ESV :	*Exact Sequence Variant*
GBH :	Herbicide à base de glyphosate / *Glyphosate-based herbicide*
GES:	Gaz à effet de serre
GOX :	Glyphosate oxydoreductase
H_2 :	Dihydrogène / Dihydrogen
H_2O:	Eau / *Water*
LET:	Lieu d'enfouissement technique
MELCC:	Ministère de l'Environnement et de la Lutte contre les Changements Climatiques
MO:	Matière organique
N:	Azote / *Nitrogen*
N_2 :	Diazote / *Dinitrogen*
N_{org} :	Azote organique / Organic nitrogen
N_2O:	Oxyde nitreux / *Nitrous oxyde*
NH_2-R :	Azote assimilé (amine) /*Assimilated nitrogen (amine)*
NH_3:	Ammoniac / *Ammonia*

NH_4^+:	Ammonium
NO_2^-:	Nitrite
NO_3^-:	Nitrate
NOB :	Bactérie Oxydantes des Nitrites/ Nitrite-Oxidizing Bacteria
O_2:	Oxygène / *Oxygen*
P :	Phosphore / Phosphorous
PCoA :	Analyse en coordonnées principales / *Principal Coordinate Analysis*
POEA :	Polyoxyéthylène amine / *Polyoxyethylene tallow amine*
ppm :	Partie par million / *Parts per million*
SD :	Écart-type / *Standard deviation*
w/v :	ration poids/volume / *weight/volume ratio*

En vérité, le chemin importe peu,
la volonté d'arriver suffit à tout.

- *Albert Camus*

Remerciements

La liste des personnes formidables ayant croisé mon chemin au cours des cinq dernières années est probablement assez longue pour me faire une jupe, mais je me suis promis de m'en tenir à une seule page.

D'abord un grand merci à Frédéric pour m'avoir offert ce projet unique et pour son support durant toutes ces années. Ton soutien, ton écoute et ta confiance ont certainement forgé la scientifique que je suis aujourd'hui. J'ai souvent vanté notre relation que je considère égalitaire et inclusive et l'ouverture qu'on se permet d'avoir l'un envers l'autre.

Merci à mes collaborateurs de plus ou moins longue date et ceux qui ont participé au succès de ce doctorat. En particulier Nicholas et Emmanuel pour leur aide inestimable dans mon apprentissage de la métagénomique et tout spécialement à Joan pour son soutien, son sang-froid, sa bonne humeur contagieuse. Tu es un modèle incroyable et une partie de cette thèse te revient, merci pour tout. Mes nouveaux amis de l'UQAM, Matthieu et Marc, les stagiaires et aides de terrain, Susan, Anne-Frédérique, Estelle Camille et Nicolas. Et les chercheurs de l'IRBV qui sont toujours là pour donner un coup de pouce, merci, Michel, Alain et Marc.

À mes amis et collègues de l'IRBV pour ces moments prépandémie où on pouvait socialiser et avoir du plaisir, Antoine, Maya, Alexis, Alizée, Maxime, Dominic, Ahmed, Noël, Eszter, Adrien, Ariane, Amandine et Kym et tous les autres et aux copains de l'AECBUM et du MIL qui sont loin des yeux, mais si près du cœur.

Le parcours vers l'obtention d'un PhD est un cheminement que les personnes à l'extérieur du secteur académique ont parfois du mal à saisir, mais leur présence n'est pas moins essentielle pour autant. Merci à papa et maman pour leur support éternel, à mes best Mérani et Annie-Claude *pour you know what* et à mon copain Olivier ♥

Merci également aux partenaires financiers qui ont rendu le tout possible, le FRQNT, Mitacs, Ramo, la Fondation Espace pour la Vie, l'IRBV et le Département de Sc. biologiques.

Image tirée du Guide technique pour le compostage sur site en ICI
par Alexis Fortin et Louise Héneault-Éthier (Fortin & Héneault-Éthier, 2011)

Introduction

1.1 Mise en contexte

Comme beaucoup d'autres enjeux environnementaux découlant de l'activité humaine, la gestion des déchets s'impose comme un fardeau important pour les municipalités et les gouvernements au cours des prochaines années. Ce phénomène n'est pas nouveau, mais il prend de l'ampleur en raison de la nécessité de réduire les émissions de gaz à effet de serre (GES) et de l'atteinte de la capacité des lieux d'enfouissement technique (LET) au Québec. Les estimations les plus récentes révèlent qu'environ 5.8 millions de tonnes de déchets devront être éliminées chaque année dans la province (MELCC, 2020b). Les matières organiques (MO), un terme général qui comprend les déchets alimentaires, les déchets verts, le papier, le carton, le bois et les biosolides d'origine municipale, représentent 60 % de ces déchets, soit près de 3.5 millions de tonnes. À l'échelle mondiale, on estime qu'en 2025, plus de 2 200 millions de tonnes de résidus organiques seront générées annuellement par la collecte des déchets solides municipaux (Campuzano & González-Martínez, 2016).

L'enfouissement de la MO demeure le mode de gestion le plus courant, mais cette pratique n'est pas sans failles, même dans les LET où des systèmes d'atténuation sont en place pour réduire les impacts environnementaux. Le premier enjeu, et le plus flagrant au Québec est l'atteinte à court et à moyen terme des limites d'enfouissement de plusieurs LET d'envergure. Le site de Lachenaie, où les déchets de la majorité des Québécois sont enfouis, devrait atteindre sa capacité d'ici 2029 (La Presse +, « Ça déborde »), tandis qu'à l'heure d'écrire ces lignes, un décret gouvernemental devrait permettre aux opérateurs du LET de Saint-Nicéphore à Drummondville d'agrandir le site qui atteindra sa pleine capacité d'ici la fin de l'été 2021 (La Presse, « Québec contourne la Cour supérieure pour agrandir un site d'enfouissement »). Les normes environnementales restrictives et la faible acceptabilité sociale ne permettent toutefois pas d'envisager la création de nouveaux LETs pour combler les besoins grandissants des ménages québécois. L'enfouissement des MO était aussi responsable de près de 6 % des émissions totales de GES au Québec en 2017 (MELCC, 2020). Ces GES, tel le méthane (CH_4), le dioxyde de carbone (CO_2) et l'oxyde nitreux (N_2O) proviennent essentiellement de la décomposition anaérobique de la MO lorsque celle-ci a une forte teneur en humidité et que le taux d'oxygène est faible. Le potentiel de

réchauffement planétaire du CH_4 et du N_2O, sur une période de 100 ans, est respectivement 25 et 298 fois plus élevé que celui du CO_2 (Lou & Nair, 2009). Finalement, la décomposition anaérobique et la percolation d'eau à travers l'amoncellement de déchets génèrent la production de lixiviat; un liquide acide chargé en divers contaminants organiques et inorganiques (Kjeldsen et al., 2002). Le lixiviat s'infiltre ensuite dans les sols environnants, risquant de contaminer les eaux de surface et souterraines.

Toutefois, des options pour détourner la MO des sites d'enfouissement existent, mais restent relativement peu exploitées au Québec. Le plus récent Plan d'action (2019-2024) découlant de la Politique québécoise de gestion des matières résiduelles (MELCC, 2019) et plus concrètement le programme de traitement des matières organiques par biométhanisation et compostage (MELCC 2020) vont dans ce sens en ciblant des objectifs qui devraient permettre réduire la quantité de matières organiques destinées à l'élimination, réduire les émissions de GES et soutenir le développement des débouchés pour les composts, digestats et autres matières résiduelles fertilisantes.

La biométhanisation est bien adaptée à la transformation des résidus alimentaires, des biosolides municipaux et des fumiers étant donné l'accessibilité des molécules carbonées. Les résidus végétaux riches en lignine, de par leur composition, leur structure récalcitrante et leur ratio C :N élevé sont plus difficilement hydrolysables durant le processus de biométhanisation et nécessitent une étape de prétraitement, ce qui encourage leur traitement par compostage (Chen et al., 2008; Sawatdeenarunat et al., 2015). Les résidus verts proviennent principalement de l'entretien des arbres de rue et des espaces verts. Ils comprennent les copeaux d'arbres morts, les résidus d'élagage et les tontes de gazon (Vasarevičius et al., 2011). Outre sa présence en tant que résidu à traiter, la biomasse riche en lignine provenant des arbres (biomasse lignocellulosique) est souvent utilisée comme agent structurant pour équilibrer la teneur élevée en azote et en humidité des résidus alimentaires (Haug, 1993). Elle a donc une place particulière dans les sites de compostage municipaux en raison de son abondance et de son importance dans le processus de compostage et cette thèse sera ainsi consacrée à l'étude approfondie des facteurs intrinsèques et extrinsèques pouvant influencer la dégradation biologique de la biomasse

lignocellulosique et le recrutement des microorganismes responsables de sa décomposition lors du compostage.

1.2 Synthèse générale de la littérature

1.2.1 Qu'est-ce que le compostage?

Le compostage est issu d'un savoir-faire millénaire, développé à l'origine pour gérer les déjections animales et fertiliser les sols agricoles (Guttmann, 2005), et vise à recréer ce cycle de manière contrôlée. Ce processus biologique a depuis été largement étudié et sa définition varie légèrement selon les auteurs. Le concept de base est cependant universellement reconnu et regroupe différentes notions comme les conditions nécessaires à l'obtention d'un produit final stabilisé et propre à l'utilisation. Le compostage est un processus bio-oxydatif par lequel la matière organique se décompose dans des conditions favorisant le développement d'une température élevée due à l'activité microbienne. S'ensuit une réduction du volume et de la masse de la MO, impliquant sa stabilisation, sa minéralisation et son humification, ce qui conduit à un produit final non phytotoxique et libre d'agents pathogènes (Barrington et al., 2002; Bernal et al., 2009; Haug, 1993; Kuo et al., 2004).

1.2.1.1 Le compostage thermophile

La présence des éléments nécessaires à la survie des microorganismes responsables de la dégradation de la MO, c'est-à-dire le carbone (C), l'azote (N), l'eau (H_2O) et l'oxygène (O_2), dans des proportions définies est suffisante pour assurer la minéralisation de la MO lors du compostage (Figure 1.1) (de Bertoldi et al. 1983; Miller et al. 1992). On considère que la majorité des ressources en azote proviennent des « matières vertes », ou humides, telles que les résidus alimentaires, les boues d'épuration, le fumier et les résidus frais provenant des jardins. Les « matières brunes » telles que les feuilles mortes, la sciure de bois, le papier journal et la paille sont riches en carbone et ont une teneur en eau beaucoup plus faible (Kuo et al., 2004; Ryckeboer et al., 2003). La mise en commun de ces éléments, associé à un taux d'humidité de 50-60 % et un maximum d'oxygène permet le développement de conditions thermophiles qui résultent d'une activité microbienne intense au début du

procédé (Haug, 1993). Ces températures élevées favorisent une décomposition rapide de la MO ainsi que l'éradication des graines de " mauvaises herbes " et des organismes pathogènes.

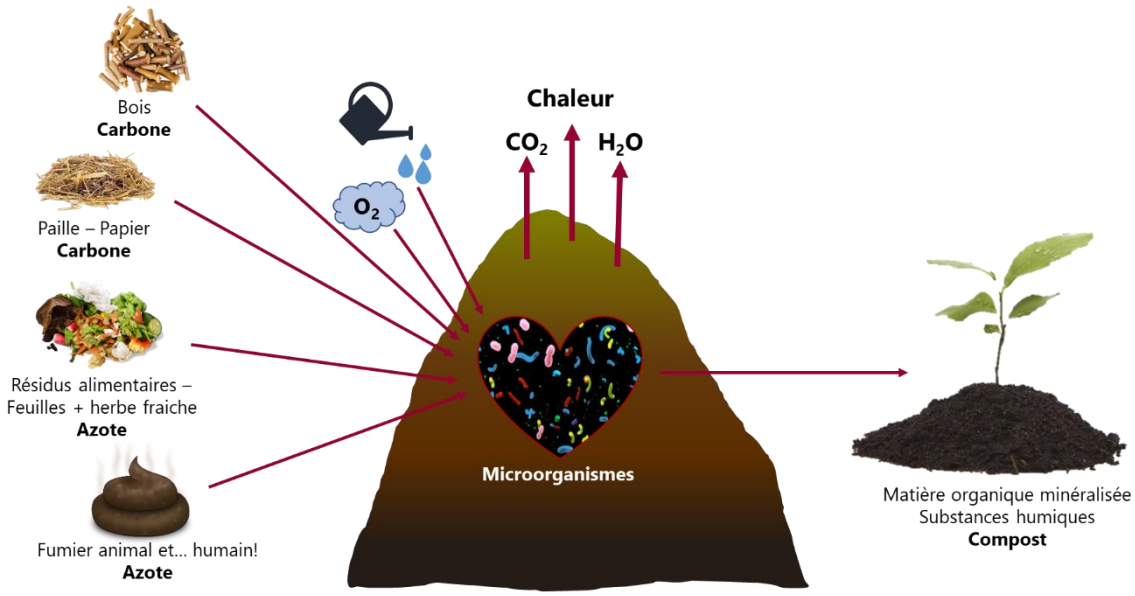

Figure 1.1 - Schéma général du processus de compostage

Le processus de compostage peut être divisé en quatre phases (01.2). La **première phase mésophile** (se déroulant entre 20 °C et 45 °C) dure quelques jours à partir du moment de la mise en place du mélange (Bernal et al. 1996; Keener et al. 2000). Durant cette phase, les bactéries et les champignons présents sur la MO commencent à dégrader les sucres simples et solubles. L'abondance du substrat entraîne une croissance très rapide du nombre de microorganismes, une augmentation de l'activité microbienne et un important dégagement de chaleur dû à la respiration des microorganismes. Au cours de cette phase, on assiste également à une augmentation de la production de CO_2 et une diminution progressive du pH causée par la dégradation de la MO et la production d'acides organiques par les bactéries et les champignons mésophiles (Cooperband, 2000; Hellmann et al., 1997).

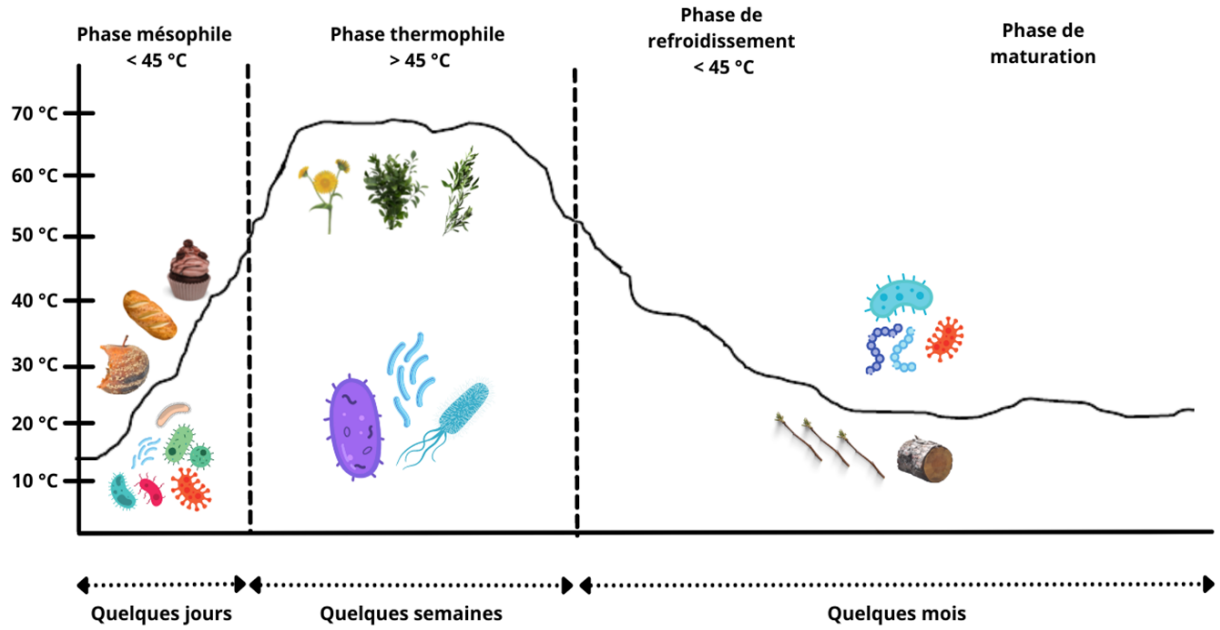

Figure 1.2 - Étapes du compostage thermophile, inspiré de Trautmann et Krasny, 1997

Lorsque la température dépasse la barre des 40-45 °C, les organismes mésophiles laissent la place à des organismes tolérants les hautes températures, c'est ce qui marque le début de la **phase thermophile.** La température durant cette phase peut atteindre plus de 80 °C et se maintiendra jusqu'à ce que le substrat devienne limitant et n'arrive plus à soutenir le métabolisme des organismes thermophiles. La décomposition de la MO atteint son paroxysme durant cette phase, alors que les lipides, les protéines, la cellulose, l'hémicellulose et une partie de la lignine sont dégradés (Chefetz et al., 1996; Hellmann et al., 1997; Ryckeboer et al., 2003). À cette étape du compostage, le pH augmente progressivement suivant la volatilisation et la consommation des acides organiques. La production d'ammoniac (NH_3) provenant de la dégradation des protéines et d'autres sources d'azote organique est également enregistrée. La diversité microbienne dans la phase thermophile est plus faible que dans la phase précédente, mais les organismes présents, principalement des bactéries, sont très abondants et dominent ainsi le processus de compostage pendant une période qui peut s'étendre sur plusieurs semaines (Tuomela et al., 2000).

La décomposition progressive de la MO s'accompagne d'une diminution des sources de carbone disponibles, de l'activité microbienne et donc de la température. La chute de la température à des valeurs de 40-45 °C annonce le début de la deuxième phase mésophile, aussi appelée **phase de refroidissement** (Crawford 1983). La baisse de la température s'accompagne d'une diminution de la production de CO_2 et d'une stabilisation du pH à un niveau proche de la neutralité. La MO est recolonisée par des microorganismes capables de dégrader le carbone présent dans la matière récalcitrante comme la cellulose et la lignine (Tiquia et al., 1996). Progressivement, la température diminue pendant une longue période de maturation pour se stabiliser à température ambiante. Le compost entre alors dans une **phase de maturation**, au cours de laquelle les éléments précurseurs de l'humus apparaissent. La phase de maturation peut persister sur une très longue période (plusieurs mois) en fonction du type de matériau contenu dans le tas de compost. L'objectif principal de la phase de maturation est de permettre l'hydrolyse complète de la matière première (Cooperband, 2000; Haug, 1993).

1.2.1.2 Facteurs influençant le processus de compostage

Plusieurs facteurs interviennent dans le processus de compostage et permettent de maintenir les conditions idéales pour la décomposition complète de la MO. Les facteurs abiotiques qui affectent le processus de compostage peuvent être divisés en deux groupes : ceux qui dépendent de la composition et de la nature de la matière première utilisée, comme l'équilibre nutritif, le pH, la taille des particules, la porosité et la présence de contaminants; et ceux qui sont contrôlés par les opérateurs, comme la concentration en O_2, la température et la teneur en humidité (de Bertoldi et al., 1983; Haug 1993; Das et al., 1997). Ces éléments ont un effet direct sur les facteurs biotiques, à savoir les organismes qui colonisent le compost. Les organismes vivants influencent également les propriétés abiotiques de la MO, créant ainsi de multiples interactions complexes.

À titre de source de nourriture pour les microorganismes qui colonisent le compost, la nature de la MO est le principal facteur affectant la diversité de la microflore (Vargas-García et al., 2010). Comme les microorganismes possèdent une gamme d'enzymes spécialisées qui

leur permettent de dégrader des composés spécifiques, l'abondance des différentes composantes de la biomasse végétale telles que la cellulose, les hémicelluloses et la lignine, ainsi que leur disponibilité et leur accessibilité dans la plante, influenceront le recrutement des microorganismes pendant le compostage.

L'origine et la nature de la MO sur les sites de compostage sont très diversifiées, tout comme le sont les sources potentielles de contamination et les types de contaminants rencontrés. Les principales sources de contamination sont les eaux usées et les boues d'épuration, les fumiers issus de l'élevage de tous types d'animaux (source d'éléments trace provenant de l'alimentation du bétail), et divers types de biomasse (bois traité, matériaux de construction et de démolition) (Nzihou & Stanmore, 2013). Ces composés impactent également les microorganismes par leur toxicité et l'effet de sélection que cela peut avoir sur les espèces peu tolérantes.

Bien que tous les facteurs abiotiques soient déterminants dans les processus de décomposition de la MO, la nature du substrat et la présence de contaminants sont inhérentes au compostage et représentent un défi pour les opérateurs qui doivent jongler avec une matière hétérogène dont la composition change en fonction des saisons et des activités agricoles, industrielles et commerciales environnantes. La composition de la matière lignocellulosique et la présence de contaminants, notamment la présence de glyphosate et son effet sur la décomposition de la MO et les successions bactériennes au cours des différentes phases du compostage ont fait l'objet d'une attention particulière dans cette thèse.

1.2.2 La composition de la biomasse lignocellulosique

Les glucides représentent la source de carbone la plus importante chez la plante et sont également les principaux constituants de la structure des cellules végétales. Ils sont classés en trois groupes, soit les monosaccharides, les disaccharides et les polysaccharides. Plus un sucre est complexe, plus le niveau d'hydrolyse requis pour le rendre assimilable par la plante elle-même ou les microorganismes sera important. La cellulose, les hémicelluloses et la lignine sont les principaux composants de la paroi cellulaire végétale (01.3). Leurs

proportions et leur disposition dans la plante varient selon les espèces, mais de manière générale, la biomasse lignocellulosique contient environ 40 % de cellulose, 30 % d'hémicellulose et 25 % de lignine (Pérez et al., 2002). La proportion restante comprend des composés extractibles et solubles comme différents sucres simples, l'amidon, les protéines, la chlorophylle ainsi que diverses molécules organiques comme les composés phénoliques, les terpénoïdes et les alcaloïdes qui sont issus du métabolisme secondaire des plantes (Brereton et al., 2017; Serapiglia et al., 2009).

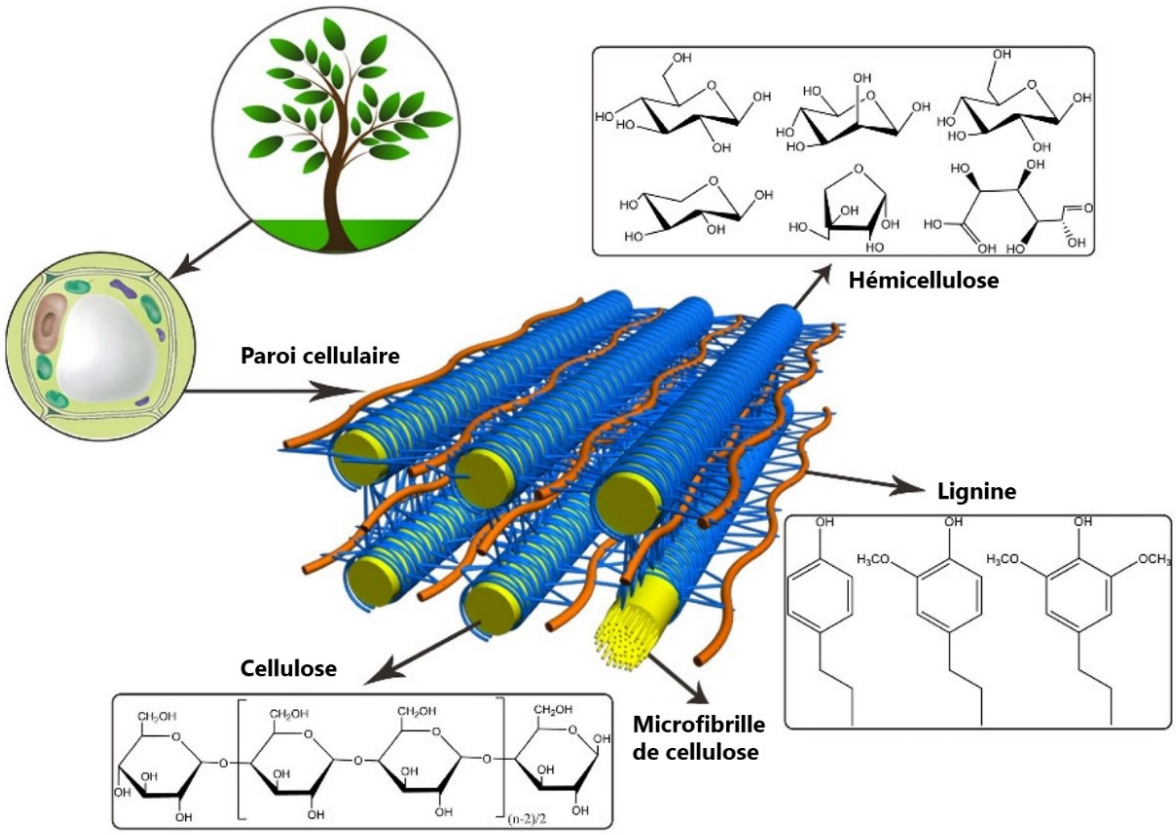

Figure 1.3 - Composition chimique de la lignocellulose, adaptée de Song et al. (2017)

La cellulose, l'hémicellulose et la pectine sont des hydrates de carbone structurels composés principalement ou exclusivement de glucose, de xylane et d'acide galacturonique respectivement (Déjardin et al., 2010; Mohnen, 2008; Scheller et al., 2010). Alors que la cellulose est considérée comme un composant très récalcitrant en raison de sa longue

structure cristalline fibrillaire, les hémicelluloses et la pectine possèdent de courtes chaînes latérales ramifiées à partir d'un squelette, ce qui les rend plus facilement hydrolysables, car elles n'ont pas tendance à former d'agrégats entre elles (Saha, 2003). La lignine est le deuxième polymère le plus abondant après la cellulose, mais sa composition diffère grandement de cette dernière. La lignine est un composé phénolique formé de trois monomères (alcools conifférylique, gaïacylique et syringylique) qui varient fortement en fonction du niveau phylogénétique de la plante (Amidon et al., 2011). Elle s'insère dans la paroi cellulaire et lui confère sa rigidité et son imperméabilité. Sa structure nécessite des enzymes de dégradation spécialisées afin d'être métabolisée par les microorganismes (Brown & Chang, 2014).

Les proportions de chacune des composantes et l'anatomie de la plante ont un impact sur la vitesse à laquelle elle se décompose. Une forte proportion de sucres solubles fournira le carbone nécessaire pour initier l'activité microbienne conduisant à l'élévation de la température lors du compostage ou du stockage de la MO, tandis que des quantités élevées de lignine nécessitent l'action de plusieurs enzymes pour accéder au carbone disponible ce qui créera inévitablement un délai avant l'apparition de la phase thermophile. L'âge de la plante et le type d'organe vont donc influencer la composition de la biomasse qui va ensuite affecter la décomposition.

1.2.3 Le compost comme vecteur de contamination

Le compostage des résidus organiques, en plus de jouer un rôle important dans la gestion des déchets et de réduire le volume envoyé à la décharge, produit également du compost qui peut être utilisé comme engrais. Le compost est une excellente source de MO, de nutriments et de microorganismes, qui sont essentiels au maintien des cycles biogéochimiques et des conditions de croissance des plantes (Loveland & Webb, 2003). Cependant, l'épandage de compost peut également comporter des risques pour l'environnement en raison de la présence de contaminants qui peuvent être présents dans la MO d'origine. La contamination peut être présente sous forme de corps étrangers (par exemple, plastique ou verre), biologique (agents pathogènes) ou chimique (éléments trace,

détergents, médicaments, colorants, pesticides, produits pétroliers, etc.) (Brändli et al., 2005; Ghanem et al., 2007; Nzihou & Stanmore, 2013; Oleszczuk, 2008)

Les résidus végétaux sont moins susceptibles d'être contaminés que le fumier ou les boues d'épuration, mais peuvent néanmoins contenir des composés chimiques, tels que des herbicides qui sont largement utilisés dans les zones agricoles, mais aussi pour l'aménagement paysager domestique et municipal et pour la maîtrise de la végétation en bordure des routes (Brändli et al., 2005; Büyüksönmez et al., 1999).

1.2.3.1 Le glyphosate, l'omniprésent

Le glyphosate (N-[phosphonométhyl]glycine), un phosphonate synthétique de la famille des organophosphates est l'herbicide le plus largement répandu au niveau mondial en termes de superficie traitée et de quantité totale appliquée (Soumis, 2018). Ce constat est attribuable à l'utilisation généralisée des herbicides à base de glyphosate (HBG) tels que le RoundUp™ (Zhan et al., 2018). Le glyphosate est un herbicide systémique, ce qui signifie que lorsqu'il est pulvérisé sur la plante, il est absorbé par les feuilles et les tiges, puis pénètre dans la plante par diffusion et s'accumule dans la sève, puis est rapidement distribué dans tous les tissus métaboliquement actifs (Duke & Powles, 2008). Les HBG peuvent également être utilisés pour le désherbage en présemis et comme dessiccateur pour accélérer la vitesse de maturation des grains avant la récolte. En plus du glyphosate, la formulation du RoundUp™ et d'autres HBG contiennent des surfactants qui permettent une meilleure adhésion aux feuilles au moment de l'application, tel le polyoxyéthylène amine (POEA), un tensioactif non ionique identifié comme toxique pour certaines bactéries, organismes aquatiques et poissons (Navarro & Martinez, 2014; Song et al., 2012; Tsui & Chu, 2003).

Le glyphosate agit en inhibant l'enzyme 5-énolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase) de la voie du shikimate, qui est indispensable à la production d'acides aminés aromatiques essentiels chez les plantes (Gomes et al., 2014). Les mammifères, y compris les humains, ne synthétisent pas d'acides aminés aromatiques et les obtiennent plutôt de leur alimentation, les microorganismes par contre utilisent la voie du shikimate comme les plantes pour synthétiser leurs acides aminés aromatiques (Aristilde et al., 2017).

Les facteurs qui influencent la dégradation des HBG pendant le compostage sont les mêmes que ceux qui conditionnent leur dégradation dans les sols. Les conditions environnementales, telles que le pH ou la capacité d'échange cationique (CEC) du sol ou la température et le niveau d'oxygène, ont une incidence significative sur le devenir et la dégradation des pesticides (De Jonge et al., 2001; Muskus et al., 2019; Nguyen et al., 2018). Le glyphosate peut être dégradé via des voies biotiques ou abiotiques telles que l'adsorption, la thermolyse et la photodégradation, mais la dégradation microbienne est considérée comme la voie la plus importante (Borggaard & Gimsing, 2008)

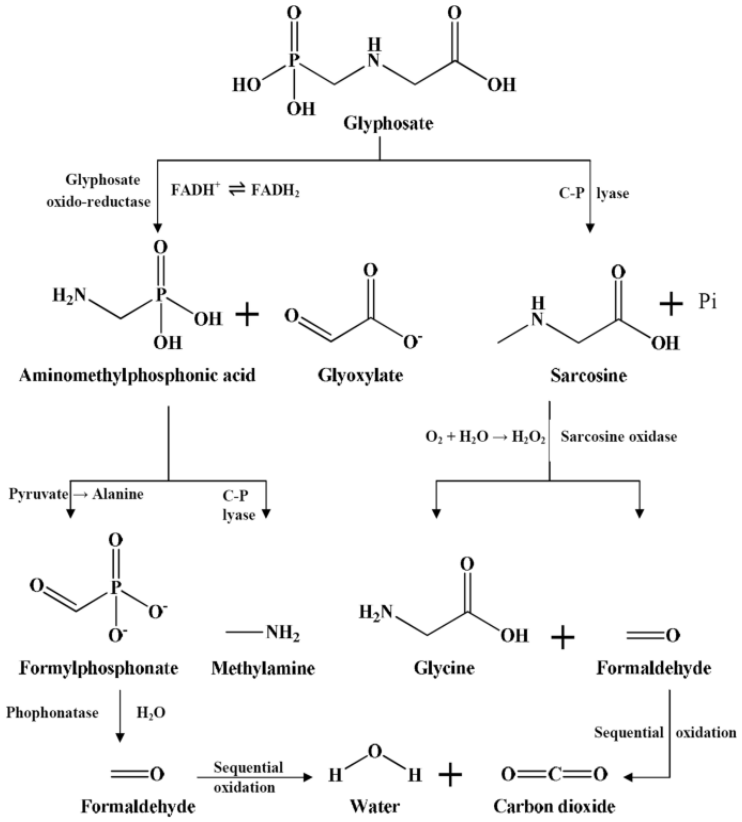

Figure 1.4 - Voies de dégradation biologique du glyphosate (Singh et al., 2020)

Le glyphosate peut être facilement dégradé par deux voies métaboliques bactériennes, à savoir la voie de l'AMPA (acide aminométhylphosphonique) et celle de la sarcosine (Figure 1.4). Cela est possible grâce à 1) une oxydase, la glyphosate oxydoréductase (GOX), qui rompt la liaison carboxyméthylène-azote (C-N) du glyphosate et le convertit en quantités

stœchiométriques d'AMPA et de glyoxylate et/ou 2) l'action de la C-P lyase, qui clive la liaison carbone-phosphore (C-P) pour produire de la sarcosine et du phosphate. Les deux voies de dégradation utilisent la C-P lyase pour cliver la liaison C-P de l'AMPA lorsque celui-ci est produit (Feng et al., 2020; Zhan et al. 2018)

L'AMPA est le métabolite principal et majeur de la voie de dégradation du glyphosate. En raison de son adsorption importante sur les particules de sol, il a tendance à y persister pendant plusieurs mois et devient une source secondaire de contamination dans l'environnement (Silva et al., 2018a). L'AMPA est clivé pour produire du phosphate inorganique et de la méthylamine, qui est ultimement minéralisée en CO_2 et NH_3. La dégradation du glyphosate et la production d'AMPA lors du compostage n'ont été étudiées que partiellement (Lashermes et al., 2010; Lashermes et al., 2012). Les résultats publiés suite à ces études ont montré une faible minéralisation du glyphosate tandis que la production et/ou la dégradation de l'AMPA n'a pas été quantifiées. Ces travaux n'ont pas non plus mesuré l'effet du glyphosate sur le processus de compostage et l'évolution des propriétés physico-chimiques de la matière en décomposition. Plusieurs questions demeurent quant aux risques potentiels liés à la présence d'AMPA dans le compost mature et à l'impact que le glyphosate peut avoir sur la minéralisation du carbone et de l'azote et sur les communautés bactériennes responsables de la décomposition de la MO.

1.2.4 Les bactéries, maîtres du compost

Les bactéries sont les principales agentes du processus de compostage. Elles sont nombreuses, variées et orchestrent la décomposition de la MO dans un environnement artificiel unique. Au fur et à mesure que la matière se décompose et que les températures varient, différentes espèces prédominent et se succèdent, chacune étant adaptée à un environnement particulier (Gajalakshmi & Abbasi 2008; Ryckeboer et al. 2003; Satyanarayana et al., 2013). Le type de bactéries trouvées lors de la dégradation de la MO varie en fonction de plusieurs paramètres. Elles peuvent être plus ou moins abondantes selon les matières premières utilisées, la procédure de compostage employée, le niveau d'aération du compost, la disponibilité des nutriments, le niveau de maturation de la MO,

etc. (Antunes et al., 2016; Nakasaki et al., 1993; Neher et al., 2013; Wang et al., 2016). Néanmoins, certains groupes de microorganismes sont présents de manière relativement constante, car ils sont associés à des rôles fondamentaux qui interviennent dans la dégradation de la MO. Certaines fonctions telles que la conversion de la cellulose, des hémicelluloses et de la lignine ainsi que la transformation de l'azote, la production de méthane et la dégradation des contaminants organiques sont souvent assurées par des groupes spécifiques de microorganismes.

1.2.4.1 Le cycle du carbone

Les microorganismes capables de dégrader la cellulose produisent une série d'enzymes aux fonctionnalités distinctes, qui agissent en synergie. Les endoglucanases hydrolysent les liaisons internes, créant de nouvelles extrémités sur la chaîne de glucanes, les cellobiohydrolases, pour leur part, agissent sur les extrémités de la chaîne, libérant des molécules de cellobiose, et enfin, les β-glucosidases fragmentent le cellobiose, produisant deux molécules de glucose (Béguin & Aubert, 1994). Le xylane étant le sucre le plus abondant dans les hémicelluloses, les enzymes responsables de sa dégradation sont souvent nommées xylanases et sont très variées (Saha, 2003). Les produits de l'hydrolyse de la cellulose et des hémicelluloses sont disponibles comme sources de carbone et d'énergie pour les microorganismes vivant dans l'environnement où ils sont dégradés. Les actinobactéries (*Thermobifid*a sp., *Thermomonospora* sp. et *Thermobispora* sp.) se trouvent souvent en abondance lorsque l'oxygène est disponible, tandis que les Firmicutes (*Clostridium* et *Bacillus*) tolèrent mieux les conditions anaérobiques (Pérez et al., 2002). Comme la température optimale pour l'action des cellulases et des xylanases se situe entre 40 et 80 °C, la dégradation des glucides pendant la phase thermophile du compost est omniprésente (Satyanarayana et al., 2013).

La dégradation de la lignine diffère de celle des glucides par la nature des réactions, qui sont principalement oxydatives plutôt qu'hydrolytiques. La lignine est une molécule complexe, insoluble et de poids moléculaire élevé qui est souvent considéré comme le principal élément récalcitrant de la paroi cellulaire. Bien que la dégradation de la lignine soit souvent

considérée comme étant principalement effectuée par les champignons de la pourriture blanche (Tuomela et al., 2000), le nombre de bactéries connues pour dégrader la lignine est en constante augmentation (Brown & Chang, 2014; Bugg et al., 2011). Les laccases et peroxydases bactériennes sont principalement produites par des membres des phylums Actinobactéries, α- et γ-Protéobactéries (Janusz et al., 2017). Comme pour la dégradation des glucides, les températures élevées favorisent la dégradation de la lignine (Tuomela et al., 2000). Au terme de l'hydrolyse des hydrates de carbone complexes et de la lignine en sucres et molécules simples, une partie du carbone libéré se trouvera immobilisée dans la biomasse microbienne, l'une sera entièrement minéralisée et relâchée sous forme de CO_2 ou CH_4 selon les conditions du milieu, tandis que le carbone restant sera stabilisé pour former des molécules stables s'apparentant à l'humus (Haug, 1993).

1.2.4.2 Le cycle de l'azote

La présence d'azote sous sa forme organique et inorganique est essentielle pour le maintien des processus biologiques lors du compostage. La majeure partie de l'azote retrouvé dans un mélange de MO fraiche est de type organique (N_{org}) et provient des protéines et des peptides simples contenus dans la MO (01.5) (Haug, 1993). Lors du processus de compostage, l'azote organique est dégradé en ammonium par une grande variété de microorganismes, notamment des bactéries et des champignons (*Aspergillus* sp., *Penicillium* sp., *Bacillus* sp., *Pseudomonas* sp., *Clostridium* sp. etc.) (Tiquia et al., 2002). L'azote atmosphérique peut également être transformé en ammonium par les bactéries du genre *Azotobacter*. La concentration la plus élevée de NH_4 est produite pendant la phase thermophile où la dégradation de la MO et la demande en oxygène sont à leur maximum (de Bertoldi et al., 1983). Le pH est habituellement supérieur à 7.5 et la nitrification ne se produit que rarement, car les températures élevées inhibent l'action des microorganismes responsables de la nitrification (Tiquia et al., 2002). L'ammonium ainsi formé subit ensuite différentes transformations en fonction de l'état du mélange en cours de compostage. Par exemple, il peut être dissout puis immobilisé par les microorganismes du mélange, qui l'utilisent comme source d'azote et le transforment à nouveau en azote organique.

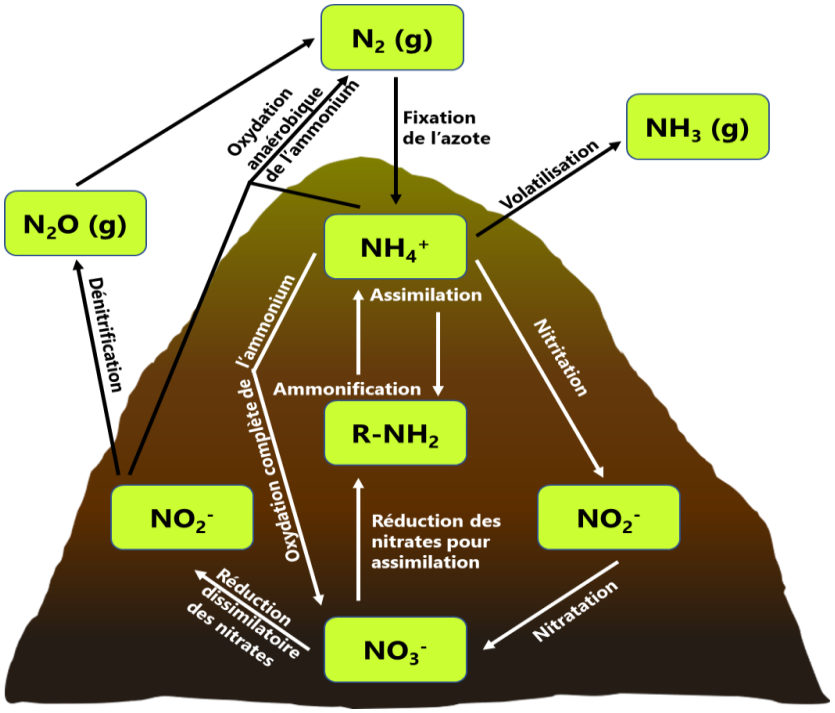

Figure 1.5 - Cycle de l'azote lors du compostage

Alternativement, la nitrification, détectée par la formation de NO_3^-, se produit lorsque la température descend en dessous des valeurs thermophiles (40 °C), l'intensité du processus dépendant de la quantité de NH_4^+ disponible pour les bactéries nitrifiantes (Bernal et al., 1996; Sánchez-Monedero et al., 2001; Tiquia et al., 2002) . Cette réaction est divisée en deux étapes d'oxydation, soit la nitritation qui transforme le NH_4^+ en NO_2^- (bactéries oxydantes de l'ammonium (AOB) : *Nitrosomonas* sp., *Nitrosospira* sp., *Nitrosococcus* sp., *Nitrosovibrio* sp. etc.) et la nitratation (bactéries oxydantes des nitrites (NOB) : *Nitrospira* sp., *Nitrobacter* sp. etc.) qui convertit le NO_2^- en NO_3^- (Fukumoto & Inubushi, 2009; Fukumoto et al., 2003; Maeda et al., 2011). La nitrification se produit principalement durant la maturation, conduisant à un faible rapport NH_4^+/NO_3^- dans le compost mature (Bernal et al., 2009).

Il arrive que certaines formes d'azote inorganique soient perdues lors du processus de compostage. Ces pertes ont un impact négatif sur le compostage, car elles diminuent la concentration en nutriments et donc la qualité du compost et génèrent des problèmes environnementaux et de santé humaine (Maeda et al., 2011). Les pertes d'azote peuvent se

produire par volatilisation (NH_3), lixiviation et dénitrification (N_2O ou N_2). La formation de microsites anaérobiques cause un manque d'oxygène et peut amener les organismes (*Pseudomonas* sp.*, Clostridium* sp., *Bacillus* sp., *Flavobacterium* sp. etc.) à utiliser le nitrate comme source d'oxygène, ce qui entraîne la dénitrification et l'arrêt de la nitrification (Parkinson et al., 2004). Parallèlement, l'azote peut se volatiliser sous forme d'ammoniac et se dégager lorsque le mélange est à haute température avec un pH supérieur à 7.5. Les pertes par lessivage peuvent être facilement réduites en contrôlant la teneur en humidité de la masse compostée. Les pertes en NH_4^+ peuvent être particulièrement élevées au début du processus tandis le lessivage du NO_3^- survient dans la dernière phase du compostage, lorsque la nitrification est à son maximum (Parkinson et al., 2004). Le rapport entre les formes inorganiques d'azote a été utilisé comme critère d'évaluation de la maturité du compost. À la fin du processus, la concentration de nitrates devrait être supérieure à celle de l'ammonium, indiquant que le procédé a été préparé dans des conditions d'aération adéquates. Un niveau élevé de NH_4^+ indique un compost non stabilisé. Un rapport NH_4/NO_3 inférieur à 0,16 a été établi comme indice de maturité pour les composts de toutes origines (Bernal et al., 2009). En général, la concentration totale de N augmente pendant le compostage en raison de l'effet de concentration (Bernal et al., 1996).

1.2.4.3 Interaction entre méthanogène et méthanotrophes

Les organismes méthanogènes sont présents dans un large éventail d'habitats où ils sont les derniers intervenants dans la dégradation anaérobie de la MO. Ils sont largement distribués dans la nature et colonisent différents environnements en fonction de leur tolérance à différentes conditions de température, de pH et de salinité. Les marais, les rizières, les sources hydrothermales, les systèmes digestifs des animaux et les décharges sont historiquement connus pour abriter ces organismes (Conrad, 2007; Ferry, 1993), mais lorsque les conditions optimales sont mises en place pour leur croissance, les sites de compostage peuvent être un habitat idéal pour les méthanogènes (Chen et al. 2014; Fukumoto et al. 2003; Jäckel et al., 2005; Kim et al. 2018; Thummes et al., 2007).

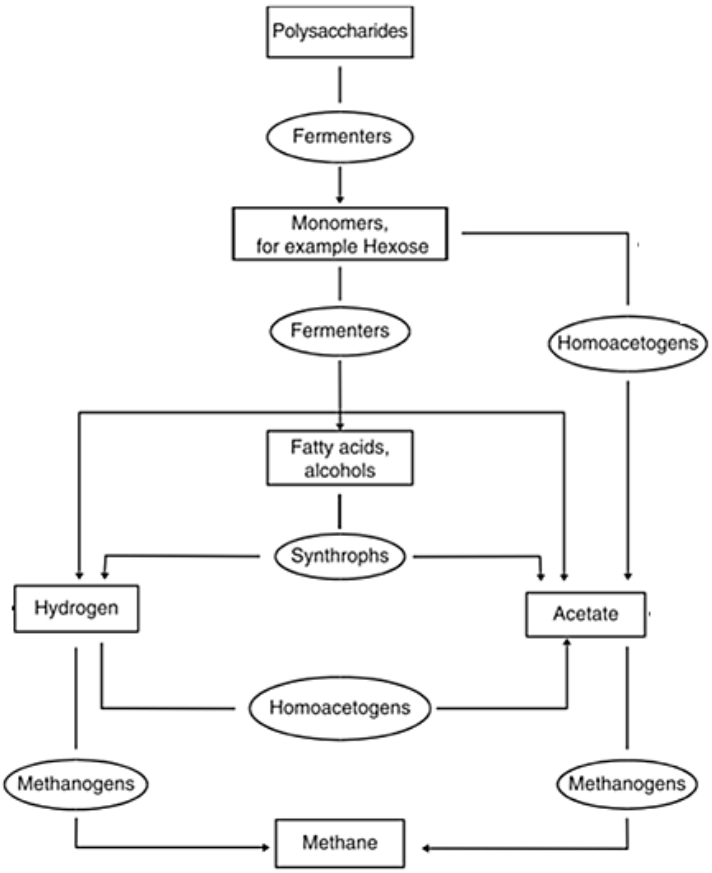

Figure 1.6 - Voie de dégradation anaérobie de la matière organique (polysaccharides) en méthane. Les intermédiaires sont représentés dans les boîtes, les microorganismes dans les ovales (Conrad, 2007).

Les microorganismes méthanogènes sont tous strictement anaérobiques, et appartiennent au phylum Euryarchaeota du domaine Archaea (Ferry, 1993). Ils sont caractérisés par leur production de CH_4 à partir de substrats simples tels que l'hydrogène (H_2), le monoxyde de carbone (CO), le formate, et certains alcools (isopropanol, éthanol), qui sont fournis par des microorganismes fermenteurs et homoacétogènes (Figure 1.6) (Garcia, 1998). Il existe deux grands groupes de méthanogènes ; les méthanogènes acétotrophes et les méthanogènes hydrogénotrophes (Conrad, 2007; Costa & Leigh, 2014). Les membres de deux genres de méthanogènes seulement sont capables de cataboliser l'acétate, à savoir *Methanosarcina* et *Methanosaeta*, qui appartiennent à l'ordre des Methanosarcinales. Les méthanogènes hydrogénotrophes convertissent le CO_2 avec H_2 en CH_4. Ce type de

catabolisme se retrouve chez la plupart des taxons méthanogènes, comme les membres des Methanopyrales (c.-à-d. *Methanopyrus* sp.), Methanococcales (i.e. *Methanococcus* sp.), Methanobacteriales (c.-à-d. *Methanobacterium* sp.), Methanomicrobiales (c.-à-d. *Methanomicrobium* sp.) et Methanocellales (c.-à-d. *Methanocella* sp.) (Buan, 2018). Plusieurs genres sont également adeptes des hautes températures, tels que *Methanoculleus* et *Methanosarcina* qui ont été observés dans le compost (Thummes et al., 2007; Yamamoto et al., 2011).

Parallèlement, les bactéries méthanotrophes sont des organismes aérobiques qui se distinguent par leur capacité à utiliser le méthane comme unique source de carbone et d'énergie. Les bactéries méthylotrophes, quant à elles, peuvent utiliser plusieurs composés monocarbonés, dont le méthane, le méthanol, les amines méthylées, les halométhanes et les composés soufrés méthylés, comme sources de carbone et d'énergie et assimiler le formaldéhyde comme principale source de carbone organique (Hanson & Hanson, 1996). Les méthanotrophes sont divisés en deux groupes selon certains critères morphologiques et physiologiques, soit le type I et le type II, qui appartiennent respectivement aux gamma- et alpha-Proteobactérie, et qui sont tous dotés de l'enzyme méthane monooxygénase qui leur permet d'oxyder le méthane et de libérer de l'énergie. La liste des méthanotrophes taxonomiquement décrits comprend actuellement 15 genres, qui appartiennent aux familles Methylococcaceae (c.-à-d. *Methylomonas, Methylobacter, Methylococcus, Methylocaldum, Methylomicrobium, Methylosphaera, Methylosarcina, Methylohalobius, Methylothermus* et *Methylosoma*), Methylocystaceae (*Methylosinus* et *Methylocystis*), Beijerinckiaceae (*Methylocella* et *Methylocapsa*) et Methylacidiphilaceae (*Methylacidiphilum*) (Camp et al., 2009; Dedysh, 2009).

Quelques études ont relevé la présence de méthanotrophes dans le compost (Chen et al. 2014; Halet et al., 2006) on utilise même le compost comme recouvrement dans les certains sites d'enfouissement pour capter le méthane produit par la fermentation de la MO (Elshorbagy & Mohamed, 2000; Tanthachoon et al., 2008). La présence et l'abondance des méthanogènes et des méthanotrophes dans le compost jouent un rôle prépondérant dans

la libération et la captation des GES et nécessitent une attention particulière pour minimiser les impacts négatifs du compostage.

1.2.4.4 Dégradation bactérienne du glyphosate

Les bactéries sont les principales responsables de la dégradation du glyphosate dans l'environnement. La transformation du glyphosate en AMPA a été largement observée dans les sols et bien que peu d'étude présente la dégradation du glyphosate dans le compost (Figure 1.4), l'activité microbienne intense et la diversité de microorganismes devrait accélérer la décomposition de l'herbicide comparativement à ce qui est observé dans les sols (Büyüksönmez et al., 1999). Quelques souches de bactéries capables de dégrader le glyphosate ont été isolées à partir d'échantillons environnementaux contaminés. La plupart des espèces signalées utilisent le glyphosate comme seule source de phosphore. Quelques exceptions utilisent le glyphosate comme source d'azote ou de carbone (Singh et al., 2020). Les souches de bactéries capables de dégrader le glyphosate comprennent notamment *Achromobacter* sp. MPK 7A, *Achromobacter* sp. SW9, *Agrobacterium radiobacter* SW9, *Alcaligenes* sp. GL, *Aminobacter aminovorans* strain KCTC 2477, *Arthrobacter atrocyaneus* ATCC 13752, *Arthrobacter* sp. GLP-1, *Bacillus cereus* CB4, *Comamonas odontotermitis P2*, *Enterobacter cloacae*, *Flavobacterium* sp. GD1, *Geobacillus caldoxylosilyticus* T20, *Lysinibacillus sphaericus*, *Ochrobactrum anthropi GPK 3*, *Ochrobactrum intermedium* Sq20, *Pseudomonas pseudomallei* 22, *Pseudomonas putida*, *Pseudomonas* sp. 4ASW, *Pseudomonas* sp. PG2982, *Pseudomonas* sp. LBr, *Sinorhizobium meliloti* et *Streptomycete* sp. (Firdous et al., 2020; Gorodylova et al., 2021; Hove-Jensen et al., 2014; Ouided & Abderrahmane, 2013; Singh et al., 2020; González-Valenzuela & Dussán, 2018.; Zhan et al., 2018).

Ochrobactrum sp. et *C. odontotermitis* possèderaient l'enzyme GOX pour dégrader le glyphosate en AMPA tandis que *S. meliloti, A. radiobacter, P. pseudomallei* utilise la C-P lyase pour produire de la sarcosine et du phosphate (Hove-Jensen et al., 2014). *Bacillus cereus* CB4, *O. anthropi* GPK 3, et *Pseudomonas* sp. LBr, possède les enzymes GOX et C-P lyase et peuvent produire de la sarcosine et de l'AMPA à partir du glyphosate (Feng et al.,

2020; Singh et al. 2020). Seulement quelques espèces ont été associées à la dégradation de l'AMPA, tel *Escherichia coli, S. meliloti*, *Arthrobacter* sp. GLP-1, *A. atrocyaneus* ATCC 13752, *Pseudomonas* sp. strains 4ASW and 7b, *Pseudomonas* sp. LBr, *Bacillus megaterium* 2BLW, et *Pseudomonas* sp. (Hove-Jensen et al., 2014; Zhan et al., 2018).

La forte majorité des études qui ont porté sur l'effet du glyphosate sur l'activité, la diversité et la succession microbienne dans le sol n'ont pas mesuré d'effet négatif permanent (Gomez et al., 2009; Imparato et al., 2016; Lane et al., 2012; Lupwayi et al., 2009; Lupwayi et al., 2004; Lupwayi & Blackshaw, 2012; Mijangos et al., 2009a; Ratcliff et al., 2006; Zabaloy et al., 2012), alors que plusieurs ont observé une augmentation de l'activité microbienne (Busse et al., 2001; Gomez et al., 2009; Haney et al., 2002; Haney et al., 2000; Lane et al., 2012; Lupwayi et al., 2004; Means et al., 2007; Mijangos et al., 2009a; Ratcliff et al., 2006) suite à l'utilisation du glyphosate comme source de carbone, d'azote ou de phosphore par les bactéries présentes. Newman et al. (2016) ont mesuré une diminution de l'occurrence des membres du phylum Acidobactérie tandis que davantage de membres de Protéobactéries étaient présents dans la rhizosphère de soya et de maïs après l'application de glyphosate. Au cours de la même expérience, les gènes reliés au métabolisme des hydrates de carbone et des acides aminés étaient moins exprimés tandis que les gènes impliqués dans la respiration et le métabolisme des protéines étaient plus exprimés (Newman et al., 2016). Ces études suggèrent que l'impact du glyphosate sur l'activité microbienne lors du compostage serait également négligeable.

1.3 Cadre conceptuel de la thèse

Cette thèse vise à mettre en lumière le rôle de la composition de la biomasse lignocellulosique et la présence de glyphosate dans les résidus organiques sur l'évolution des paramètres physicochimiques, la minéralisation des sucres structuraux et de la lignine, le cycle de l'azote et la dynamique de succession des bactéries et des archées au cours des principales phases du compostage thermophile. Cet objectif général est étudié à travers quatre différents chapitres.

Le **Chapitre 2** porte sur la transformation de la biomasse lignocellulosique lors de la mise en tas pendant une période prolongée sous des conditions hivernales. Le design expérimental est basé sur l'utilisation d'un cultivar de saule arbustif (*Salix miyabeana* 'SX67') cultivé en culture intensive sur courte rotation dont des individus ont été récoltés à deux années de croissance différentes. Les objectifs étaient ainsi d'évaluer comment, l'âge des tiges récoltées et la période d'entreposage influencent les propriétés finales de la biomasse. L'utilisation d'une matière « pure » permet de suivre adéquatement la transformation de la matière et d'associer toute différence survenant pendant la période de stockage à la composition de la biomasse ligneuse. L'hypothèse selon laquelle les tiges de différents âges n'auraient pas la même composition initiale et que les tiges plus jeunes subiraient une décomposition moins importante en raison de l'abondance de composés phénoliques ayant des propriétés antimicrobiennes dans l'écorce sera testée. La seconde hypothèse testée stipule qu'une hausse des températures et qu'une diminution des teneurs en sucres structuraux sera enregistrée pendant, et à la fin de la période d'entreposage respectivement.

Le **Chapitre 3** a pour objectif d'évaluer l'impact du glyphosate sur la décomposition de la MO lors du compostage. À partir d'une expérience menée en milieu contrôlé, où du glyphosate de grade analytique (AG) et un herbicide à base de glyphosate (GBH) ont été ajoutés à un mélange de résidus verts récolté au Jardin botanique de Montréal, l'effet sur l'évolution des propriétés physicochimiques du mélange sera investigué. La dégradation du glyphosate, la production d'AMPA et l'accumulation de ce dernier sont également au cœur des questionnements de ce chapitre. Selon les informations présentes dans la littérature, il est supposé que la présence de glyphosate ajoutée à des concentrations proches des valeurs appliquées en zone agricole ralentira la minéralisation du carbone présent dans la biomasse végétale et que cet impact sera plus important pour le GBH que pour le glyphosate de grade analytique. Il est aussi attendu que le profil de température sera modifié, avec une hausse de température moins importante. Une dégradation moyenne du glyphosate et l'accumulation d'AMPA sont attendues.

Le **Chapitre 4** a été élaboré dans le but de faire une caractérisation précise des bactéries et un suivi des variations de leur abondance ainsi que de celles des principales composantes de la biomasse et des différentes formes d'azote lors du compostage. Pour se faire, trois tas de compost correspondant à des niveaux de maturation différents ainsi que la matière première et le compost mature produit ont été échantillonnés au Jardin botanique de Montréal. Les différentes formes d'azote inorganiques ainsi que les teneurs en cellulose, hémicellulose et lignine ont été associées aux espèces des bactéries et d'archées présentes à chaque étape afin de tirer un portrait global des transformations de la MO et des espèces de bactéries potentiellement impliquées dans ces transformations. Globalement, il est attendu qu'une proportion importante de la cellulose et des hémicelluloses soit dégradée durant la phase thermophile, que la lignine soit principalement dégradée durant la phase de maturation et que la nitrification ait lieu après la phase thermophile. Parallèlement, il est supposé que plusieurs bactéries responsables de la décomposition de la cellulose et des hémicelluloses soient abondantes durant la phase thermophile, que les bactéries responsables de la dégradation de la lignine seront présentes durant la phase de maturation et que les bactéries responsables de la nitrification seront abondantes durant la phase de refroidissement et de maturation.

Finalement, le **Chapitre 5** porte sur l'impact du glyphosate sur les communautés de bactéries présentes lors du compostage. Tirée de la même expérience que celle présentée au chapitre 3, cette partie vise à évaluer si le glyphosate impacte la diversité et l'abondance des bactéries à différentes phases du compostage. L'hypothèse principale stipule que la diversité microbienne sera moins importante en présence de glyphosate en raison d'un effet de sélection causé par le glyphosate. Il est également attendu que les bactéries présentes dans le compost contaminé au glyphosate de grade analytique (AG) seront partiellement différentes de celles présentes dans le compost sans glyphosate.

Cristaux de glace se formant à la surface d'un tas de copeaux de saule

Chapitre 2 - Impact de l'âge des tiges et de l'entreposage hivernal à ciel ouvert sur la transformation des copeaux de saule

Impact of stem age and open-air winter storage on the transformation of willow chips

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Keywords: Willow, wood chips, winter storage, biomass composition, self-heating, degradation

Submitted in Biomass and Bioenergy,

JBAB-D-21-00999

Abstract

Willow (*Salix*) biomass production has been expanding dramatically in recent years with the development of phytoremediation and wastewater treatment projects and plans to build bio-refineries are increasing the future options for valorizing this biomass. In this context, winter storage of biomass after harvesting is unavoidable, due to limited shelter capacity. Questions about the impact of outdoor storage during winter and stem age at harvest time led to the establishment of an experiment on the site of Ramo®, a biomass-based products producer located in Quebec, Canada. Two- and three-year-old *Salix miyabeana* 'SX67' stems were chipped and piled after leaf fall in November 2018. Temperature was measured during the experiment, while physicochemical parameters and biomass composition were analyzed at the beginning and the end. The experiment showed that stem age influenced the initial biomass composition, with the two-year-old stems containing more extractives and the three-year-old stems containing more structural sugars. Heat production varied with stem age, which was reflected in the final biomass composition. A loss of extractives and an increase in lignin was measured in both stem types, while an increase in glucan, xylan and mannan was also measured in the two-year-old chips. This new information complements existing data on mass loss during wood chip storage and provides essential knowledge for future decision-making related to the development of lignocellulose value chains from bioenergy crops.

2.1 Introduction

Initially developed for bioenergy, fast-growing shrub willows (*Salix sp.*) are produced in an intensive short rotation coppice (SRC) system optimized to maximize yield. Harvesting is generally done every 2, 3 or 4 years and can be carried out over a 25-year period (Karp, 2014). Decisions about optimal harvest intervals are strongly influenced by considerations related to the type of machinery available and the operation cost (Gouker et al., 2021). Older and larger stems may require specialized, expensive machinery, while younger stems can often be collected with a smaller, less expensive harvester. Although the yield of a single

harvest of three-year-old stems is greater than that of two-year-old stems (Kopp et al., 1997; Szczukowski et al., 2002), the difference tends to be negligible when the yield is calculated over several harvesting cycles (Gouker et al., 2021). Finally, the biomass composition of these fast-growing species changes substantially between years of growth, with proportions of cellulose and hemicellulose tending to increase with age, while lignin generally declines (Gouker et al., 2021; Szczukowski et al., 2002).

The range of applications for willow biomass has diversified with newly acquired insights into its particular properties, such as a high proportion of accessible cellulose (Karp, 2014). The cultivar 'SX67', of the commercial Asian genotype *Salix miyabeana* has been among the cultivars with the highest yield for the last fifteen years (Grenier et al., 2015; Labrecque & Teodorescu, 2005; Tharakan & Volk, 2005; Volk et al., 2011). Local producers use this genotype to produce ramial chipped wood, mulch, living fences and noise barriers. It is also used in various phytoremediation, wastewater treatment and riparian buffer strip projects (Brereton et al., 2020; Faubert et al., 2021; Grenier et al., 2015; Hénault-Ethier et al., 2017; Pitre et al., 2010; Jerbi et al., 2020) that aim in part to repurpose the biomass produced on these marginal lands. For example, wastewater treatment projects using irrigated willow plantations have produced a considerable amount of biomass yield that can be recovered as required (Jerbi et al., 2020; Sas et al., 2021). Willow biomass also has current and projected potential for valorization in co-firing (Šyc et al., 2012), second-generation biofuels, phenolics extraction (Brereton et al., 2017) and bulk chemical production (Krzyżaniak et al., 2014).

Since the species's harvest time can range from fall (Jirjis, 2005) to spring (Whittaker et al., 2016), a certain proportion of the harvested biomass must be stored in order to have year-round access to the material. Storage also commonly serves as a technique to reduce the moisture content of wood chips following harvest (Garstang et al., 2002; Pettersson & Nordfjell, 2007). Heaping the organic material often provides the conditions necessary for biological activity to develop, as observed in composting (Haug, 1993). When adequate moisture content and soluble carbon is present, heat will build up if the pile is sufficiently large. Factors such as pile height and shape, chip size, level of compaction, and surrounding

climatic conditions also influence wood chip degradation (Bedane et al., 2011; Jirjis, 2005; Pettersson & Nordfjell, 2007; Whittaker et al., 2016). Wood chips stored in heaps can undergo a number of modifications as a result of three distinct mechanisms, namely living cell respiration, biological degradation, and thermo-chemical oxidative reactions (Krigstin & Wetzel, 2016). These mechanisms, which can occur in succession depending on the storage conditions, modify the wood chips in different ways. The most commonly reported changes are a loss of dry mass, a decrease in moisture content, or change in chemical composition (Bedane et al., 2011; Garstang et al., 2002; Jirjis, 2005; Muklada et al., 2021; Pettersson & Nordfjell, 2007; Whittaker et al., 2016).

In North America, willow wood chips are often stored outdoors between November and April and are thus subjected to winter conditions. These particular environmental conditions may influence the changes that the biomass can undergo during a typical storage in southern Quebec. This study was designed to address questions about the combined effects of stem age at harvest and storage of freshly ground willow chips through an experiment conducted in an open-air pile over a typical overwintering storage period.

2.2 Materials and Method

2.2.1 Study site and experimental set-up

The experiment was conducted at Saint-Roch-de-l'Achigan, QC, Canada (45°48'56.5 N - 73°39'08.8 W) on the property of the agricultural company Ramo®, a willow grower. The equivalent of 18 m^3 (around 5.6 Mg) of two- and three-year-old stems of *Salix miyabeana* 'SX67' were harvested and chipped on November 14, 2018, after leaf fall. The mean biomass fresh weight per tree was 5.9 ± 0.4 kg for the three-year-old and 4.2 ± 0.3 kg for the two-year-old trees. The willow wood chips were arranged directly on the ground. One pile contained wood chips from two-year-old stems, the other wood chips from three-year-old stems. Each pile was about 1.5-2.0 m high, covering a total area of about 3m x 3m. The experiment ended on May 14, 2019, for a total duration of 6 months of overwintering. The

average temperature at the study site between November and May is -3.5 °C, with snow accumulation reaching 170 cm on average (Environment Canada, historical data).

2.2.2 Sampling

Four samples were collected from each pile at the beginning of the experiment and at the end of the six-month storage period. A subsample was used for moisture content measurement and subsequent analysis (carbon, total nitrogen, and pH). The remaining samples were stored at -20 °C for NH_3/NH_4^+ and NO_2^--NO_3^- analyses. Four net bags (23 cm x 23 cm) filled with about 500g of the wood chips were inserted at the center of each pile, at about 50 cm from the ground. Three of the four bags contained a temperature recorder (iButton® Type G, ALPHA MACH, Ste-Julie, Canada) that recorded the pile internal temperature at 4-hour intervals.

2.2.3 Wood composition analysis

Prior to compositional analysis, 1.5 g of ODW milled and sieved biomass was doubly extracted with water and ethanol according to the NREL protocol (Sluiter et al., 2008), using a Dionex® Accelerated Solvent Extractor (ASE200) (the biomass in 11 ml cell size at 100 ° C under a pressure of 100 bar during 3 static cycles of 7 min for each extraction). The extracted biomass was then analyzed for structural carbohydrates and lignin in accordance with (Sluiter et al., 2012). All sugars were quantified using a high-performance liquid chromatography 'HPLC' system (Shimadzu Corporation, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column and refractive index detector. The HPLC data was corrected for the standard anhydro i.e. the contribution of water to the molecule weight of sugars between the monomer and the polysaccharide form (Serapiglia et al., 2009).

2.2.4 Physicochemical analysis

The moisture content was determined by drying at 105 °C for 24 h. The pH was analyzed in a 1:10 (w/v) water extract of oven-dried samples. Total carbon (C), nitrogen (N) and C:N ratio were determined on oven-dried samples by dry combustion at 950 °C using a vario MICRO cube analyzer (Elementar, Langenselbold, Germany). Total mineral nitrogen

(NH_3/NH_4^+ and NO_2^-/NO_3^-) was extracted with 2.0M KCl (Carter & Gregorich, 2006) and measured by colorimetry on a QuikChem® 8500 Series 2 FIA System (Lachat Instruments, Milwaukee, WI) according to the method 12-107-06-1B (NH_3/NH_4^+), 12-107-04-1-B (NO_2^-/NO_3^-).

2.2.5 Statistical analysis

All statistical analyses for the physicochemical and biomass composition measurements were carried out in GraphPad Prism 8.4.3. A 2-way ANOVA with Tukey's multiple comparisons *post hoc* test was performed between initial (2-YO-I vs. 3-YO-I) and final (2-YO-F vs. 3-YO-F) 2-year-old and 3-year-old wood chips and between the initial and final measurements of each pile (2-YO-I vs. 2-YO-F and 3-YO-I vs. 3-YO-F). A multiple t-test analysis was used to assess the presence of significant temperature difference between the two piles. Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%. Each row was analyzed individually, without assuming a consistent SD. For every analysis, $p < 0.05$ was considered statistically different, denoted graphically as * $p < 0.05 > 0.01$, ** $p < 0.01 > 0.001$, *** $p < 0.001 > 0.0001$.

2.3 Results

2.3.1 Temperature profile

The overall mean temperature in the center of the two-year-old pile was significantly higher ($p < 0.001$), with a value of 25.9 °C ± 17.2, while the mean temperature inside the three-year-old pile was 18.2 °C ± 13.6 (02.1). The highest temperature was recorded in the two-year-old wood chip pile (average of 55.8 °C on December 17). The mean temperature was significantly higher in the two-year-old pile at the beginning of the experiment (-3.83 vs -5.12, **), after 4 weeks (45.17 vs. 8.83, ****), 6 weeks (47.00 vs. 20.33, ***), 8 weeks (45.33 vs. 24.00, ***), 20 weeks (9.17 vs. 3.17, ****), 22 weeks (8.83 vs. 1.00, ****) and 24 weeks (15.50 vs. 9.50, **). The outdoor temperature on the harvest day (November 14) ranged between 13.3 °C and -6.9 °C (Environment Canada, historical data).

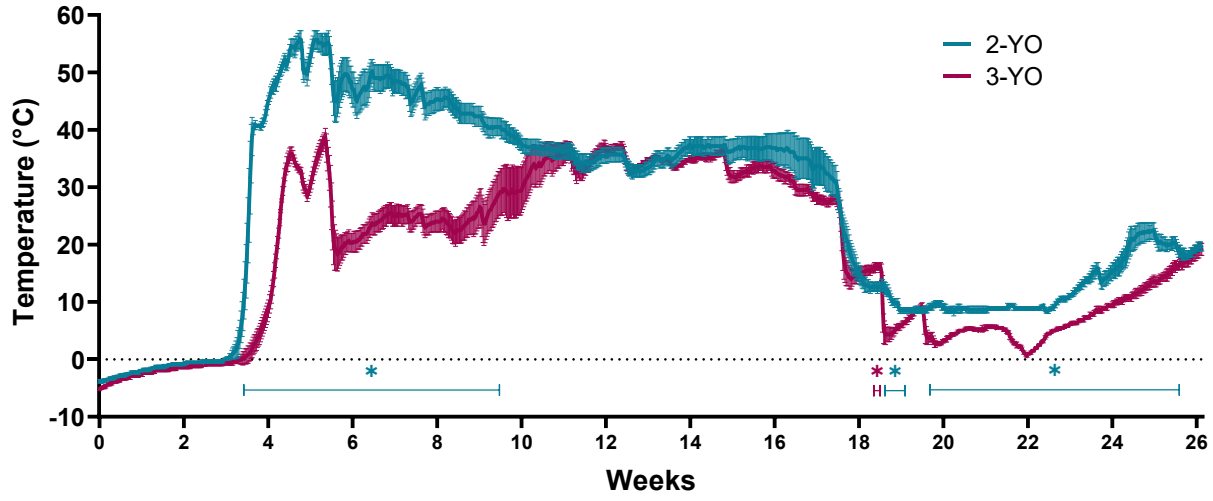

Figure 2.1 - Temperature profile measured at 4-hour intervals between November 14, 2018, and May 14, 2019, in the two-year-old (2 YO) and three-year-old (3 YO) 'SX67' willow chip piles. The stars indicate the periods when the temperature difference between the piles was significant. The colour of the stars corresponds to the different piles and indicates which pile was the hottest during the given period.

2.3.2 Biomass composition analysis

The proportions of structural sugars (glucan, xylan, galactan, arabinan and mannan), lignin (acid soluble and acid insoluble lignin) and extractives were measured in the two wood chip piles, at the beginning and at the end of the experiment. The proportion of glucan was higher in 3-YO-I compared to 2-YO-I, with amounts reaching 39.9 % ± 0.31 and 35.2 % ± 1.68 respectively (Figure 2.2 A). An increase in glucan was measured between 2-YO-I and 2-YO-F, rising from 35.2 % ± 1.7 to 39.0 % ± 1.5, while glucan content in the 3-year-old pile was stable, with 3-YO-I at 39.9 % ± 0.3 and 3-YO-F at 36.8 % ± 2.1. Xylan levels were also higher in the 2-YO-F compared to 2-YO-I, with values of 12.8 % ± 0.86 and 11.3 % ± 0.43 respectively, while no difference between the two piles and between 3-YO-I and 3-YO-F was observed, as they had respective values of 12.6 ± 0.3 and 12.6 ± 0.4 (Figure 2.2 B). Mannan level was higher in 3-YO-I compared to 2-YO-I, with values of 1.51 % ± 0.1 and 1.08 % ± 0.1 respectively, while it increased between 2-YO-I and 2-YO-F to reach 1.53 % ± 0.11 (Figure 2.2 C). No change in galactan or arabinan content was observed, with overall values rising only from 1.22 to 1.49 and from 0.57 to 0.84 respectively (Figure 2.2 D, E). The total sugar content was significantly higher in 2-YO-F compared to 2-YO-I, with values of 55.4 % ± 2.4

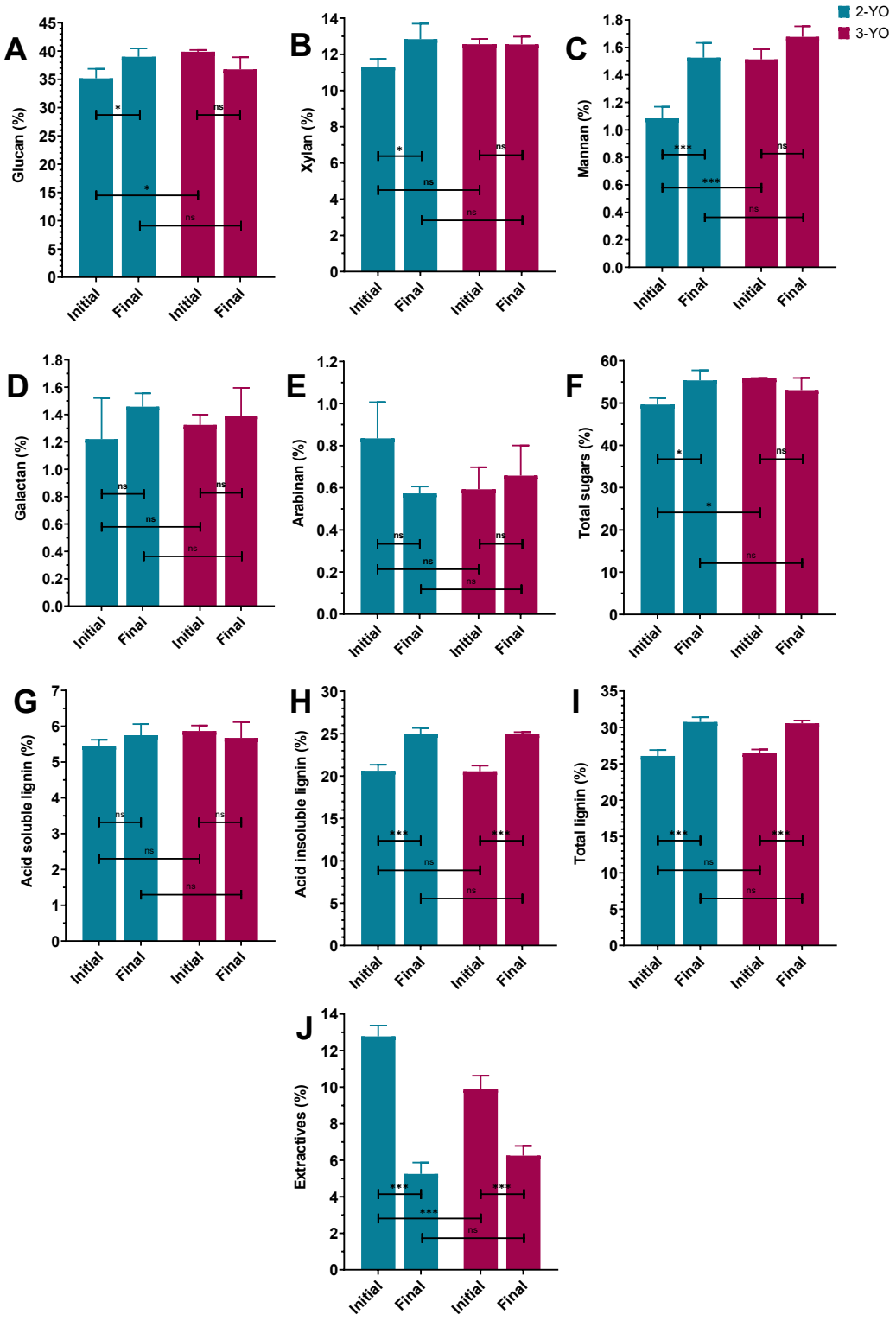

Figure 2.2 - Biomass composition measured (n = 4) in the 2-year-old (2 YO) and 3-year-old (3 YO) piles, at the beginning (Initial) and at the end of the storage period (Final). Significant differences are identified with stars (* P < 0.05 > 0.01, ** P < 0.01 > 0.001, *** P < 0.001 > 0.0001)

and 49.7 % ± 1.5 respectively, and in 3-YO-I compared to 2-YO-I, with values of 55.9 % ± 0.1 and 49.7 % ± 1.6 respectively (Figure 2.2 F). No differences were observed in the biomass composition at the end of the storage period between the two wood chip piles.

No change was observed in acid soluble lignin (ASL) content. Measured values were of 5.45 % ± 0.2 for 2-YO-I, 5.75 % ± 0.3 for 2-YO-F, 5.87 % ± 0.2 for 3-YO-I and 5.68 % ± 0.4 in 3-YO-F (Figure 2.2G). Acid insoluble lignin (AIL) and total lignin content were higher at the end of the experiment (Figure 2.2 H, I). AIL contents increased from 20.6 % ± 0.7 in the 2-YO-I to 25.0 % ± 0.7 in 2-YO-F and from 20.6 % ± 0.7 in 3-YO-I to 24.9 ± 0.3 in 3-YO-F. Total lignin values increased from 26.1 % ± 0.8 in 2-YO-I to 30.8 % ± 0.6 in 2-YO-F and from 26.4 % ± 0.5 in 3-YO-I to 30.6 % ± 0.4 in 3-YO-F. Finally, the proportion of extractives was higher in 2-YO-I compared to 3-YO-I with values of 12.8 % ± 0.6 and 9.9 ± 0.72 respectively (Figure 2.2 J). A significant decrease was measured in both piles between the beginning and the end of the experiment, while 2-YO-F dropped to 5.25 ± 0.62 and 3-YO-F reached 6.25 ± 0.53.

2.3.3 Physicochemical measurement

Changes in physicochemical properties were observed following storage of the wood chips. Notably, moisture content was significantly higher in 2-YO-F and 3-YO-F, with values of 61.3 % ± 2.38 and 57.9 % ± 2.42 respectively, compared to 2-YO-I and 3-YO-I, which had values of 48.2 % ± 0.1 and 47.9 % ± 0.3 respectively. No difference was measured between 2-YO-I and 3-YO-I or between 2-YO-F and 3-YO-F (Figure 2.3 A). The pH was also significantly higher in 2-YO-F and 3-YO-F, with values of 7.73 ± 0.08 and 5.93 ± 0.16 respectively, compared to 2-YO-I and 3-YO-I, which had values of 5.1 ± 0.1 and 4.96 ± 0.02 respectively (Figure 2.3B). The pH was also higher in the 2-YO-F compared to the 3-YO-F. A significant decrease in NH_3/NH_4^+ was measured between the 2-YO-I and the 2-YO-F, as well as between the 3-YO-I and the 3-YO-F, with initial values of 30.3 µg N/g ± 7.0 and 19.7 µg N/g ± 7.6 respectively, and final values below the detection limit of the instrument (LOD > 0.35 µg N/g) (Figure 2.3 C). All recorded values for the NO_2^-/NO_3^- analysis were below the detection limit of the instrument (LOD > 0.05 µg N/g) and therefore do not appear on

Figure 2.2. Total nitrogen content was significantly higher in 2-YO-F, with a recorded value of 0.75 % ± 0.04, compared to 3-YO-F, which had a total nitrogen content of 0.50 % ± 0.05, while no difference was observed between 2-YO-I and 3-YO-I, as the values reached 0.579 ± 0.07 and 0.516 ± 0.05 respectively (Figure 2.3 E). No difference was observed between the total nitrogen values measured in 2-YO-I and 2-YO-F, or between 3-YO-I and 3-YO-F. There was no significant difference in the total carbon content or the C:N between the two piles at either sampling time, with values ranging from 45.2 to 46.3 for total carbon, and from 62.1 and 94.4 for the C:N (Figure 2.3 D, F).

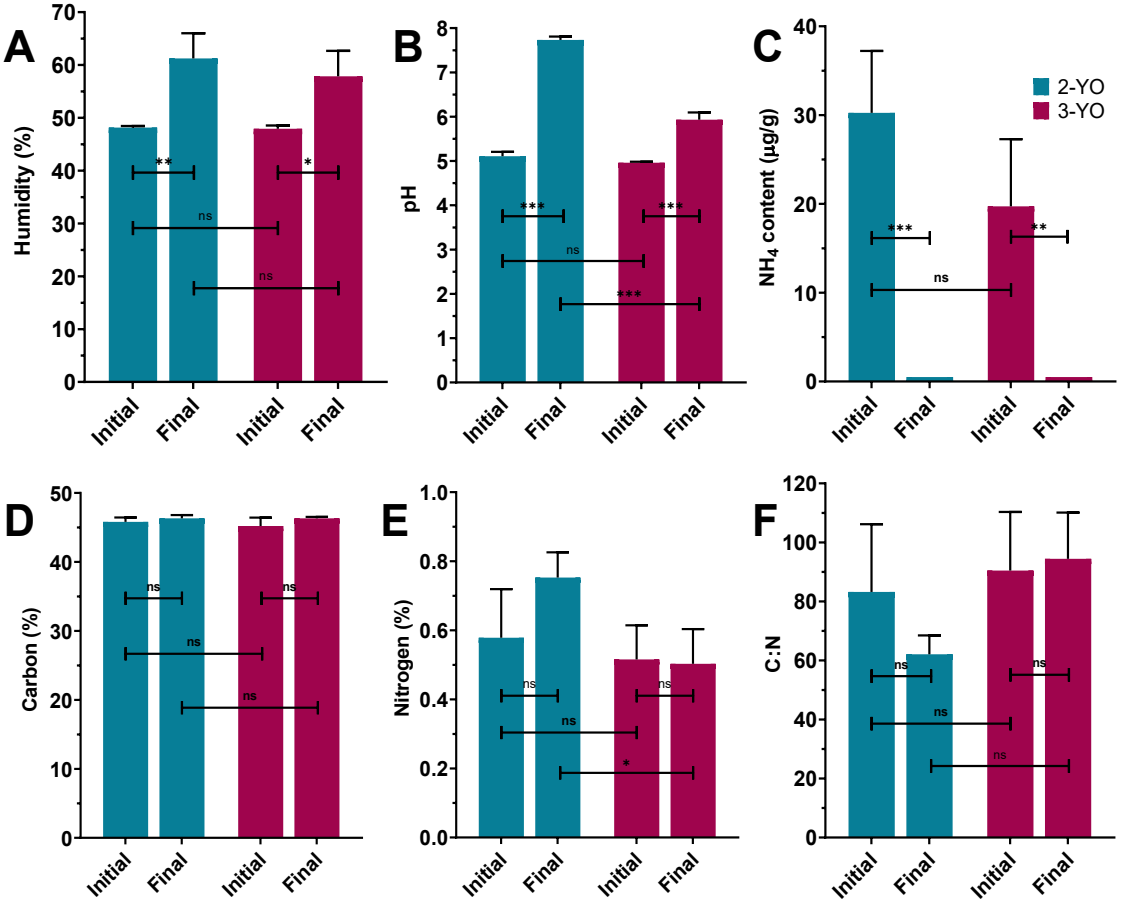

Figure 2.3 Physicochemical parameters measured (n = 4) in the 2-year-old (2 YO) and 3-year-old (3 YO) piles, at the beginning (Initial) and at the end of the storage period (Final). Significant differences are identified with stars (* P < 0.05 > 0.01, ** P < 0.01 > 0.001, *** P < 0.001 > 0.0001)

2.4 Discussion

While outdoor winter storage subjects wood chips to freezing ambient temperatures, it does not appear to interfere with heat production. The reduced air flow caused by the snow cover on the heaps and the insulation effect may have reduced air convection and dissipation, thus allowing temperatures to remain largely higher than ambient values. In general, the storage period had a more significant effect on post experiment willow chip composition than stem age.

2.4.1 Initial biomass composition and self-heating during storage depends on stem age

The physicochemical parameters were comparable between the two- and three-year-old 'SX67' stems used in the experiment (Figure 2.1), while compositional differences in the proportion of different structural sugars, extractives and lignin were observed (Figure 2.3). A higher proportion of total extractives compounds was measured in the two-year-old stems ($p < 0.001$), whereas the three-year-old stems contained more glucan ($p = 0.014$), mannan ($p < 0.001$) and total sugars ($p = 0.012$).

The extractives measured in this experiment are derived from a double extraction with water and ethanol, which allows the removal of inorganic material, non-structural sugars, nitrogenous material, chlorophyll, waxes, and other minor components (Sluiter et al., 2008). The elements mentioned are notably present in large quantities in the young twigs and bark to meet the need for growth. Since the bark to wood ratio is greater in the stems with a smaller diameter, it is expected that more extractives compounds would be found in the two-year-old stems. Willow bark is also rich in terpenoids, condensed tannins, and phenolic compounds, although they have not been measured in this study (Adler et al., 2005; Serapiglia et al., 2009; Tahvanainen et al., 1985; Whittaker et al., 2016).

Glucan and mannan are mainly present in cellulose (Synytsya & Novak, 2014) and hemicelluloses (Moreira & Filho, 2008) respectively. Their role is mainly structural, as they are found in the cell walls of woody species. Cell wall thickening, a process often associated

with aging (Sennerby-forsse, 1989), could explain their greater proportion in three-year-old stems, a phenomenon that was also observed by Gouker et al., (2021). The higher proportion of glucan and mannan would therefore result in a higher proportion of total sugars.

The compositional values measured in the three-year-old stems are similar to those measured by Ray et al., (2012), who used the same analytic procedure. On three-year-old stems from 35 different cultivars, these researchers obtained slightly higher values of glucan and lignin than those found in this study, while xylan, galactan, arabinan and mannan values were lower in the three-year-old stems of the cultivars measured here. Serapiglia et al., (2009) performed thermogravimetric measurements of biomass composition on two-year-old stems of the cultivar 'SX67' and obtained glucose and xylose values generally higher than those obtained in this study, while the amount of extractive and lignin were higher in our study. The high extractive values obtained in this experiment concur with the work of Brereton et al., (2017) who found high concentrations of condensed tannins and flavonols in individuals of the same cultivar grown at the same site, although stem age was not mentioned in the study.

The nearly systematic temperature increase measured in wood chip piles can be due to three distinct mechanisms, namely 1) living cell respiration, 2) biological degradation or 3) thermo-chemical oxidative reaction (Krigstin & Wetzel, 2016). Several factors such as pile height and size, particle shape and environmental conditions can influence heating, but, in our case, differences in biomass composition are potentially responsible for the observed patterns. Living cell respiration occurs in freshly cut wood chips, where the newly exposed parenchymal cells start using this oxygen for their metabolism which generate heat. The former mechanism, along with early biological degradation, is mediated by the presence of readily available soluble sugars in the cells (Ferrero et al., 2009), while physical and chemical heat production requires very high temperatures and are therefore less frequent (Everard et al., 2013).

Most of the phenol compounds found in trees are present in their glycosylated form (Julkunen-Tiitto, 1985). These glycosylated phenolic compounds are present in high abundance in willows, and are found in greater proportion after leaf fall (Boeckler et al., 2011). These compounds are abundant in willow bark and have been shown to be highly degraded in ensiled *Salix acmophylla* chips (Muklada et al., 2021). They can also serve as a source of sugars for microorganisms possessing the necessary enzymes for their de-glycosylation (Sánchez-Monedero et al., 1999). These same compounds were previously found in significant amount in the 'SX67' cultivar grown at this particular site (Brereton et al., 2017). The greater abundance of extractives compounds and bark that may contain glycosylated phenolics could thus have supported greater biological activity in the two-year-old chip pile, leading to greater heat production. Conversely, the three-year-old stems contained a higher proportion of structural sugars and a lower amount of soluble sugars, which would have reduced the accessibility to simple carbon sources needed to sustain biological activity and thus heat production. (Karp, 2014; Sennerby-forsse, 1989).

The choice of coppice age (and, indirectly, compositional differences) at harvest would thus have an impact on the self-heating process, with younger stem leading to higher temperature in wood chip storage piles, which may eventually promote greater biomass degradation.

2.4.2 Compositional and physicochemical changes after overwintering

Changes in biomass composition and physicochemical properties of the wood chips measured between the beginning and end of storage suggest that some biological activity took place during this period.

Humidity, pH and NH_3/NH_4^+ content varied significantly for both piles between the beginning and the end of the storage period. No change in total carbon content and C: N ratio was measured, while differences in total nitrogen content were observed between the samples from the two- and three-year-old piles collected at the end of the storage period (Figure 2.3). In addition, a significant decrease in extractive content was measured for both

types of wood chips, while an increase in acid insoluble lignin and total lignin was also observed. Meanwhile, an increase in glucan, xylan, mannan and total sugars was measured in the two-year-old chip pile.

Humidity most likely increased due to snow cover melting in springtime. Since moisture content has been identified as a major factor in the development of microorganisms in wood chip pile (Everard et al., 2013; Jirjis, 2005; Lin & Schmidt, 1991), the snow factor should be considered if the pile is harvested later in the spring, when outside temperatures are higher.

The pH was heavily impacted by winter storage in both wood chip piles, although the increase in pH was greater in the two-year-old pile. The different biochemical phenomena taking place during the transformation of organic matter cause variations in pH. This marked increase in pH can result from the degradation of acidic compounds and the release of hydroxyl (OH^-) molecules from the Fenton's-like reaction (Arantes et al., 2012). The significant decrease in extractives during the storage period could imply a decrease in the amount of acidic phenolic compounds, such as hydroxycinnamic, benzoic and shikimic acid (Brereton et al., 2017), which could have led to an increase in pH. Concurrently, the early stages of wood decomposition often involve the action of brown-rot fungi, which have the ability to release hydrogen peroxide. Hydroxyl radicals randomly attack the constituents of the wood cell wall, causing a non-enzymatic breakdown of the lignocellulose network with internal cleavage of the cellulose molecules and modification of the lignin (Arantes et al., 2011). The OH^- ions thus produced could have contributed to the increase in pH recorded between the beginning and end of the storage period.

Ammonium completely disappeared during winter storage, most likely consumed by microorganisms. Since inorganic forms of nitrogen were not observed at the end of the storage period, the total nitrogen measured must be present in its organic form, i.e., immobilized in microorganism cells or as a compound within the undecomposed biomass constituents. The absence of a decrease in total carbon concentrations is explained by the absence of structural carbohydrates and lignin loss. A previous study on dry mass loss

during outdoor storage of willow wood chips came to the same conclusion, after temperatures rose sharply over a long storage period, with no variation measured in total carbon content (Whittaker et al., 2016).

Cellulose and lignin are recalcitrant components of the plant cell wall and account for 61.3 % and 66.4 % of the total carbon in the two- and three-year-old wood chip respectively (Figure 2.2). Given the conditions under which the wood chips were stored, it is very likely that the consumption of these components was not initiated. Soluble and available sugars, such as starch and reducing sugars, were thus probably involved in the microbial activity and heat production. Furthermore, if brown-rot fungi did grow in large numbers in the piles, it is possible that cellulose and lignin were spared, since they have an incomplete cellulase system and do not possess the enzymes necessary to degrade lignin (Arantes et al., 2012; Martínez et al., 2011).

Cellulose, hemicelluloses and lignin were not only spared by the biological activity during storage, as their proportions even increased in some cases. This phenomenon could be explained by a concentration effect resulting from a decrease of the extractive content, which caused a loss of mass. In this case, storage had an effect similar to the pre-treatment step applied to biomass during cellulose isolation: by removing the extractive and sparing the glucans, storage acted *de facto* as a natural pretreatment. The increase in the proportions of certain structural sugars and lignin in the two-year-old stems is of particular relevance, as these components are of interest for uses including biofuel and bioenergy production. On the other hand, phenolic compounds present in willows are increasingly being investigated for a possible important role in the pharmaceutical and cosmetic fields (Brereton et al., 2017; Sas et al., 2021). It would therefore be relevant to conduct an analysis of phenolic compounds that may be lost during storage in order to guide optimization in storage techniques.

2.5 Conclusion

Mass loss is generally cited as the number one issue in the storage of lignocellulosic biomass. This study demonstrates that storage can also affect the concentrations of

structural sugars, lignin, and extractives. Woody biomass could be managed according to the components to be valorized, using storage as a form of pre-treatment, or avoiding it in the case where easily degradable extractives are targeted. Since there are different ways to convert each of these components, the storage method, and the age of the stems at harvest will have to be taken into consideration in order to maximize the valorization potential according to the needs of the industry and thus reduce the costs and increase the profits. For instance, in the case where extractives would be valorized, it would be advisable to use younger stems and to avoid storing the biomass for a long period of time. Conversely, the age of the stems grown for biofuel production would be a less important criterion, while storage could facilitate the pre-processing step.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

V. Grenier: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition; **A. Jerbi:** Formal analysis, Writing - Review & Editing; **F. E. Pitre:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

Acknowledgements and funding

Special thanks to Nicolas Pinceloup and Susan Boucher for their help with experimental setup and sampling. We are grateful to the Ramo® team for their help with harvest and pile management. Finally, thanks to Karen Grislis for her work on English revision. This research was made possible by financial support from the Fonds de recherche du Québec – Nature et technologies (FRQNT), Mitacs Acceleration program and Ramo® to the first author scholarship (PhD program).

Essais de compostage en milieu contrôlé

Chapitre 3 – Dissipation et impact du glyphosate lors du compostage de résidus organiques

Dissipation and impact of glyphosate during composting of organic wastes

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Version révisée transmise au Journal of Environmental Quality

JEQ-2021-07-0199-TR.R1

Abstract

The addition of organic matter (OM) containing glyphosate during compost production, through the introduction of contaminated plant residues or sewage sludge, presents a risk of hindering the proper OM breakdown carried out by microorganisms and causing the accumulation of glyphosate or aminomethylphosphonic acid (AMPA). In order to measure the impact of glyphosate and glyphosate-based herbicide (GBH) on OM decomposition as well as the dissipation of glyphosate to AMPA during composting, a controlled environment experiment was conducted using mesocosm scale vessels. Analytical grade (AG) glyphosate (150 mg kg^{-1}) and GBH (VisionMAX™) equivalent to the amounts applied in agricultural areas (300 mg kg^{-1}) were added to a mixture of green residues, which were then composted for 112 days. Sampling after 2, 7, 28 and 112 days showed a negligible impact of glyphosate and GBH on physicochemical properties of the mixture (temperature, OM %, pH, total carbon and nitrogen and C:N ratio), ammonification, nitrification and phosphate content. No differences between AG glyphosate and GBH treatments were measured. Glyphosate levels decreased significantly after two days, to reach 53.1 % and 71.1 % of the initial content (T0) for AG glyphosate and GBH treatments respectively, and glyphosate dissipation was almost complete or total after 112 days of composting. AMPA could not be detected at any time during the experiment, regardless of the treatment. Our results show that conditions for OM decomposition were maintained despite the addition of glyphosate and suggest that only trace amounts of glyphosate or AMPA are likely to be present in mature compost.

3.1 Introduction

It is estimated that more than 2,200 million ton of organic residues will be generated worldwide from municipal solid waste collection as of 2025 (Campuzano & González-Martínez, 2016). Composting is an integral part of the solution to reduce the negative impacts on human health and the environment resulting from the production of greenhouse gases and the pollution of soils and waterways associated with landfilling of organic matter (OM) (Alam & Ahmade, 2013; Lou & Nair, 2009; Taiwo, 2011). Composting, described as a

controlled, aerobic, biological process that achieves thermophilic conditions through the activity of microorganisms, is prescribed for a multitude of organic residues, such as municipal solid waste (MSW), manures, municipal and agricultural green waste, and sludge from wastewater treatment plants (Haug, 1993).

The diversity of the OM received by composting facilities leads to heterogeneity in the quality of the resources, which often carry a wide range of organic and inorganic contaminants (Brändli et al., 2005). Due to their extensive use, herbicides are among the organic contaminants that may be present during composting (Brändli et al., 2005; Büyüksönmez et al., 1999). As glyphosate-based herbicides (GBH) are widely applied around the world, their ubiquity in the environment raises concerns (Benbrook, 2016) since they were detected in and on various plant residues (Rampoldi et al., 2011), as well as in sewage sludge (Ghanem et al., 2007; Struger et al., 2015).

A potent herbicide, glyphosate (N-[phosphonomethyl]glycine) causes the inhibition of the 5-enolpyruvylshikimate- 3-phosphate synthase, leading to the depletion of essential aromatic amino acids required for plants, bacteria and fungi growth (Bentley, 1990; Haney et al., 2002). The effect of glyphosate and GBH on plants is very well documented (Gomes et al., 2014), but the impact on microorganisms has been much less studied. Nevertheless, it has been reported that the presence of glyphosate could cause microbial unbalance in the rhizosphere of different plants (Mijangos et al., 2009; Newman et al., 2016; Zobiole et al., 2011) and in the gut microbiome of rats, quails, bees and beetles (Gómez-Gallego et al., 2020; Mesnage et al., 2019; Motta et al., 2018; Ruuskanen et al., 2020). In addition, one of the surfactants in GBH, the polyethoxylated tallow amine (POEA), can be toxic to species of bacteria and protozoa (Tsui & Chu, 2003). Since composting is essentially a biological process driven by microorganisms, a disruption of the compost microbiome caused by glyphosate could have deleterious effects on the biogeochemical cycles of carbon, nitrogen and phosphorous and would thus alter the degradation of organic matter (Chávez-Ortiz et al., 2022; Panettieri et al., 2013; Ratcliff et al., 2006). While many studies have investigated the presence and degradation of organic contaminants during composting (Barker & Bryson, 2002; Brändli et al., 2007; Büyüksönmez et al., 2000), little information is available on the

effect these contaminants may have on the decomposition of organic matter during the process.

Glyphosate dissipation mechanisms that occur during composting are similar to those in soils, the most important one being the biological degradation, known to produce aminomethylphosphonic acid (AMPA), a noxious metabolite considered recalcitrant in soils (Büyüksönmez et al., 1999; Silva et al., 2018) and phytotoxic even for glyphosate-resistant crops (Saunders & Pezeshki, 2015; Smedbol et al., 2020). Glyphosate can be readily degraded by two bacterial metabolic pathways, namely the AMPA (aminomethylphosphonic acid) and sarcosine pathways. This is achieved through 1) an oxidase, glyphosate oxidoreductase (GOX), which breaks the carboxymethylene-nitrogen (C-N) bond of glyphosate and converts it into stoichiometric amounts of AMPA and glyoxylate and/or 2) the action of C-P lyase, which cleaves the carbon-phosphorus (C-P) bond to produce sarcosine and phosphate. Both degradation pathways use C-P lyase to cleave the C-P bond of AMPA when it is produced (Feng et al., 2020; Zhan et al., 2018).

Several authors have documented the presence of pesticides in plant residues and compost (Brändli et al., 2007; Taube et al., 2002), while some have reported herbicide dissipation during composting (Fogarty and Tuovinen 1991; Frenich et al. 2005; Kupper et al. 2008), but few have addressed the case of glyphosate. The only studies on glyphosate dissipation during composting were conducted by Lashermes and collaborators (Lashermes et al., 2010; Lashermes et al., 2012) and aimed to measure the mineralization of radioactive glyphosate by quantifying ^{14}C-CO_2 produced in small-scale laboratory tests without reporting its effect on compost properties. Therefore, a number of questions remain regarding the effect of glyphosate and GBH on OM decomposition rate, glyphosate dissipation, and the potential accumulation of AMPA during composting.

Here, a composting experiment was conducted using green waste as well as horse and sheep manure in a controlled environment to test the following hypotheses: 1) AG glyphosate and GBH (VisionMAX™) have an impact on the standard evolution of physicochemical properties during OM composting and 2) glyphosate degrade considerably, potentially leading to an accumulation of AMPA at the end of the 112-day composting period.

3.2 Materials and methods

3.2.1 Compost preparation and handling

A controlled composting experiment was performed using mesocosm-sized containers built from 19-liter cylindrical buckets into which holes were drilled for aeration and drainage. The OM used consisted of plant residues from the exhibition greenhouses and outdoor gardens of the Montreal Botanical Garden, litter from the City of Montreal Police Department's stables and sheepfold bedding from an eco-grazing experiment conducted at the Montreal Botanical Garden site in July 2019. The mixture was prepared to obtain a C:N ratio of 25-30:1 and a moisture content of approximately 60 %. A total of 4 kg of fresh OM was added to each vessel. Three experimental conditions were tested in quintuplicate (n = 5) to assess the level of glyphosate dissipation and its impact on OM decomposition: untreated Control, AG glyphosate and GBH (VisionMAX™). Analytical grade glyphosate (N-(Phosphonomethyl) glycine 96 % purity (Sigma-Aldrich Chemical Co., St. Louis, MO) was used at a final concentration of 150 mg kg^{-1} per vessel. The treatment with VisionMAX™ Silviculture Herbicide (Monsanto Canada) was applied using 540 g acid equivalent (a.e./l) applied at the recommended rate of 2.75 l/ha. The recommended field rate application was converted based on the density of the mix (250 kg/m^3) and a depth of 2 mm (Equation 1) (Poon, 2010), resulting in a content of 300 mg kg^{-1} per vessel.

$$\boldsymbol{Value\ in\ mg\ kg^{-1} = Value\ in\ \frac{kg}{ha} \div \left(1\frac{kg}{10^6}\right) \div \left(bulk\ density\ \left(\frac{kg}{m^3}\right)\right) \div (depth(m) \times \frac{10^4\ m^2}{ha})} \quad \textbf{(1)}$$

AG glyphosate and VisionMAX™ were diluted with deionized (DI) (Millipore, Billerica, MA) water to a final volume of 100 mL and were then added alternately with the organic residues before the vessels were closed and mixed thoroughly, while the Control treatment received the same amount of DI water. The vessels were wrapped in mineral wool to reduce heat diffusion and were placed on a table inside a greenhouse at the Montreal Botanical Garden. The composting vessels were mixed once a week and watered to maintain a humidity level of approximately 60 %. The experiment used a randomized complete design with 5 replicates per treatment for a total of 15 vessels analyzed.

3.2.2 Sampling and physicochemical properties

A suite of properties were tracked over the course of the experiment. Temperature was monitored using data loggers (iButton®, Alpha Mach, Canada) set to record temperature every 2 hours. The iButtons were inserted in the middle of each vessel and at 3 different places in the greenhouse for ambient temperature monitoring. Compost samples were collected from the vessels for physicochemical (OM, pH, total carbon, total nitrogen and C:N ratio, ammonia (NH_3/NH_4^+), nitrite/nitrate (NO_2^--NO_3^-) and orthophosphate (PO_4^{3-}), glyphosate and AMPA analysis immediately after loading (T0) and after mixing at 2, 7, 28 and 112 days of composting (T2, T7, T28 and T112 respectively). Each sample was subsequently split into 3 subsamples. Two compost samples were frozen at -20 °C (for NH_3/NH_4^+, NO_2^--NO_3^-, PO_4^{3-}, glyphosate and AMPA analysis) and one compost sample was dried (oven dried at 105 °C for 24 hours) for pH, OM, total carbon, total nitrogen and C:N ratio analysis. Oven-dried samples were milled at a particle size of < 2 mm before being stored in the dark until analysis. The pH was analyzed in a 1:10 (w/v) water extract of oven dried samples. Total carbon (C) and nitrogen (N) were determined on oven dried samples by dry combustion at 950 °C using the varioMICROcube analyser (Elementar, Langenselbold, Germany). OM content was assessed by determining mass loss on ignition at 600 °C to a constant weight.

Total mineral nitrogen (NH_3/NH_4^+ and NO_2^--NO_3^-) was extracted with 2.0M KCl (Carter & Gregorich, 2006) and PO_4^{3-} was extracted using Bray N°.2 solution (Wuenscher, Unterfrauner, Peticzka, & Zehetner, 2015) and measured by colorimetry on QuikChem® 8500 Series 2 FIA System (Lachat Instruments, Milwaukee, WI) using method 12-107-06-1B (NH_3/NH_4^+), 12-107-04-1-B (NO_2^--NO_3^-) and 12-115-01-1-N (PO_4^{3-}).

3.2.3 Glyphosate and AMPA measurements

Measurements of glyphosate and AMPA contents were performed. A total of 2.5 g of freeze-dried and sieved (< 2 mm) compost was placed in a 50 mL tube and extracted with 40 mL of a 0.125 M NH_4OH + 0.05 M KH_2PO_4 solution (Alferness & Iwata, 1994). The samples were then vortexed for 30 seconds, tumbled for 45 minutes at 200 rpm and then centrifuged for

20 minutes at 3500 rpm. The filtered samples (0.2 µm) were then diluted (1/10) and 40 µL was used for the samples treated with glyphosate, while 20 µL was used for samples treated with VisionMAX™. All samples were then loaded into a 1.5 mL injection vial. A solution of DL-2-amino-3-phosphonopropionic acid (APPA) at 10 µg/mL was used as an internal standard. A volume of 10 µL of this solution was added to each sample before drying under nitrogen flow (N_2). A five-point standard curve was prepared in a glyphosate-free compost matrix at concentrations of 0, 0.5, 1.0, 1.5 and 2.0 µg/L of glyphosate and 0, 1, 2, 3 and 4 µg/L of AMPA for the Control samples and at 0, 10, 20, 40 and 80 µg/L of glyphosate and 0, 20, 40, 80 and 160 µg/L of glyphosate for the AG glyphosate and GBH treatments. Dried samples were derivatized by adding 500 µl of trifluoroethanol (TFE) and 1 ml of trifluoroacetic anhydride (TFAA), as mentioned by Börjesson and Torstensson (2000), and heated at 100 °C for 1 h. Both chemicals were acquired from Sigma-Aldrich (Oakville, Canada). After returning to room temperature, the samples were evaporated to dryness under N_2 flow. The samples were dissolved in 1 mL of isopropyl acetate before 1 µL was injected.

Glyphosate and AMPA quantification were performed on a Varian GC 3800 gas chromatograph equipped with a Rxi®-5Sil MS capillary column (Restek, Pennsylvania, USA) (30 m × 0.25 mm ID, 0.25 µm). Injector and detector were held at 280 °C and 300 °C, respectively. Hydrogen was used as the carrier gas, with a column flow rate of 1.4 ml min^{-1}. Initial oven temperature was set at 70 °C and held for 1 min followed by a 1 °C min^{-1} increase up to 84 °C, a 4 °C min^{-1} increase up to 120 °C and finally up to 250 °C, at an increase of 80 °C min^{-1} and held for 7 min for a total run time of 32.63 min. Calibration curves shown good linearity for both analytes (r^2 = 0.99 and r^2 = 0.97 for glyphosate and AMPA, respectively). The detection limit (LOD) and quantification limit (LOQ) for AMPA was 0.03 µg/g and 0.09 µg/g respectively and, for glyphosate, 0.02 µg/g and 0.05 µg/g respectively.

3.2.4 Statistical analysis

All statistical analyses were carried out in GraphPad Prism 8.4.3. Results were expressed as the average of five replicates for the OM, pH, total carbon, total nitrogen and C:N analysis (n = 5) and four replicates for temperature, NH_3/NH_4^+, NO_2^--NO_3^-, PO_4^{3-} and glyphosate/AMPA analysis of the AG glyphosate and GBH treated compost (n = 4). A RM (repeated measures)

two-way analysis of variance (ANOVA) with the Geisser-Greenhouse correction was carried out to detect the effects of treatments and sampling time. Tukey's multiple comparisons test with individual variances computed for each comparison was performed as *post hoc* test ($p < 0.05$). Multiple t-test with assumed homoscedasticity and correction using the Holm-Šídák method was performed on glyphosate and AMPA content values.

3.3 Results

3.3.1 Temperature profile

The temperature inside the composting vessels (Control, AG glyphosate, and GBH) was monitored at 2-hour intervals during the entire experiment. The temperature was significantly higher in the Control than in the Glyphosate treatment at the beginning of the composting process, respectively after 4 hours and 6 hours (Figure 3.1 B). A steep temperature increase was observed in all three treatments, reaching a maximum of 57.5 °C for the Control, 55.8 °C for the AG glyphosate treatment and 56 °C for the GBH treatment after 26 hours of composting. The duration of the thermophilic phase (> 45 °C) was slightly longer for the Control, with an average total thermophilic phase duration of 56 hours, while the AG glyphosate treatment was in thermophilic condition for 48 hours and the GBH treatment for 50 hours. After 7 days of experimentation, and until its conclusion at 112 days, the temperature remained stable and no other differences in temperature were recorded between treatments (Figure 3.1 A).

3.3.2 Physicochemical properties

The different composts developed in a similar way, regardless of the treatment (Table 3.1). The complete results of the analysis of variance (Sum of square (SSq), df, Mean squares (MS) and F-values) are available in Supplementary Table S3.1. The OM decrease was significant after 112 days for all treatments, with a 13.8 % decrease from T0 for the Control (Table 3.1), a 12.0 % decrease for the AG glyphosate treatment, and a 11.9 % decrease for the GBH (Figure 3.2 A).

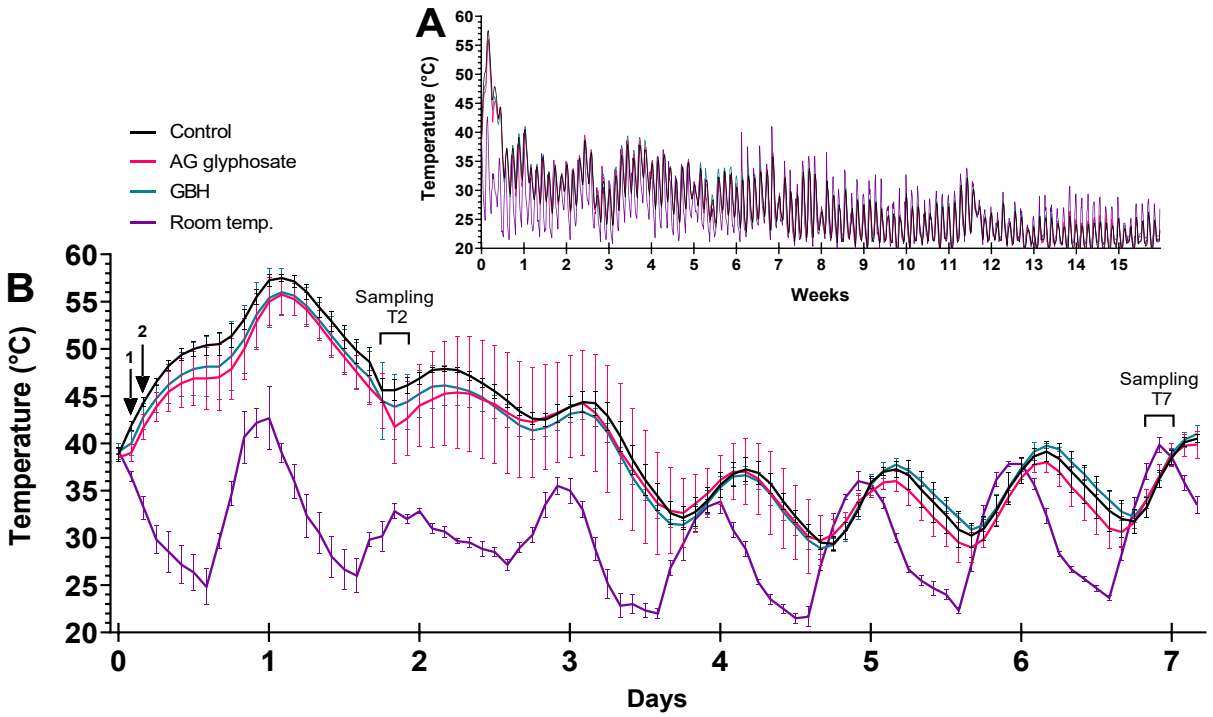

Figure 3.1 - Temperature profile during the whole composting process (1l2 days, A) and the first week of composting (B). Temperature was recorded every 2 hours in composting vessels (n = 4) and inside the greenhouse (Room temp., n = 3) ± SD for the Control, analytical grade glyphosate (AG glyphosate) and glyphosate-based herbicides (GBH). Temperature after 4 hours (1) and 6 hours (2) was significantly different between Control and AG glyphosate (adjusted p-values of 0.008 and 0.047 respectively. No other significant differences between treatments were observed.

A significant increase in pH was measured for the Control between T0 and T7 with values ranging from 9.08 to 9.45, while values remained stable for the AG glyphosate and GBH treatments (Figure 3.2 B; Table 3.1). The decrease in pH was significant for all three treatments between T0 and T1l2 with values going from 9.08 to 7.71 for Control, 9.19 to 7.88 for AG glyphosate and 9.29 to 7.71 for GBH. No significant changes in total carbon percentages were measured for any treatment (Figure 3.2 C; Table 3.1). Values at T0 decreased from 41.7 % of the total carbon for Control, 40.8 % for AG glyphosate, and 41.2 % for GBH treatment to 38.7 % for Control, 40.1 % for AG glyphosate, and 39.4 % for GBH treatment at T1l2. The percentage of total nitrogen increased significantly for all three treatments (Figure 3.2 D; Table 3.1) between T0 and T1l2, from 1.61 % to 2.68 % for Control, from 1.52 % to 2.56 % for AG glyphosate treatment, and from 1.69 % to 2.48 % for GBH treatment. Finally, a significant decrease in the C:N ratio was measured between T0 and T7

for the Control, decreasing from 27.2 to 22.9 (Figure 3.2 E; Table 3.1). The decrease between T0 and T112 was significant for all 3 treatments, with values decreasing from 27.2 to 14.48 for Control, from 27.0 to 15.9 for AG glyphosate treatment and 24.7 to 16.0 for GBH treatment.

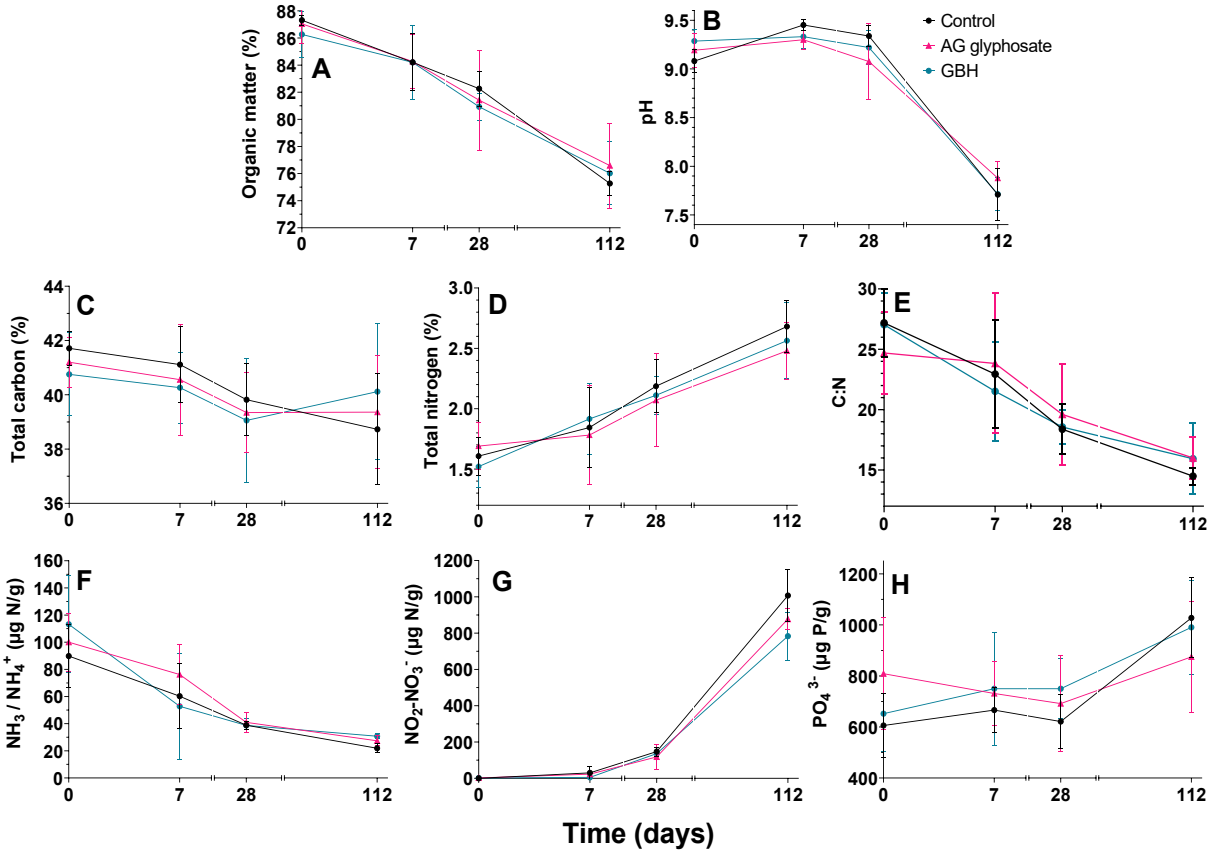

Figure 3.2 - Physicochemical measurements (O.M., pH, C, N, C: N at n = 5 and NH_3/NH_4^+, NO_2^--NO_3^- and PO_4^{3-} at n = 4) on Control, AG glyphosate and GBH treatment measured throughout the composting process (0, 7, 28 and 112 days). All values represent mean ± SD

The NH_3/NH_4^+ content in the GBH treatment significantly differed from the Control at T112, with final content of 30.7 µg N/g and 21.9 µg N/g, respectively (Figure 3.2 F). A significant increase in NO_2^--NO_3^- content was measured between T7 and T28 for the Control and GBH treatments, going from 30.1 µg N/g to 147.2 µg N/g and from 5.52 µg N/g to 136.4 µg N/g, respectively (Figure 3.2 G; Table 3.1).

	Time-points compared	O.M.	pH	C	N	C: N	NH_3/ NH_4^+	NO_2^--NO_3^-	PO_4^{3-}
Control	T0 vs. T7	0.095	*0.004*	0.611	0.403	*0.036*	0.619	0.43	0.915
	T7 vs. T28	0.199	0.374	0.517	0.257	0.302	0.386	*0.003*	0.776
	T28 vs. T112	*0.004*	*< 0.001*	0.358	0.114	0.053	*0.033*	*0.004*	*0.034*
	T0 vs. T112	*< 0.001*	*< 0.001*	0.112	*0.004*	*< 0.001*	*0.022*	*0.002*	0.137
AG glyphosate	T0 vs. T7	*0.006*	0.649	0.941	0.070	0.101	0.290	*0.044*	0.907
	T7 vs. T28	0.325	0.513	0.777	0.534	0.410	0.121	0.2	0.948
	T28 vs. T112	0.251	*0.001*	0.833	0.304	0.520	0.150	*< 0.001*	0.335
	T0 vs. T112	*0.017*	*< 0.001*	0.884	*0.020*	*0.021*	*0.013*	*< 0.001*	0.492
GBH	T0 vs. T7	0.681	0.947	0.917	0.980	0.994	0.291	0.71	0.889
	T7 vs. T28	0.277	0.419	0.239	0.615	0.361	0.866	*0.001*	> 0.999
	T28 vs. T112	*0.010*	*< 0.001*	> 0.999	0.304	0.257	0.286	*0.006*	0.258
	T0 vs. T112	*0.010*	*< 0.001*	0.219	*0.020*	*0.021*	0.052	*0.004*	*0.030*

Table 3.1 - Results (adjusted p-values) of repeated measures two-way ANOVA and Tukey's post hoc multiple comparisons test for physicochemical measurements O.M., pH, C, N, C:N (n = 5) and NH_3/NH_4^+, NO_2^--NO_3^- and PO_4^{3-} (n = 4) on Control, AG glyphosate and GBH treatment between different time-points. Values in italics are significant. No significant differences were observed between treatments at any sampled time-point.

The increase in content between T28 and T112 was significant for all three treatments, with values increasing from 147.2 µg N/g to 1007.1 µg N/g for Control, from 118.1 µg N/g to 877.1 µg N/g for AG glyphosate treatment, and from 136.4 µg N/g to 783.4 µg N/g for GBH treatment. A significant increase in PO_4^{3-} content was recorded for the Control between T28 and T112, with values going from 605.8 µg P/g to 1027.5 µg P/g, while the increase was significant between T0 and T112 for the GBH treatment, with PO_4^{3-} going from 652.3 µg P/g to 990.5 µg P/g (Figure 3.2 H). However, PO_4^{3-} content did not vary significantly over the 112 days of composting for the AG glyphosate, with T0 values of 808.8 µg P/g and final values of 874.5 µg P/g.

3.3.3 Glyphosate and AMPA content

The presence of glyphosate in the compost was directly measured over the course of the experiment and showed a rapid degradation, irrespective of the treatment. As expected, glyphosate and AMPA measurements were under the detection limit (LOD) for the Control (Figure 3.3). Significant glyphosate degradation was observed between T0 and T2 for both

treatments, with a decrease in glyphosate levels of 29.5 % for the AG glyphosate treatment and 49.4 % for the GBH treatment compared to the initial amount of glyphosate applied (Table 3.2). The decrease was also significant between T2 and T7 for both treatments, going from a remaining glyphosate content of 50.6 % to 19.6 % for the AG glyphosate treatment and from a remaining content of 68.1 % to 20.2 % for the GBH treatment. The decrease was also significant between T7 and T28 for the AG glyphosate treatment, dropping from 19.6 % of remaining glyphosate to 4.8 %. The entire amount of the glyphosate applied in the AG glyphosate treatment was degraded by day 112 of composting, while 2.0 % of the initially added glyphosate remained in the GBH treatment. The remaining glyphosate content was significantly higher in the AG glyphosate treatment compared to the GBH treatment at T2. AMPA measurements were under LOD (0.03 µg/g) for both treated groups and every sampling time.

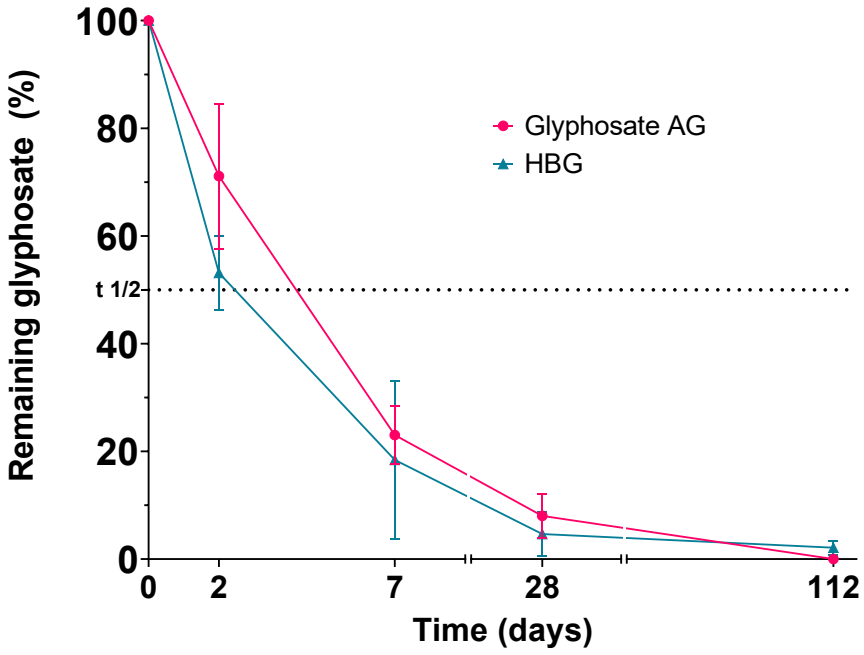

Figure 3.3 - Remaining glyphosate content (%) from analytical grade glyphosate (AG glyphosate) and glyphosate-based herbicide (GBH) treatment (n = 4) measured throughout the composting process (0, 7, 28 and 112 days).

		Glyphosate content
AG glyphosate	T0 vs. T2	*0.027*
	T2 vs. T7	*0.002*
	T7 vs. T28	*0.013*
	T28 vs. T112	0.092
	T0 vs. T112	< 0.001
GBH	T0 vs. T2	*0.003*
	T2 vs. T7	*0.022*
	T7 vs. T28	0.274
	T28 vs. T112	0.544
	T0 vs. T112	*< 0.001*

		Glyphosate content
AG glyphosate vs. GBH	T0	> 0.999
	T2	*0.021*
	T7	0.983
	T28	0.983
	T112	0.983

Table 3.2 - Results (adjusted p-values) of repeated measures two-way ANOVA and Tukey's post hoc multiple comparisons test for comparison of glyphosate content in AG glyphosate (n = 5) and GBH (n = 4) treatment comparison between consecutive time-point and Multiple t-test comparisons significance determined using the Holm-Šídák method for difference in remaining glyphosate content between AG glyphosate (n = 5) and GBH (n = 4) treatment at different time-points.

3.4 Discussion

3.4.1 Temperature profile

The temperature increase was comparable for all treatments suggesting a very negligible impact of glyphosate and GBH ingredients on microbial activity, as it has been previously documented regarding the effect of glyphosate on soil microbial activity (Al-Rajab & Schiavon, 2010; Bonfleur et al., 2015; Haney et al., 2000; Lupwayi et al., 2009; Mijangos et al., 2009; Ratcliff et al., 2006; Wardle & Parkinson, 1991). The presence of a xenobiotic compound during microbial activity measurements can induce a lag phase characterized by the initial adaptation of microbial communities to the stressful environmental conditions (Dörfler et al., 1996) as reported by Lashermes et al. (2012), although it was not observed in this study, nor in other studies investigating the effect of glyphosate on microbial activity (Al-Rajab & Schiavon, 2010; Haney et al., 2000). The duration of the thermophilic phase, ranging from 48 hours to 56 hours, is relatively short but consistent with the results obtained by Horiuchi et al. (2003), Magalhaes et al. (1993) and Xu et al. (2010), who also conducted tests in small-scale composters (Mason & Milke, 2005; Petiot & de Guardia, 2004). Thus,

the presence of glyphosate or GBH adjuvants seemingly had no impact on the establishment of thermophilic conditions during composting, even with a glyphosate content higher than that commonly occurring in agricultural soils (≥ 0.05 mg kg^{-1} across Europe (Silva et al., 2018b)).

3.4.2 Compost properties over time

The addition of glyphosate to the developing compost mixtures did not seem to affect their physicochemical properties. The modest OM loss (12-14 %) was consistent with previously reported findings in short term composting studies (Francou et al., 2005). The alkaline pH measured at the beginning of the experiment (Figure 3.2 B) could be due to high urea levels in horse and sheep litters which tend to increase the pH of the medium when degraded (Sánchez-Monedero et al., 2001; Zhu et al., 2013) whereas acidic conditions are generally reported at the initiation of composting (Nakasaki et al., 1993; Smårs et al., 2002). The loss of total carbon at the end of the experiment was not significant (Table 3.1) and was similar among the different treatments (Figure 3.2 C), possibly due to the short thermophilic phase and limited composting time (Pérez et al., 2002). Nitrogen content increased significantly between the beginning and end of the experiment (Table 3.1), a phenomenon widely reported to be related to the concentrating effect inherent to the decrease in total compost mass (Bernal et al., 1996). Finally, the C: N ratio, whose decrease is characteristic of the increasing compost maturity and stability, declined significantly between T0 and T112 for all groups (Figure 3.2 E). The values measured at T112 (14.48 for Control, 15.90 for Glyphosate treatment and 16.00 for GBH treatment) are very close to the values considered as corresponding to a mature compost (< 15) (Bernal et al., 1998), suggesting that the generated compost in our trial can almost be considered mature.

The NH_3/NH_4^+ content did not differ between treatments but decreased significantly over the course of the experiment (Figure 3.2 F). Since no leachate was collected during the experiment, this result suggests that ammonium was primarily used by the microorganisms as a source of nitrogen and was oxidized in the nitrification process, although loss through volatilization of NH_3 is also possible. These observations are in disagreement with studies

showing a stimulation of ammonification in a forest soil (Stratton & Stewart, 1991) and in a soil planted with forage plants (Mijangos et al., 2009) in presence of glyphosate. Here, nitrification, i.e. NO_2^--NO_3^- production, was also unaffected by the presence of glyphosate (Figure 3.2 G). The significant increase in nitrite and nitrate between the end of the first and the fourth month of composting was similar for all treatments. The NO_2^--NO_3^- increase coincided with a pH drop during the same period, hence it is conceivable that composting conditions had a more important role in nitrification processes than the presence of glyphosate. Indeed, the absence of glyphosate effect on nitrification was previously observed in soils (Marsh and Davies 1978; Müller et al. 1981; Stratton 1990; Stratton and Stewart 1991). The presence of glyphosate did not cause any variation in phosphate content through time (Figure 3.2 H). Furthermore, there was no noticeable change within the AG glyphosate treatments, while a significant increase in PO_4^{3-} content was measured in the Control treatment between T28 and T112 and the GBH treatment between T0 and T112 (Table 3.1). Presumably, the phosphate molecules released during composting were used directly by microorganisms present in the active mixture.

3.4.3 Glyphosate and AMPA content

Glyphosate content decreased significantly and rapidly in both treatment groups (Figure 3.3, Table 3.2), leading to no detectable levels in the AG glyphosate treatment and about 2 % of the initial amounts in the GBH treatment by the end of the composting process. This rapid decrease was observed alongside the highest measured temperature in what is referred to as the thermophilic phase. The recorded decrease was greater than what can be observed in soils, where 85-90 % of glyphosate is reported to have a half-life ranging from 8.36 to 9.12 days (Singh et al. 2020), a possible consequence of the intense microbial activity.

Degradation of glyphosate can be achieved through abiotic and biotic pathway, e.g., adsorption, photolysis, thermolysis, and biodegradation with catabolic enzymes (Singh et al., 2020). The main glyphosate degradation pathway in soils is microbial (Lopes Catão & López-Castillo, 2018). Given both the intense microbial activity and microorganisms diversity in compost (Antunes et al., 2016), biotic degradation was most likely the major

route of glyphosate degradation. In soils, glyphosate is primarily converted into AMPA by the action of the glyphosate oxidoreductase (GOX), while the C-P lyase, produces sarcosine and phosphate (Feng et al., 2020; Zhan et al., 2018). A number of bacteria capable of metabolizing glyphosate through the action of either of these enzymes have been isolated from contaminated soil samples. Most reported species use glyphosate as the sole source of phosphorus. A few exceptions use glyphosate as a nitrogen or carbon source (Singh et al., 2020). The identified organisms include members of the genera *Achromobacter, Agrobacterium, Alcaligenes, Arthrobacter, Comamonas, Enterobacter, Flavobacterium, Geobacillus, Ochrobactrum, Pseudomonas, Sinorhizobium* and S*treptomyces* all of which have also been previously observed in compost (Antunes et al., 2016; Ryckeboer et al., 2003).

The particular conditions encountered during composting compared to soil are not particularly conducive to the observation of other mechanisms. For example, Lashermes et al., (2010) measured the glyphosate adsorption coefficient (*Kd*) for different feedstocks. Analyses performed on branches, hedge trimming, grass clipping, and mixed leaves gave *Kd* values ranging from -1 to 3 L kg^{-1} DM. This study concluded that the vast majority of glyphosate was probably available for microbial degradation. Aslam et al., (2013) also concluded that glyphosate was weakly adsorbed on maize mulch. The high pH measured during the first months of the experiment would have further decreased the adsorption due to the repulsive forces between absorbents and glyphosate (Herath et al., 2016). Furthermore, the extraction solution used in this study is known to have a recovery rate of 90 % to 100 % in soil, crops, and water (Alferness & Iwata, 1994). Similarly, the various abiotic degradation pathways may not have been favoured during the experiment either. Indeed, the absence of UV light entering the closed vessels and the low temperature in the composters are unlikely to allow photodegradation reactions or thermolysis (degradation at 230 °C (Chen et al., 2012)). Fenton-like reactions are known to act on glyphosate degradation (Feng et al., 2020) and thus could occur during composting. However, acidic conditions are required for these reactions to take place (Arantes et al., 2012; Pignatello et al., 2006), and while they may have been present at isolated locations, it is unlikely they occurred widely among all the composting vessels.

Glyphosate dissipation was not significantly different between the GBH and the AG glyphosate treatments (Table 3.2). This finding suggests that ingredients from the GBH formulation, such as the non-ionic polyoxyethyleneamine (POEA) surfactant, which was previously reported to be toxic to mouse fibroblast-like cells, certain bacteria, aquatic organisms and fish (Navarro & Martinez, 2014; Song et al., 2012; Tsui & Chu, 2003), have no apparent deleterious effect on the overall processes affecting glyphosate dissipation in this experiment.

The decrease of glyphosate content observed in the study is also higher than the ones reported in similar studies on mulch and crop degradation and composting (Cassigneul et al., 2016; Lashermes et al., 2012; Rampoldi et al., 2011). Rampoldi et al. (2011) reported a mineralization rate of glyphosate of 41.7 ± 12.4 % from in corn residues and 27.8 % ± 11.4 in soybean residues after storage at 28 °C for 56 days. A similar study from Cassigneul et al. (2016) looked at glyphosate mineralization during the laboratory decomposition of cover crop mulches stored in hermetically sealed jars incubated at 20 °C for 84 days. The reported mineralization varied from 13.0 to 15.8 % depending on the cover crop studied. Finally, Lashermes et al. (2012) assessed glyphosate mineralization during composting of a mixture of sewage sludge, branches, grass clippings, hedge trimmings and leaves for 92 days and recorded 33 % of ^{14}C present as CO_2. The experimental conditions in the two former studies were very different from those encountered during composting, where high microbial activity and higher temperatures can enhance degradation. Lashermes and collaborators (2012), concluded that the low glyphosate mineralization could be due to the inability of the microorganisms to degrade the compound. The authors also observed the formation of non-extractable residue (NER) at the beginning of the process, which they believe to be a potential consequence of intense microbial activity. Glyphosate and its metabolites could be either incorporated into the microbial biomass or bound to the OM as a result of enzymatic oxidation reactions (Charnay et al., 2004). It should be noted that the measurement methods used here allows for the extraction and direct analysis of glyphosate through chromatography (GC-ECD) while the cited studies monitor the mineralization of glyphosate through the production of ^{14}C-CO_2. While ^{14}C-CO_2 measurements only consider the

proportion of glyphosate that has been fully mineralized (i.e., CO_2 production), our method considers partial glyphosate mineralization through its degradation into metabolites such as the AMPA.

AMPA content was also measured during the experiment in order to monitor potential accumulation following glyphosate degradation. In all samples collected and for both the AG glyphosate and GBH treatments, content values were below the detection limit of the instrument. The AMPA pathway is considered the dominant route in soils (Hove-Jensen et al., 2014), so it is likely that AMPA was indeed produced, but was degraded almost instantaneously by various microorganisms that would have used it as a source of C, N, or P (Borggaard & Gimsing, 2008).

3.5 Conclusion

The study presented in this paper is the first to estimate the effect of glyphosate and a GBH on the evolution of physicochemical parameters during composting. Composting seems to proceed independently of the presence of glyphosate and the adjuvants present in the GBH formulation. A significant and quick glyphosate dissipation occurred during the first days of the process and culminated at the end of the experiment with a complete dissipation for the glyphosate-containing treatment and a 98 % dissipation for the GBH treatment, while AMPA could not be detected at any time during the experiment, regardless of the treatment. The large-scale deployment of composting as the primary method for treating plant residues and municipal solid waste in several major metropolitan areas requires a thorough understanding of all the issues related to the presence of contaminants in the process and the safety of the compost produced. Although this study does not prove beyond doubt the safety of the compost produced in the presence of glyphosate, the results demonstrate the absence of extractable glyphosate and AMPA at the end of the experiment. Subsequent analyses of the diversity, abundance and dynamics of the bacterial communities may provide information on the tolerance and/or susceptibility of certain groups and the degradation pathways exploited.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

V. Grenier: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition **M. Moingt:** Methodology, Investigation, Writing - Review & Editing **M. M. Lucotte:** Resources, Writing - Review & Editing **F. E. Pitre:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

Acknowledgements and funding

Special thanks to Suzanne Boucher for her help in the preparation of the experiment, sampling and physicochemical analyses. We are grateful to Estelle Lacasse, Camille M. Chédeville and Joan Laur for their help with sampling. Our thanks to Joan Laur, Eszter Sas and Noël Fagoaga for their help in the preparation of the organic residue mixture. Finally, thanks to Karen Grislis for her work on English revision. This research was made possible by financial support from the Fonds de recherche du Québec – Nature et technologies (FRQNT), Mitacs Acceleration program and the Space for Life Foundation to the first (Ph.D. scholarship) author.

Supplementary data

		O.M.				pH			
Source of variation	***df***	**SS**	**MS**	**F-value**	**p-value**	**SS**	**MS**	**F-value**	**p-value**
Time	3	979.2	326.4	68.79	*<.001*	25.12	8.373	410.3	*<.001*
Treatment	2	2.659	1.330	0.3757	0.695	0.0122	0.0061	0.0800	0.924
Time x Treatment	6	9.253	1.542	0.3250	0.919	0.4236	0.0706	3.459	*0.008*

		C				N			
Source of variation	***df***	**SS**	**MS**	**F-value**	**p-value**	**SS**	**MS**	**F-value**	**p-value**
Time	3	37.59	12.53	5.142	*0.016*	7.771	2.590	39.04	*<.001*
Treatment	2	0.9556	0.4778	0.1055	0.901	0.0581	0.0290	0.3098	0.79
Time x Treatment	6	9.508	1.585	0.6504	0.689	0.1951	0.0325	0.4899	0.811

		C:N				NH_3/NH_4^+			
Source of variation	***df***	**SS**	**MS**	**F-value**	**p-value**	**SS**	**MS**	**F-value**	**p-value**
Time	3	994.8	331.6	34.98	*<.001*	38413	12804	30.48	*<.001*
Treatment	2	1.013	0.5063	0.0316	0.969	612.8	306.4	0.7842	0.485
Time x Treatment	6	43.81	7.302	0.7702	0.598	1810	301.7	0.7182	0.638

		$NO_2^-NO_3^-$				PO_4^{3-}			
Source of variation	***df***	**SS**	**MS**	**F-value**	**p-value**	**SS**	**MS**	**F-value**	**p-value**
Time	3	6439240	2146413	549.1	*<.001*	646364	215455	9.882	*<.001*
Treatment	2	33996	16998	3.789	0.064	28317	14159	0.3275	0.729
Time x Treatment	6	70024	11671	2.986	*0.023*	161845	26974	1.237	0.319

Tableau S3.1 - ANOVA results for time and treatment effect on each measured parameter measurement (O.M., pH, C, N, C:N (n = 5) and NH_3/NH_4^+, NO_2^--NO^{3-} and PO_4^{3-} (n = 4)). *df* = degree of freedom, SS = sum of square and MS = mean square

Plateforme du compostage du Jardin botanique de Montréal

Chapitre 4 – Dynamique de succession des communautés bactériennes et archéales pendant le compostage de résidus verts

Replacement dynamics of bacterial and archaeal communities during green waste composting

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In preparation for submission in Environmental Microbiology

Abstract

In an attempt to report changes in bacterial communities during composting, the ANCHOR bioinformatics pipeline was used to process 16S ribosomal RNA sequences from 5 compost windrows of different maturity levels. Compost properties changed rapidly over time, while a total of 2,335 ESVs were differentially abundant between phases. General compost community diversity increases over time, from 830 ESVs found in Litter to 2,131 in the mature Compost which shows the highest bacterial species richness. Structural carbohydrates and lignin degraders were abundant at the beginning of the thermophilic phase, especially members of the Firmicute and Actinobacteria phyla. Species capable of ammonification and denitrification were present throughout the composting process, whereas only a limited number of nitrifying bacteria were identified and were concentrated in the final phases. Furthermore, 95 % of all Archaea were identified as methanogens with prevalence during the late thermophilic phases. They were relatively less abundant during the cooling phase, while methanotrophs were observed at a higher relative abundance. Finally, a great proportion of the abundant species in the mature Compost were plant growth-promoting bacteria (PGPB) and soil thermophilic bacteria (STB). This study highlights the role of bacteria in carbon and nitrogen transformation and the complex synergistic relationship between organisms of different phyla to complete organic matter mineralization during composting.

4.1 Introduction

With the rapid increase in global waste production (Chen et al., 2020), composting has emerged as an alternative to landfilling of municipal solid waste (MSW). The disposal of organic wastes has negative impact on human health and the environment notably by the release of greenhouse gas and pollution of soil, groundwater and surface water (Lou & Nair, 2009; Taiwo, 2011). Composting has the potential to be an environmental route for disposal of green wastes, taking place in environments controlled to maintain thermophilic conditions from the sequential activity of a variety of bacteria and fungi to break down the

complex organic structures, such as plant cell walls, into more readily assimilated compounds (Cragg et al., 2015). The growth of these microorganisms is governed by the environmental conditions such as temperature, aeration, moisture and the substrate composition, which in turn will affect the speed and rate of decomposition of the organic matter (Gajalakshmi & Abbasi, 2008).

Green waste represents a significant fraction of the municipal solid waste (MSW) sent to composting and originate mainly from the maintenance of street trees and green spaces such as municipal parks and gardens, and include dead tree chips, pruning and grass clippings (Reyes-Torres et al., 2018; Wei et al., 2017). In addition to their presence as MSW, lignocellulosic biomass is often used as a bulking agent, providing significant dry matter and C content to balance the high N and moisture content of food residues and biosolids (Haug, 1993). Lignocellulosic biomass thus holds a critical place on municipal composting sites which is also reflected in the large number of scientific publications addressing its degradation during composting (Huang et al., 2010; Tuomela et al., 2000).

The complexity and diversity of the substrates along with the changes in temperature and oxygenation conditions within a given system requires the action of equally complex and diverse communities of microorganisms to mineralize all the organic matters (Bustamante et al., 2012; Mello et al., 2016; Ryckeboer et al., 2003; Zhang et al., 2011). The collective action of the microorganisms to release all forms of carbon in the biomass is both sequential and synergistic and involve multiple species. Nevertheless, the ubiquity and abundance of cellulose, hemicellulose and lignin requires the presence of specialized degraders at all stages of composting. Mesophilic (*Kribbella, Actinoplanes* and *Stackebrandtia*) and thermophilic (*Mycobacterium*, *Thermobifida*, *Thermomonospora* and *Thermobispora*) actinobacteria are often found in abundance in aerobic environment while Firmicutes (*Clostridium*, *Symbiobacterium*, *Bacillus and Geobacillus*) are mostly associated with anaerobic conditions (Antunes et al., 2016; Ryckeboer et al., 2003; Wang et al., 2016). *Rhodothermus marinus* and *Sphaerobacter thermophilus*, thermophilic members of the Bacteroidetes and Chloroflexi phyla respectively were also observed in compost environment containing lignocellulose (Antunes et al., 2016).

Further carbon transformation during composting can include methane production led by methanogenic archaea (Chen et al., 2014; Jäckel et al., 2005; Lee et al., 2010; Thummes et al., 2007; Yamamoto et al., 2011). Several genera fond of high temperatures such as *Methanoculleus* and *Methanosarcin*a as well as mesophiles like *Methanothermobacter* and *Methanomicrobium* have been observed in composts (Chen et al., 2014; Jäckel et al., 2005; Lee et al., 2010; Thummes et al., 2007; Yamamoto et al., 2011). Accompanying the methanogens in a variety of environments are the methanotrophs, or bacteria with the ability to oxidize methane (MOB). Although the presence of methanotrophs in compost has been noted (Halet et al., 2006; Jäckel et al., 2005), the observation of co-occurrence of methane-producing and methane-oxidizing communities in compost has so far only been reported in compost composed of manure and straw (Chen et al., 2014). Furthermore, a recent review presenting methanotrophic and methanogenic bacterial communities from diverse ecosystems (Kumar et al., 2021) such as coastal/marine, paddy fields, desert and forest soil and their impact on environment doesn't mention compost communities, thus pointing to a gap in knowledge on this subject.

Whether present in plant material or as food waste or manure, nitrogen is essential to maintain biological processes and most of it comes from proteins and simple peptides contained in the organic matter (Haug, 1993). Mineralization of organic nitrogen, oxidation of ammonium and nitrite as well as volatilization of ammonia and denitrification are all important parts of the nitrogen cycle during composting (Körner & Stegmann, 2002). While ammonification is the predominant reaction occurring in the early thermophilic stages, nitrification is said to occur during maturation with the action of ammonium oxidizing bacteria (AOB) such as *Nitrosomonas*, *Nitrospira*, *Nitrosococcus, Nitrosovibrio*, etc. and nitrite oxidizing bacteria (NOB) such as *Nitrospira* and *Nitrobacter* (Körner & Stegmann, 2002). Anaerobic conditions can lead to nitrogen losses through volatilization (NH_3), leaching and denitrification (NO, N_2O or N_2) as microorganisms like *Pseudomonas*, *Geobacillus*, *Bacillus*, *Flavobacterium* can use nitrate as a source of oxygen, resulting in denitrification and cessation of nitrification (Verstraete & Focht, 1977).

Given the essential compositing processes associated with lignocellulosic degradation, methane and nitrogen cycling are driven by microbiota, this research aimed to capture the microbial community at four-time points, spanning two years, alongside associated changes in physicochemical properties. This should enable the tracking of species-level changes and help inform future interventional studies designed to improve the environmental disposal of organic waste.to track the species-level changes and help inform future interventional studies aimed at improving environmental disposal of organic wastes.

4.3 Materials and Methods

4.3.1 Study site, sampling, and physicochemical analyses

Three compost windrows of 22m x 5m x 3m (L x W x H) composed of horse bedding (wood chips and horse feces) and green plant residues were sampled on August 8th and 9th 2018. Material in the youngest windrow ranged from 1-6 weeks in composting age (Young), with fresh material regularly added to one end, material in a second window was 3 months in age (Middle) and material in the third windrow was 12 months in age (Aged). The windrows are mixed using a tractor two to three times a month. The three windrows (1.5, 3 and 12 months), fresh horse litter (Litter) and a mature compost pile of 24 months of age (mature Compost) were all sampled for analysis. The three windrows were each split lengthwise into four sections and each section was sampled 4 times, taking 2 samples from each side of the windrow. The 4 samples of approximately 1 kg were collected at a depth of 60 cm per section and mixed to create 4 composite samples per windrow (n = 4). Temperature was taken in each hole shovelled for sampling. Four composite samples were randomly collected at a similar depth in the horse-litter pile and the mature compost pile. Samples were split into four fractions; two were frozen (-80 °C and -20 °C) and two were dried (oven dried at 105 ° C for 24 hours and air dried for two weeks). Oven and air dried samples were milled at a particle size of <2 mm before storage in the dark until analysis. Organic matter was assessed on oven-dried samples by determination of losses on ignition at 600 °C to a constant weight. The pH was analyzed in a 1:10 (w/v) water extract of oven-dried samples. Total

carbon (C) and nitrogen (N) were determined on oven dried samples by dry combustion at 950 °C using the varioMICROcube analyzer (Elementar, Langenselbold, Germany). Total mineral nitrogen (NH_4^+ and NO_2^--NO_3^-) was extracted with 2.0M KCl (Carter & Gregorich, 2006) and measured by colorimetry on QuikChem® 8500 Series 2 FIA System (Lachat Instruments, Milwaukee, WI) using method 12-107-06-1B and 12-107-04-1-B. Nitrogen loss was calculated according to the following equation (Sánchez-Monedero et al., 2001):

$$N_{losses}(\%) = (100 - (\left(\left(Ash\ content_{initial} \times N_{final}\right) \div \left(Ash\ content_{final} \times N_{initial}\right)\right) \times 100) \quad (1)$$

Cellulose, hemicellulose and lignin content were determined with an ANKOM2000 Automated Fiber Analyzer. Briefly, cellulose content was estimated as the difference between the ADF and the acid-detergent lignin (ADL), hemicellulose content was estimated as the difference between the neutral-detergent fiber (NDF) and the acid-detergent fiber (ADF), and lignin content was estimated as the difference between the ADL and the ash content.

4.3.2 DNA extraction, 16S rRNA gene amplification sequencing and processing

Total genomic DNA was extracted from subsamples of 250 mg (wet weight) using the DNeasy Power Soil® Pro Kit from Qiagen following the manufacturer's instructions. The quantity and quality of the extracted DNA were examined using a NanoDrop 2000c from Thermo Fisher. The V5-V6 region (based on Escherichia coli) of the 16S ribosomal RNA (rRNA) was targeted for amplification by PCR using the forward primer: P609D (5'-GGMTTAGATACCCBDGTA- 3') and reverse primer: P699R (5'- GGGTYKCGCTCGTTR-3') (Klindworth et al., 2013). Amplification used the following conditions: initial denaturation 94 °C for 2 min, denaturation 94 °C for 30 s, annealing 58 °C for 30 s, extension 72 °C for 30 s, final extension 72 °C for 7 min, 4 °C hold, over 35 cycles. The resulting amplicon were sequenced via Illumina MiSeq 2500 paired end 2 X 250 pb platform at the McGill University and Genome Quebec Innovation Centre (Montreal, Canada). Reagent controls were below the detection limit for quality assurance.

The ANCHOR pipeline was used to process and annotate sequence reads (Gonzalezet al. 2019) (https://github.com/gonzalezem/ANCHOR). As a control quality step, only reads where both set of primers were exactly detected (100% identity and 100% coverage) were selected for further analysis. Briefly, sequences were aligned and dereplicated using Mothur before selection of Exact Sequence Variants (ESVs) using a count threshold of 12 across all samples. Annotation used 4 sequence repositories with strict BLASTn criteria (99 % identity and coverage): NCBI-curated bacterial and Archaea RefSeq, NCBI nr/nt, SILVA, Ribosomal Database Project (NCBI-curated bacterial and Archaea RefSeq is given a priority when at 100 % identity and coverage). Multiple, equally good (highest identity/coverage), annotation was retained and reported as *ambiguous hits*. Amplicons with low counts (< 12) are binned to high-count sequences in a second BLASTn, using a lower threshold of 98 % identity/coverage. All annotation should be considered putatively and interpreted with caution as databases contain errors and are subject to change. Extensive literature search was performed to associate potential function to annotated ESVs.

4.3.3 Differential abundance and statistical analysis

Differential abundance analysis on 16S rRNA gene amplicons was performed using DESeq2 (Love et al., 2014; Thorsen et al., 2016), which can perform well with uneven library sizes and sparsity common to 16S rRNA gene data (Gonzalez et al., 2019; Minerbi et al., 2019; Weiss et al., 2017). A false discovery rate (FDR; Benjamini-Hochberg procedure) < 0.05 was applied (Anders et al., 2013; Love et al., 2014). Raw counts were log transformed across samples (rlog function, R Phyloseq package). Sparsity and low-count cut-offs were applied whereby an ESV count in a single sample is < 90 % of the count in all samples, and ESV counts must be > 2 in 40 % of the samples (Dhariwal et al., 2017; Gonzalez et al., 2019).

Alpha diversity was measured using Shannon and InvSimpson indices within Phyloseq package (McMurdie & Holmes, 2013). Alpha diversity was compared between the different groups of samples using a t-test. Canonical Analysis of Principal Coordinates (CAP) ordination was performed based on Bray–Curtis ecological distances using Phyloseq package (McMurdie & Holmes, 2013). Dispersion ellipses were drawn using

veganCovEllipse function from Vegan package (Oksanen et al., 2008) in R (R Development Core Team, 2014). All statistical analysis for the physicochemical measurements were carried out in GraphPad Prism 8.4.3. One-way ANOVA followed by a Tukey's multiple comparisons post hoc test was used to compare physicochemical properties across successive composting phases (Litter vs. Young, Young, vs. Middle, Middle vs. Aged and Aged vs. mature Compost).

4.4 Results

4.4.1 Compost properties over time

Physicochemical measurements were performed on three windrows of 1.5, 3 and 12 months of age (Young, Middle and Aged respectively) as well as the horse litter (Litter) and mature compost (Compost) and results indicate maturation and stabilization of the organic material (Figure 4.1). The average temperature of the horse litter pile was 38.2 °C. Temperatures varied significantly ($p < 0.05$) between each successive compost phase, averaging 67.8 °C for Young, 62.1 °C for Middle, 46.1 °C for Aged and 37.0 °C for mature Compost (Figure 4.1 A). Organic matter content was of 93.1 % in the Litter and progressively reduced to 56.1 % in Young, 42.9 % in Middle, 31.7 % in the Aged and 24.4 % in the mature Compost. This decrease was significant between Litter and Young as well as between Young and Compost (Figure 4.1 B). The initial pH in the litter was at 7.37. It then increased significantly from pH 7.33 in the Young to pH 7.72 in Middle and further to significantly increase to pH 8.42 in Aged phase and pH 8.23 in the mature compost (Figure 4.1 C).

Total carbon was 43.9% in Litter and decreased significantly to 26.9 % in Young. Middle, Aged and Compost were similar, averaging 22.9 %, 22.0 % and 18. 5% carbon, respectively, with Compost being significantly lower than Young (Figure 4.1 D). Nitrogen levels were 1.37 % in Litter before decreasing significantly to 1.09 % in Young and remaining similar for Middle Aged and Composts at 1.08 %, 1.10 % and 1.04 %, respectively (Figure 4.1 E). The C:N ratio reduced significantly from 32.6 in the Litter to 22.7 in Young and then remained similar at 21.0 in Middle, 19.9 in Aged and 17.7 in the mature Compost (Figure 4.1 F).

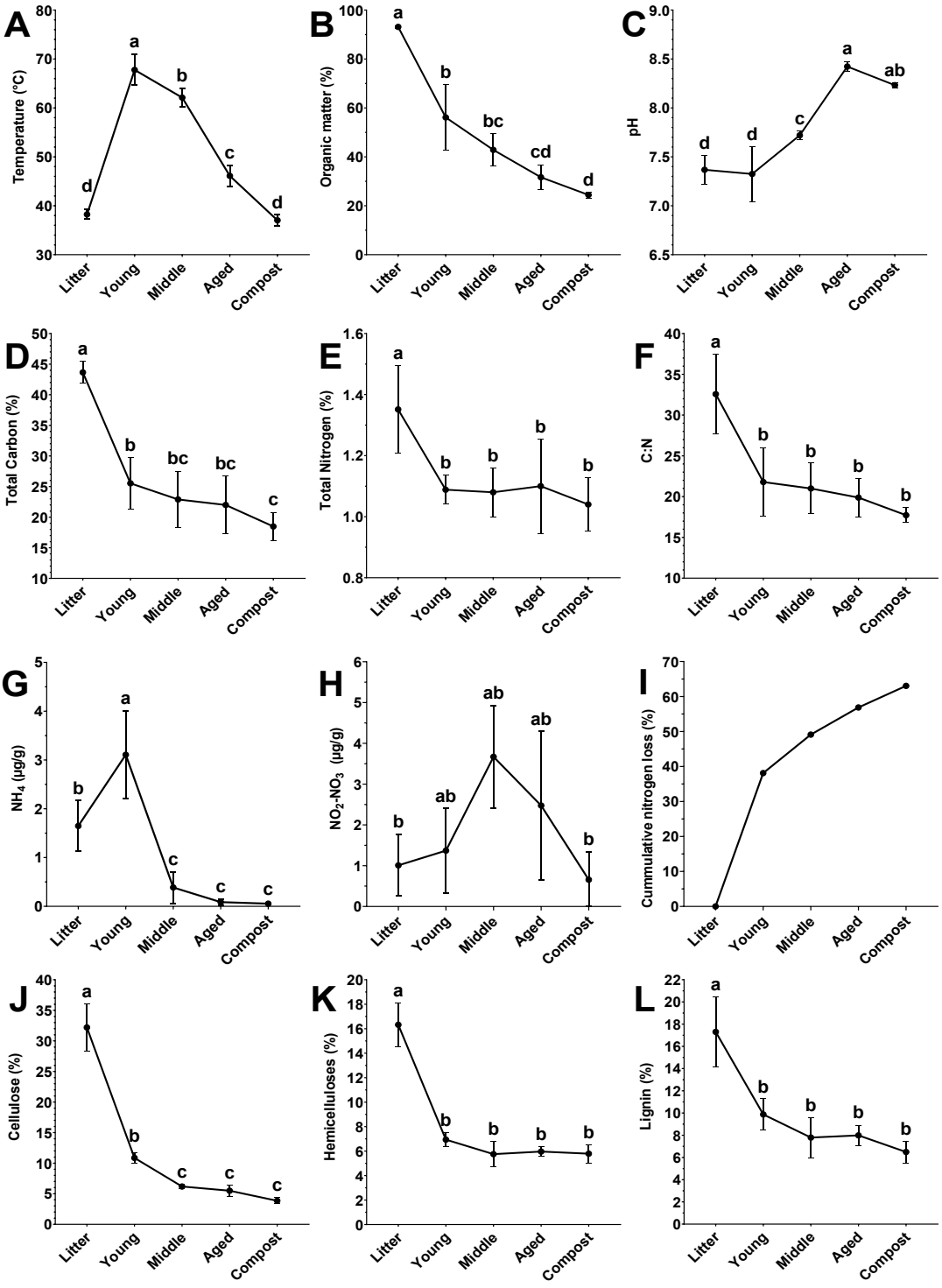

Figure 4.1 - Temperature, physicochemical properties, plant cell wall composition, inorganic nitrogen fraction and cumulative nitrogen loss of the compost throughout the different phases (Litter, Young, Middle, Aged and mature Compost). All values represent mean (n = 4) ± SD. The letters indicate significant differences between phases.

The cellulose content reached 5.50 % in the Aged phase and 3.86 % in the mature Compost but did not decrease significantly compared to Middle phase. Cellulose concentration dropped significantly from 32.20 % in the Litter to 10.86 % in the Young phase and then again to 6.19 % in Middle (Figure 4.1 G). The hemicellulose content decreased significantly from Litter to Young, from 16.33 % to 6.94 %, and remained similar at 5.75 % for Middle, 5.96 % for Aged and 5.79 % for the mature Compost (Figure 4.1 H). Lignin significantly decreased from 17.31 % in Litter to 9.87 % in Young phase. The lignin content of 7.79 % in Middle, 7.99 % in Aged and 6.49 % in the mature Compost did not vary significantly from Young (Figure 4.1 I). Ammonium (NH_4^+) content increased significantly from 1.65 µg/g in Litter to 3.11 µg/g in Young phase, and then dropped significantly from Young phase to 0.39 µg/g in Middle phase. The NH_4^+ content was 0.09 µg/g in Aged phase and 0.05 µg/g in Compost, significantly lower than Litter and Young phase. The NO_2^-/NO_3^- content was of 0.10 µg/g in Litter, 0.14 µg/g in Young phase, 0.37 µg/g in Middle phase, 0.25 µg/g in Aged phase and 0.07 µg/g in Compost and the changes were not significant between any of the phases (Figure 4.1 K). The cumulative nitrogen loss reached 38.1 % in the Young phase, 49.1 % in Middle, 56.9 % in Aged and 63.1 % in the mature Compost (Figure 4.1 L).

4.4.2 General compost community

A total of 3,133,873 amplicons were aligned with lengths (>0.1 % counts) ranging between 322 and 362 nt, and 2,612 ESVs (Exact Sequenced Variants) were identified. Of these ESVs, 517 (19.8 %) could be annotated as putative species, 694 (26.6 %) could be annotated at the level of genera, 486 (18.6 %) at higher taxonomy levels and 915 (35.0 %) were unknown. ESVs identified as putative species captured 32.3 % of raw amplicon counts (Figure 4.2 A), had an average identity of 99.8 %, including 186 ambiguous ESVs (similarity to multiple taxa) and 331 ESVs unique to one of 299 distinct species. ESVs annotated at genus level captured 25.4 % of raw counts, ESVs annotated at higher taxonomy level captured 25.3 %, and unknown ESVs captured 17.0 %. ESVs identified across all phases belonged to 26 different phyla, of which Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Chloroflexi represent 56.0 % of the total ESV diversity and shared 73.3 % of the total raw counts, while archaea represented 21 ESVs and 1.7 % of total raw counts.

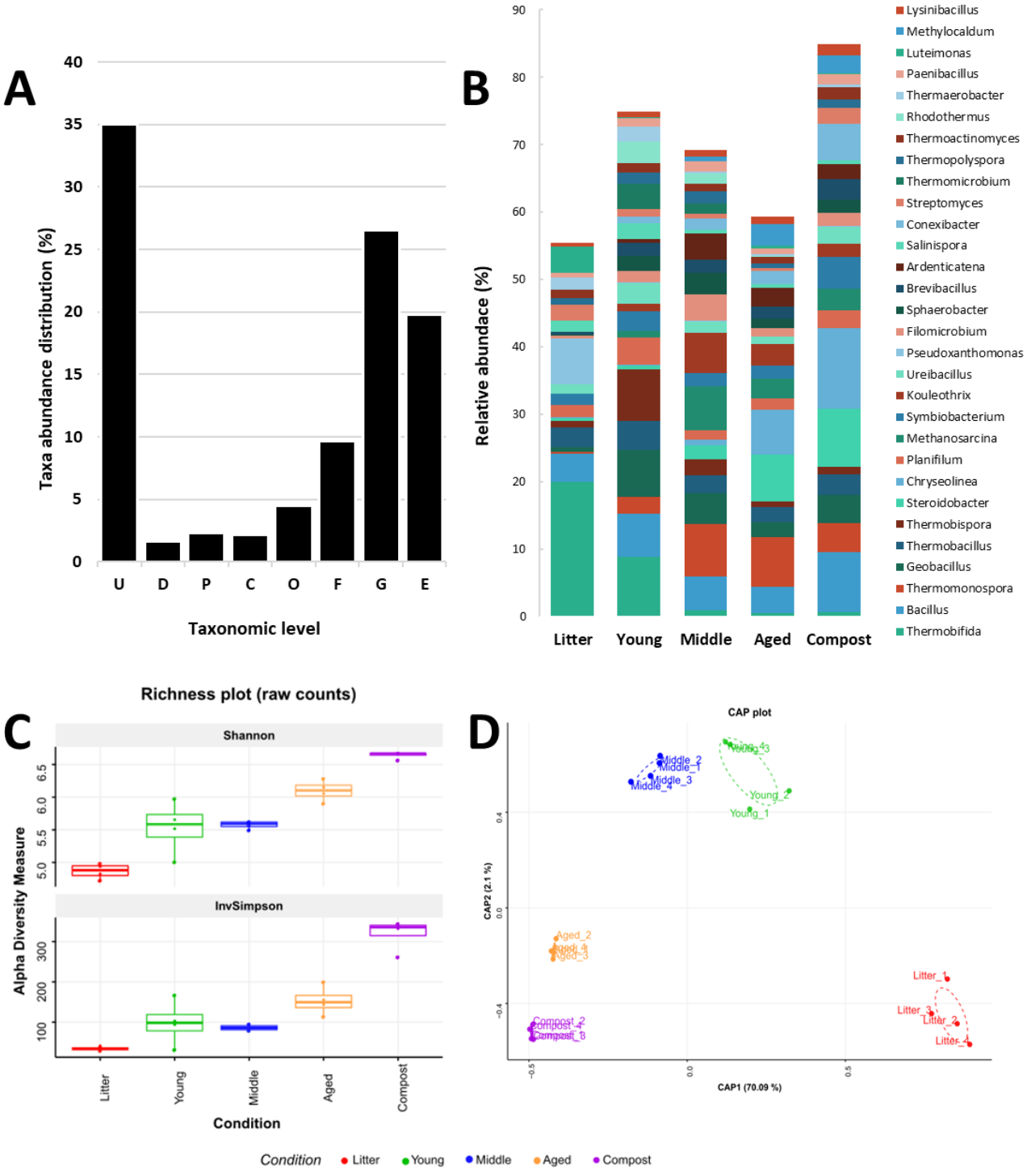

Figure 4.2 - Microbial community overview. A) Distribution of relative abundance by the level of taxonomic identification (U = Unknown, D = Domain, P = Phylum, C = Class, O = Order, F = Family, G = Genus, S = Specie). B) Genus-level taxonomic composition of the different composting phases. C) Compared alpha-diversity box plots for each sample based on two diversity estimators: Shannon and Inverse Simpson indices. D) Constrained Analysis of Principal Coordinates (CAP)

The three ESVs with highest relative abundance annotated at species levels across all samples, accounting for 5.6 % of the raw counts, were Thermobifida_fusca_2, Thermomonospora_chromogena_1 and Thermobifida_bifida_1 from the order Streptosporangiales.

The alpha diversity rarefaction curves were drawn, and all curves reached an asymptote, as the number of sequences per sample varied between 65,000 and 130,000 (Supplementary Figure 4.2). Alpha-diversity indices (Shannon and inverse Simpson) were significantly different between all groups (t-test $p< 0.05$), except for Young compared to Middle and Young compared to Aged (Figure 4.2 C). The lowest alpha diversity was found in Litter, with an average of 830 ESVs observed in all samples ($n = 4$). Diversity increased with time, with Young phase containing an average of 1630 ESVs, Middle contained 1717 ESVs, Aged 2020 ESVs and mature Compost 2131 ESVs on average, making this last phase the most diverse. Canonical Analysis of Principal Coordinates (CAP) indicated that samples separated by time with the first principal coordinate explaining 70.9 % of the variance between the samples (Figure 4.2 D). Multivariate analysis identified significant variance between each phase (PERMANOVA, $p < 0.001$).

Using differential abundance analysis, a total of 573 ESVs were identified as significantly differently abundant (DESeq2, padj <0.05) between the Litter and Young phase, 527 between the Young and Middle, 737 between Middle and Aged and 498 between Aged and Compost (Figure 4.3).

Litter contained a high proportion of Proteobacteria and Bacteroidetes compared to the first phase of composting (Young), such as species belonging to the genera *Comamonas, Cellvibrio, Daeguia* and *Sphingobacterium* (Figure 4.3 A). The genus *Thermobifida* accounts for 20 % of the ESVs identified to genus or species level in Litter, with Thermobifida_fusca_2 accounting for more than 98 % of the relative abundance. A large increase in ESVs belonging to the Firmicutes was observed in Young compared to the litter, including organisms from the genera *Paenibacillus, Bacillus, Brevibacillus, Clostridium, Planifilum*, and *Thermobacillus*, along with Thermus_thermophilus_1, a member of the

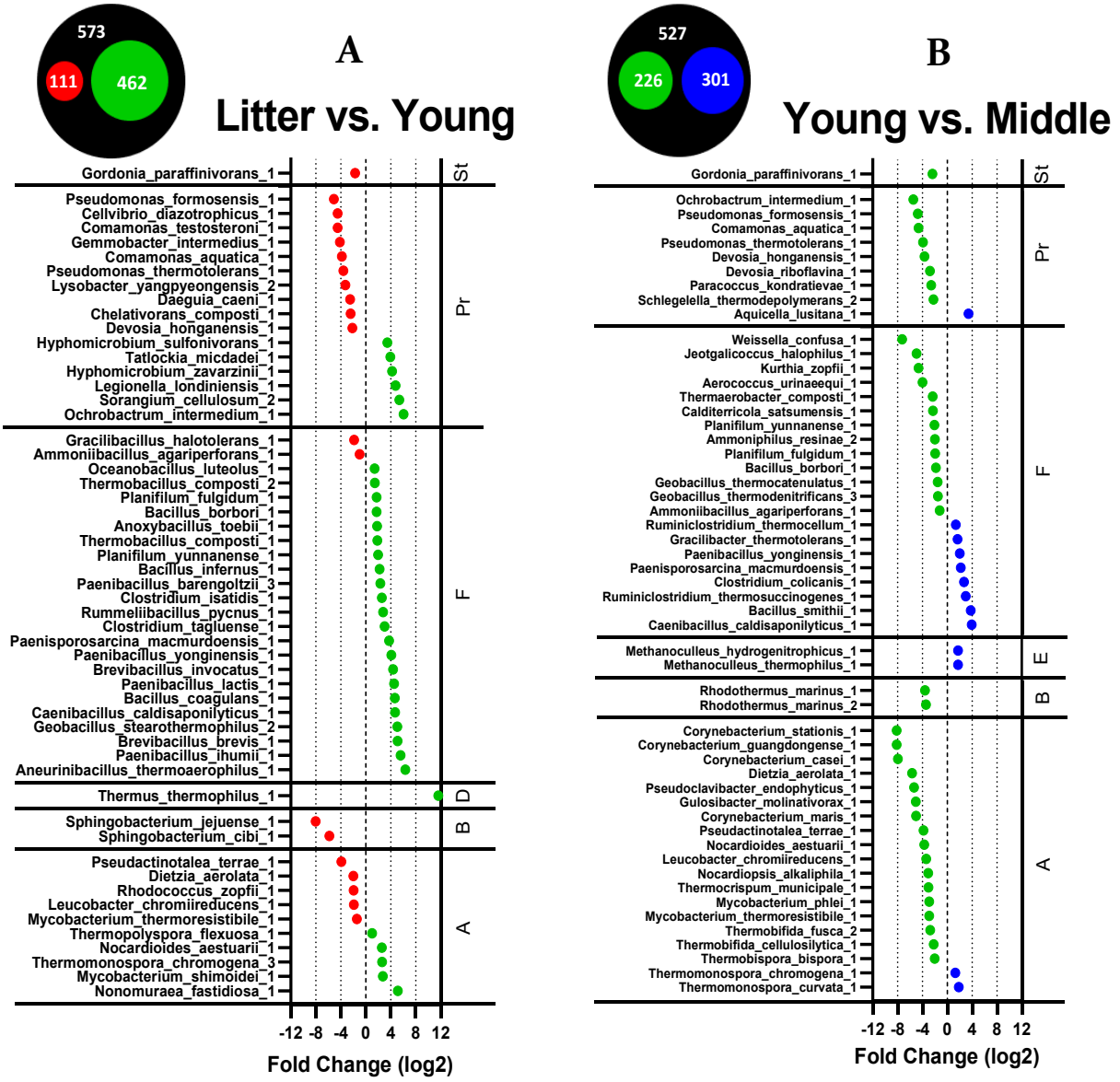

Figure 4.3 - Differentially abundant ESVs between each successive phase (A: Litter vs. Young and B: Young vs. Middle) The diagrams present the number of differentially abundant ESVs between phases as well as the proportion of higher ESVs in each phase. Fold change (FC log2) denotes relative differences in relative abundance between groups (DESeq2). In A, the red dots correspond to the most abundant bacteria in Litter and the green dots to the most abundant bacteria in Young. In B, the green dots correspond to the most abundant bacteria in Young and the blue dots to the most abundant bacteria in Middle. Species are grouped by phylum per comparison (A = Actinobacteria, B = Bacteroidetes, D = Deinoccocus Thermus, E = Euryarchaeota, F = Firmicutes, Pr = Proteobacteria and St = Streptophyta

Deinoccocus Thermus phyla who was almost 12 times more relatively abundant in Young compared to Litter (Figure 4.3 A). *Thermobifida* was the most relatively abundant genera in Young as well, with 8,87 % of ESVs identified at the genus of species level.

Several ESVs belonging to the phyla Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria had significantly lower relative abundance in Middle compared to Young (Figure 4.3 B). These included members of the genera *Corynebacterium, Mycobacterium, Thermobifida*, and *Thermobispora* from the phylum Actinobacteria*, Rhodothermus* from the phylum Bacteroidetes, *Geobacillus, Bacillus* and *Planifilum* from the phylum Firmicutes and Devosia and Pseudomonas from the phylum Proteobacteria. However, 4 ESVS identified as archaea (Methanoculleus and Methanosarcina) were more relatively abundant in Middle than in Young, as well as two ESVs from the genus Thermomonospora and two from the genus *Ruminoclostridium.* The genera *Thermomonospora*, *Methanosarcina*, *Bacillus* and *Kouleothrix* have the highest relative abundance in Middle (Figure 4.2 C). Changes between the Middle and Aged phases include the decrease in relative abundance of Firmicutes and Actinobacteria (Figure 4.4 A). There are 34 ESVs belonging to Firmicutes, i.e. members of the genera *Bacillus, Brevibacillus, Clostridium, Cohnella, Geobacillus, Paenibacillus, Symbiobacterium, Thermobacillus* and *Ureibacillus* who were present at a lower relative abundance in Aged compared to Middle while no Firmicutes increased in relative abundance in Aged compared to Middle. Several members of the Actinobacteria were present at a relatively lower abundance in Aged compared to Middle, including some belonging to the genera *Actinomadura, Saccharomonospora, Saccharopolyspora, Streptomyces, Thermobifida* and *Thermobispora*. On the other hand, an increase in relative abundance of one ESV belonging to the phylum Spirochaetes (Treponema_stenostreptum_1), one from to the phylum Ignavibacteriae (Ignavibacterium_album_1) and one archaeon (Methanobacterium_formicicum_1) as well as some Actinobacteria were observed.

Figure 4.4 - Differentially abundant ESVs between each successive phase (A: Middle vs. Aged and B: Aged vs. Compost). The diagrams present the number of differentially abundant ESVs between phases as well as the proportion of higher ESVs in each phase. Fold change (FC log2) denotes relative differences in relative abundance between groups (DESeq2). In A, the blue dots correspond to the most abundant bacteria in Middle and the orange dots to the most abundant bacteria in Aged. In B, the orange dots correspond to the most abundant bacteria in Aged and the purple dots to the most abundant bacteria in Compost. Species are grouped by phylum per comparison (A = Actinobacteria, B = Bacteroidetes, C = Chloroflexi, E = Euryarchaeota, F = Firmicutes, I = Ignavibacteriae, Pl = Planctomycetes, Pr = Proteobacteria, Sp = Spirochaetes, St = Streptophyta and T = Thaumarchaeota

The genera with the highest relative abundance in Aged are *Thermomonospora*, *Steroidobacter, Chryseolinea*, *Bacillus* and *Methylocaldum* (Figure 4.2 C).

Finally, only a few ESVs were differentially abundant between Aged and Compost and the majority were present at a higher relative abundance in Aged compared to Compost (Figure 4.4 B), although the Compost phase showed the highest bacterial specie richness of all the studied phases (Figure 4.2 D). In particular, there was a decrease in the relative abundance of 5 ESVs belonging to the Firmicutes in Compost, namely members of the genera *Brevibacillus, Lysinibacillus, Oceanobacillus* and *Ruminoclostridium* alongside two archaea, members of the Thaumarchaeota (Nitrosotenuis_cloacae_1) and Euryarchaeota (Methanobactrium_formicicum_1), a Planctomycetes (Thermogutta_terrifontis_1), an Ignavibacteriae (Ignavibacterium_album_1) and a Chloroflexi (Thermomicrobium carboxidum_1). The Compost phase is characterized by the increase in relative abundance of ESVs from the Proteobacteria phylum such as *Hyphomicrobium*, *Geobacter, Comamonas, Devosia, Pseudomonas* and *Polaromonas*, from the Actinobacteria phylum such as *Nocardioides, Actinotalea* and *Cryobacterium* and finally an ESV belonging to the Bacteroidetes, namely Flavobacterium_degarlachei_1.

A total of 607 ESVs could be annotated to genera or species with 100 % identity out of 2330 ESVs observed in this phase, which is equivalent to 241 different genera. The ten most relatively abundant were *Chryseolinea, Bacillus, Conexibacter, Geobacillus, Thermomonospora, Hyphomicrobium, Methanosarcina, Symbiobacterium, Cryobacterium* and *Brevibacillus* (Figure 4.2 C). Finally, the ESVs identified at the species level with 100 % identity without ambiguous annotation with the highest relative abundance are Thermomonospora_chromogena_1, Symbiobacterium_thermophilum_1, Sphaerobacter thermophilus_1, Ureibacillus thermosphaericus_1 and Planifilum_yunnanense_1 and accounts for 2.14 % of the total ESVs.

4.4.3 Microbial carbon dynamic

4.4.3.1 Cellulose degradation

Fifty-two differently abundant ESVs could be annotated as species associated with cellulose degradation. These included species within Firmicutes (21), Actinobacteria (19), Proteobacteria (6), Bacteroidetes (3), Chloroflexi (2) and Deinoccocus Thermus (1) (Figure 4.5). Cellulose degraders were abundant in the early thermophilic phase with fifteen ESVs with higher relative abundance in Young compared to the Litter, such as Bacillus_coagulans_1, Bacillus_borbori_1 and Nocardioides_aesturi_1 and. A total of sixteen ESVs observed at a relatively lower abundance in Middle compared to Young and twenty-four were at a lower relative abundance in Aged compared to Middle. Six ESVs were at a lower relative abundance in Middle compared to Young and two were at a higher relative abundance in Aged compared to Middle. Cellulose degraders were present in the mature Compost with four ESVs being at a higher relative abundance compared to Aged (Devosia_honganensis_1, Sorangium_cellulosum_2, Actinotalea_ferrariae_1 and Norcardioides aestuari_1) while 6 were found at a lower relative abundance in Compost compared to Aged.

4.4.3.2 Hemicellulose degradation

Forty differently abundant ESVs annotated as putative species with hemicellulose degradation potential belonged to Firmicutes (20), Actinobacteria (11), Bacteroidetes (3), Proteobacteria (2), Chloroflexi (1), Deinococcus Thermus (1) and Planctomycetes (1) (Figure 4.6). Twelve ESVs had higher relative abundance in Young compared to Litter, including Thermopolyspora_flexuosa_1, Thermobispora_bispora_1, Bacillus coagulans_1, Sorangium cellulosum_1 and three Paenibacillus species. Thermostaphylospora_chromogena_1, Thermomonospora curvata_1 and Ruminoclostridium_thermosuccinogenes_1 were found at a higher relative abundance in Middle compared to Young while 6 ESVs presented a lower relative abundance in Middle compared to Young. 19 ESVs showed a lower relative abundance in Aged compared to Middle. Kofleria_flava_1 was at a higher relative abundant

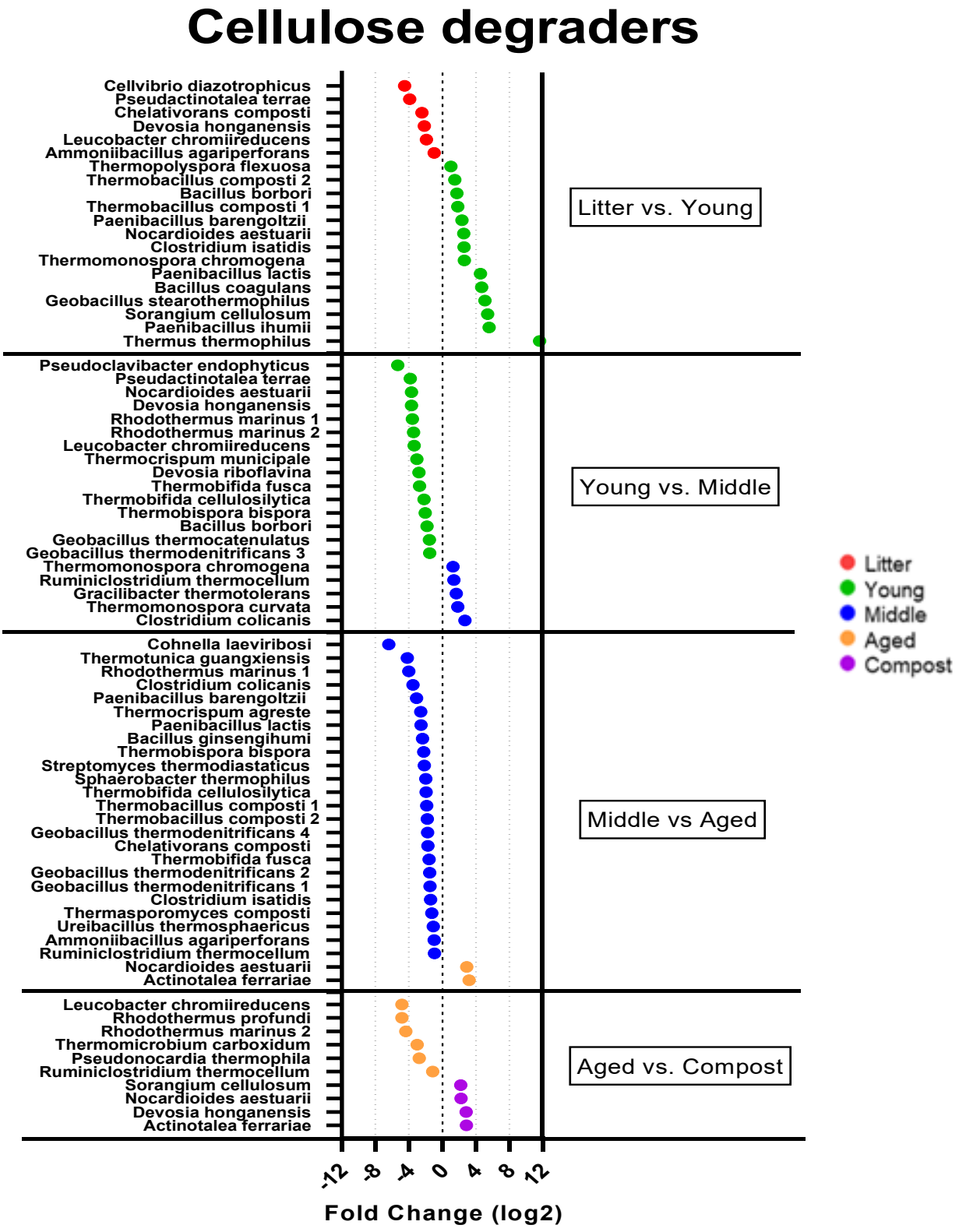

Figure 4.5 - Differently abundant putative species involved in cellulose degradation. Fold change (FC log2) denotes relative differences in relative abundance between successive sampled phase (DESeq2); Litter vs. Young, Young vs. Middle, Middle vs. Aged and Aged vs. Compost.

in Aged compared to Middle while no significant differentially abundant ESVs with putative hemicellulose degradation potential was identified between Aged and the mature Compost.

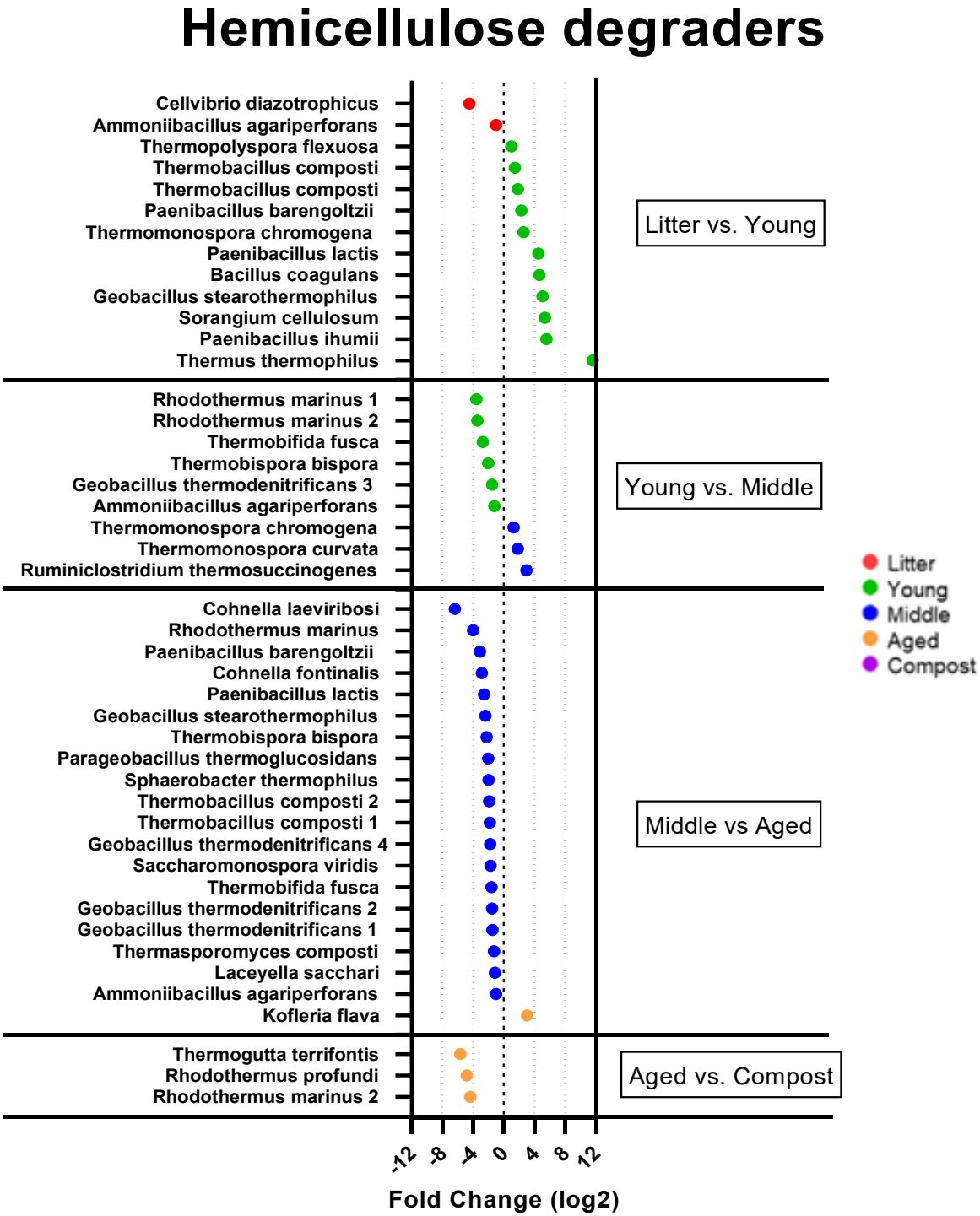

Figure 4.6 - Differently abundant putative species involved in hemicellulose degradation. Fold change (FC log2) denotes relative differences in relative abundance between successive sampled phase (DESeq2); Litter vs. Young, Young vs. Middle, Middle vs. Aged and Aged vs. Compost.

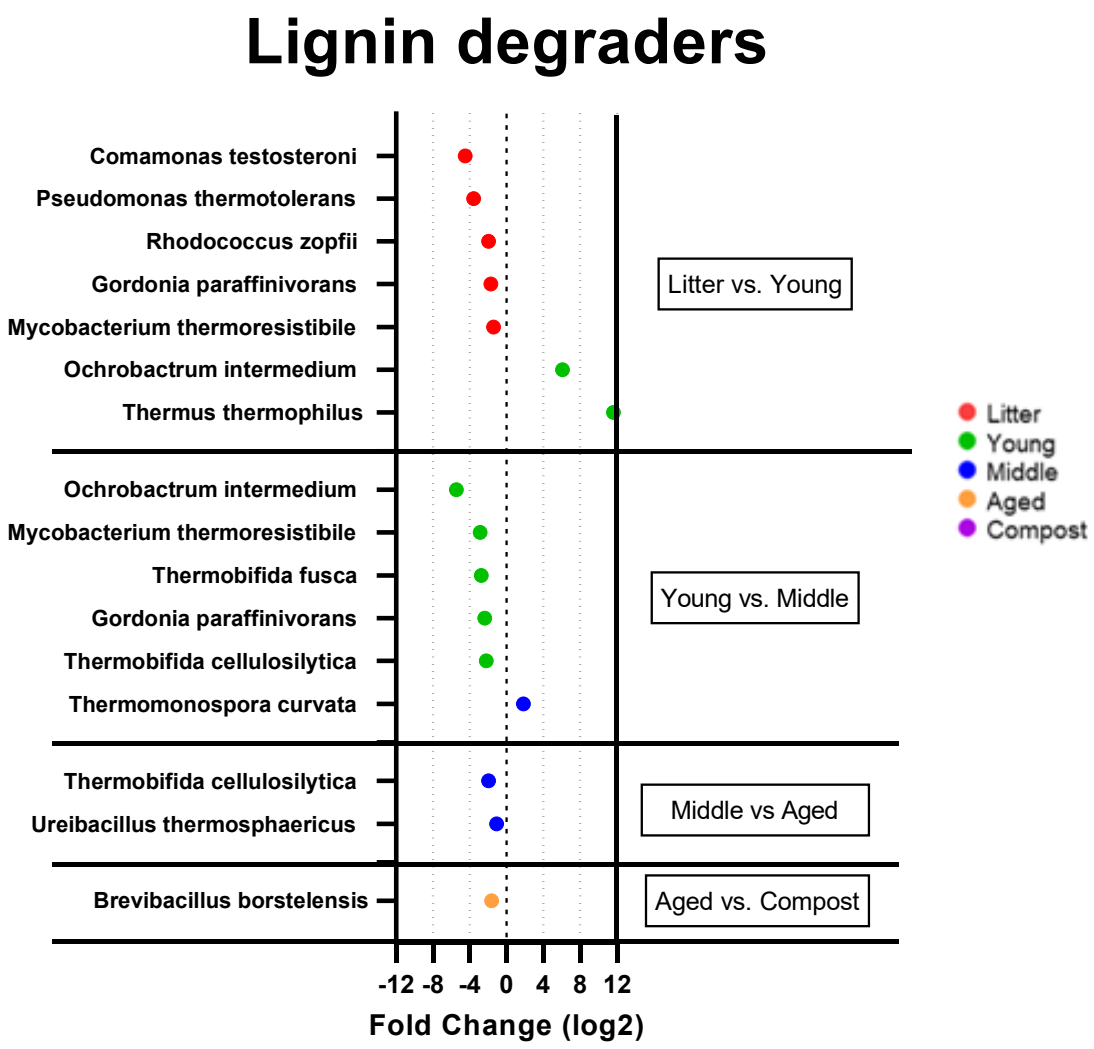

Figure 4.7 - Differently abundant putative species involved in lignin degradation. Fold change (FC log2) denotes relative differences in relative abundance between successive sampled phase (DESeq2); Litter vs. Young, Young vs. Middle, Middle vs. Aged and Aged vs. Compost.

4.4.3.3 Lignin degradation

A total of 12 ESVs annotated as putative species with lignin degradation potential were identified, belonging to the phyla Actinobacteria (6), Proteobacteria (3), Firmicutes (2) and Deinococcus Thermus (1) (Figure 4.7). Of these, five were found at a higher relative abundance in Litter compared to Young and corresponded to Mycobacterium_thermoresistible_1, Gordonia_paraffinivorans_1, Rhodococcus zopfii_1, Pseudomonas_thermotolerans_1 and Comamonas_testosteroni_1. Ochrobactrum intermedium_1 and Thermus_thermophilus_1 were at a higher relative abundance in Young

compared to Litter while Thermomonospora_curvata_1 was at a higher relative abundance in Middle compared Young. Five ESVs, namely Ochrobactrum_intermedium_1, Mycobacterium thermoresistible_1, Thermobifida_fusca_1, Thermobifida_cellulosilytica_1 and Gordonia paraffinivorans_1 were at a lower relative abundance in Young compared to Middle, Thermobifida_cellulosilytica_1 and Ureibacillus_thermosphaericus_1 were at a lower relative abundance in Aged compared to Middle and Brevibacillus_borstelensis_1 was at a lower relative abundance in Compost compared to Aged. Symbiobacterium _thermophilum_1 have lignolytic degradation potential and although it was not significantly differentially abundant, it was present in high abundance in each phase, particularly in Litter, Young and Compost.

4.4.3.4 Methanogens and methylotrophs community

Differently abundant ESVs from putative methanogens belong to five different families, namely Methanobacteriaceae (5), Methanomicrobiaceae (3), Methanosarcinaceae (3), Methanocellaceae (1) and Methanosaetaceae (1) (Figure 4.8). Two ESVs from the Methanobrevibacter genus isolated from horse faeces (GenBank: AB739324.1) were present in a higher relative abundance in Litter compared to Young. Methanocellaceae_1 was found at a higher relative abundance in Young compared to Litter while five ESVs were found at higher relative abundance in Middle compared to Young and another five ESVs in Aged compared to Middle. Methanobrevibacter_1 was found to be at a relatively lower abundance in Middle compared to Aged while no significant decrease in ESVs relative abundance was found in Aged compared to Middle. No significant increase in relative abundance was monitored in Compost compared to Aged. Four ESVs, including Methanobacterium_formicicium_1 and Methanothrix_thermoacetophila_1 were at a lower relative abundance in Compost compared to Aged.

The differently abundant ESVs identified as methylotrophs belonged to four Orders within Proteobacteria: Methylococcales (8), Rhizobiales (4), Nitrosomonadales (3), and Rhodobacterales (1) (Figure 4.8). One ESV identified as Methylomirabilaceae_1 was an exception, belonging to the candidate phylum Rokubacteria. Methyloccocales_1 had a

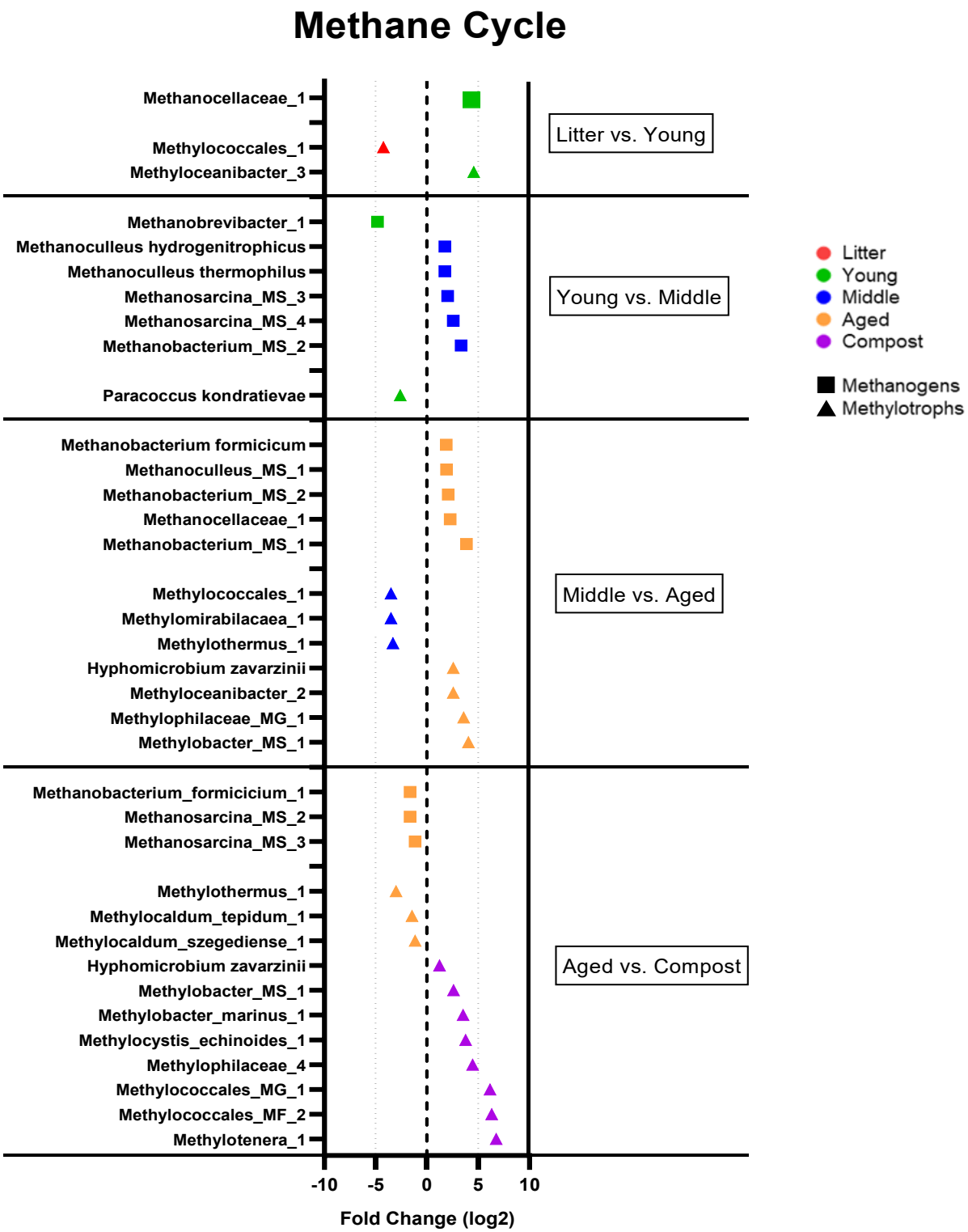

Figure 4.8 - Differently abundant putative species involved in methane production and oxidation. Fold change (FC log2) denotes relative differences in relative abundance between successive sampled phase (DESeq2); Litter vs. Young, Young vs. Middle, Middle vs. Aged and Aged vs. Compost.

higher relative abundance in Litter compared to Young, while Methyloceanibacter_3 has a higher relative abundance in Young compared to Litter. Paracoccus_kondratievae_1 was at a lower relative abundance in Middle compared to Young. Two ESVs identified as Methylocaldum_szegediense_1 and Methylothermus_1 were in high abundance in Middle compared to Young without it being significant. Four ESVs were at a higher relative abundance in Aged compared to Middle while three were at a lower relative abundance in Aged compared to Middle. In addition, Methylocaldum_marinum_1 and Methylocaldum_tepidum_1 were in high relative abundance in Aged compared to Middle without it being significant. Five ESVs, including Methylocaldum_szegediense_1 and Methylocaldum_tepidum_1 were at a lower relative abundance in Compost compared to Aged while Hyphomicrobium_zavarzinii_1, Methylobacter_marinus_1 and Methylocystis_echinoides_1 and five other ESVs were at a higher relative abundance in Compost compared to Aged.

4.4.4 Microbial nitrogen dynamics

4.4.4.1 Nitrate fixation and ammonification

Differently abundant ESVs from nitrogen cycling species were identified, including those involved in nitrogen fixation and ammonification, ammonia oxidation, nitrate oxidation and denitrification (Figure 4.9). Three differently abundant ESVs from species associated with nitrogen fixation were observed during the composting process. Cellvibrio_diazotrophicus_1 was in higher relative abundance in Litter compared to Young, Methanobacterium_formicicum_1 was in higher relative abundance in Aged compared to Middle and Methylocystis_echinoides_1 was in higher relative abundance in Compost compared to Aged.

Putative ammonifiers were the best represented group involved in nitrogen cycling, with a total of 102 differentially abundant ESVs. The ESVs belonged to the phylum Firmicutes (41), Actinobacteria (33), Proteobacteria (22), Bacteroidetes (4), Euryarchaeota (1) and Chloroflexi (1) (Figure 4.9). Of these, 12 were in higher relative abundance in the Litter compared to Young. A total of 23 ESVs were in higher relative in the Young phase compared

to Litter, while 27 were in higher relative abundance in Young compared to Middle, 40 were in higher relative abundance in Middle compared to Aged and 6 were in higher relative abundance in Aged compared to Compost. Nine identified ESVs were in higher relative abundance in Middle compared to Young, 9 were in higher relative abundance in Aged compared to Middle and another 9 were in higher relative abundance in Compost compared to Aged.

4.4.4.2 Ammonia and nitrite oxidation

Three differently abundant ESVs with putative ammonia oxidation potential were identified. Nitrosomonas_1, is in higher relative abundance in Aged compared to Middle and again in Compost compared to Aged (Figure 4.9). An ESV identified as the ammonia-oxidizing archaea *Nitrosotenuis cloacae* was also at a higher relative abundance in Aged compared to Middle but was then at a lower relative abundance in Compost compared to Aged. Nitrosomonas_2 was absent from every phase except for Compost, although the increase in abundance was not significant.

Along with the putative ammonia-oxidizing bacteria (AOBs), some previously mentioned differently abundant methane-oxidizing bacteria (MOBs) could also have the potential to oxidize ammonia. This includes Methylococcales_1 who was at a higher relative abundance in Litter compared to Young and at a lower relative abundance in Middle compared to Aged, Methylothermus_1 who was also at a lower relative abundance in Middle compared to Aged, Methylothermus_1 who was at a higher relative abundance in Aged compared to Middle and at a higher relative abundance in Aged compared to Compost. Methylocaldum_tepidum_1 and Methylocaldum_szegediense_1 were also at a higher relative abundance in Aged compared to Compost while Methylocystis_echinoides_1, Methylobacter_MS_1, Methylobacter_marinus_1, Methylococcales_MG_1 and Methylococcales_MF_2 were at a higher relative abundance in Compost compared to Aged.

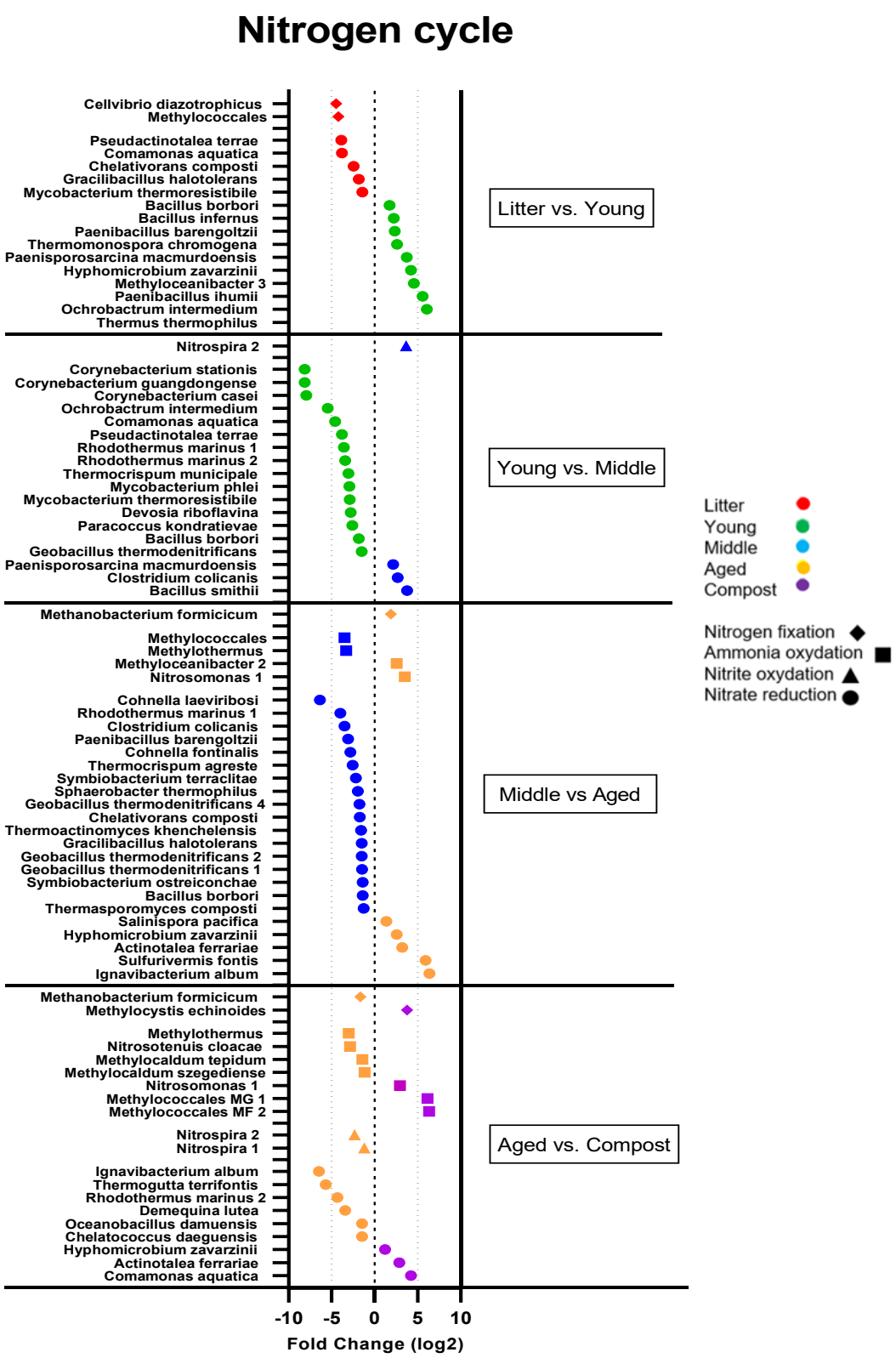

Figure 4.9 - Differently abundant putative species involved in nitrogen cycle. Fold change (FC log2) denotes relative differences in relative abundance between successive sampled phase (DESeq2); Litter vs. Young, Young vs. Middle, Middle vs. Aged and Aged vs. Compost

Three differently abundant ESV identified as putative nitrite oxidisers were present, namely Nitrospira_2 who was at a higher relative abundance in Middle compared to Young and then was at a lower relative abundance in Compost compared to Aged, along with Nitrospira_1 which was also at a lower relative abundance in Compost compared to Aged. ESV identified as Nitrospira japonica was only present in Compost, although the increase in abundance compared to Aged was not significant.

4.4.4.3 Denitrification

Forty-eight differently abundant ESVs could be annotated as species associated to nitrate reduction and denitrification. These included species within Firmicutes (19), Actinobacteria (14), Proteobacteria (11), Bacteroidetes (1), Chloroflexi (1), Ignavibacteriae (1) and Planctomycetes (1) (Figure 4.9). Five differently abundant ESVs with putative potential for nitrate reduction were at a higher relative abundance in Litter compared to Young such as Mycobacterium_theroresistible_1 and Chelativorans_composti_1. Nine ESVs, including Hyphomicrobium zavarzinii_1, Bacillus_infernus_1, Brevibacillus_brevis_1 and Geobacillus stearothermophilus_2 were at a higher relative abundance in Young compared to Litter. This was followed by a significant increase in relative abundance for 3 ESVs and a decrease for 16 ESVs in Middle compared to Young, including Corynebacterium_casei_1, Corynebacterium guangdongense_1, Corynebacterium_stationis_1, Geobacillus thermodenitrificans_3, and Mycobacterium phlei_1. Five ESVs, such as Salinispora_pacifica_1, Hyphomicrobium zavarzinii_1 and Sulfurivermis_fontis_1 were at a higher relative abundance in Aged compared to Middle while 17 were at a lower relative abundance in Aged compared to Middle, like Geobacillus_thermodenitrificans_1, _2 and _4, Thermoactinomyces_khenchelensis_1. Finally, Hyphomicrobium zavarzinii_1 and Comamonas_aquatica_1 were at a higher relative abundance in the mature Compost compared to Aged while seven ESVs had significantly lower relative abundance in Compost.

4.5 Discussion

4.5.1 Compost properties over time

The composting process studied led to the production of a compost considered mature and stable. The high temperatures measured during Young (67.8 °C) and Middle (62.1 °C) were accompanied with a significant decrease in the amount of organic matter at the beginning of the process (Figure 4.1 A, B). Indeed, the loss of organic matter over the same period reached 54 % of the initial values. The thermophilic phase, also called bio-oxidative phase, is often considered as the most active one, as the high temperatures enable to accelerate the reaction rates and the efficiency of the thermostable enzymes to reach an optimal lignocellulose degradation level (Ryckeboer et al., 2003). The substantial changes from Litter to Middle suggest that most of the organic matter decomposition occurs within the first three months under thermophilic conditions.

The three main plant structural components; cellulose, hemicelluloses and lignin were quantified at each phase to monitor their degradation (Figure 4.1 G, H and I). For all three constituents, the sharpest decrease occurred between Litter and Young, with a 57.5 % decrease in hemicellulose, 66.3 % decrease in cellulose and a 43.0 % decrease in lignin. A significant decrease between Young and Middle was observed for cellulose, but not for hemicelluloses and lignin. These results suggest that the majority of lignocellulose degradation occurred during the thermophilic stages, which is consistent with expected temperatures of 55-60 °C for optimal lignocellulolytic activity in composting (Tuomela et al., 2000). The large amounts of cellulose in the initial mixture would have required a longer active degradation period compared to hemicelluloses and lignin.

Ammonium increased in Young before a significant decrease was observed in Middle, while NO_2^--NO_3^- content increased slightly between Litter and Middle phase before decreasing between Middle phase and Compost, although nitrite and nitrate changes were not significant due to a high variability within compost phase (Figure 4.1 J). The increase in NH_4^+ content corresponds to the degradation of organic nitrogen from plant and horse

faeces material and is expected during the thermophilic phase (Bernal et al., 2009). The following decreases in NH_4^+ could be due to uptake by microorganisms, direct oxidation by ammonia oxidizing microorganisms (AOB/AOA), or through the loss by volatilization of NH_3. The slight accumulation of NO_2^--NO_3^- in the following phases and high reduction in total nitrogen suggests ammonia oxidation, volatilization and denitrification may be occurring (Figure 4.1 K).

4.5.2 General compost community

Bacterial diversity increased as composting phases progressed (Figure 4.2 C), with fewer species of higher relative abundance in early phases and a higher number of different bacterial species present at a lower relative abundance in the Compost. Differential abundance (DA) analysis also revealed that the most substantial changes in ESVS relative abundance occurred between Litter and Young (573 DA ESVs), and Middle and Aged phases (with 739 DA ESVs) (Figure 4.3). The high temperature during thermophilic phases is likely to have created strong selection and limited diversity (Ryckeboer et al., 2003), which translates into a strong difference in the relative abundance of bacteria before and after the thermophilic phase. However, previous research has suggested that limited resources of mature compost can create strong competition and limit diversity of bacteria (Antunes et al., 2016; de Gannes et al., 2013; Zhou et al., 2018) which might suggest that easily available carbon was still present by the end of the process, resulting from non-optimal composting.

Constrained ordination analysis performed using canonical analysis of principal coordinates (CAP) reveal that the groups of samples corresponding to the different sampled phases are all different from each other (Figure 4.2 D). The sample groups are all perfectly separated from each other as a function of sampled pile age (x-axis) and possibly temperature (y-axis). Samples from the thermophilic phases were clustered closer together at the y-axis level, as were samples from the Litter, Aged and Compost phases. These results reflect the major factors shaping the dynamics of bacterial communities, such as age, decomposition rate and abundance of initial plant material as well as temperature which is decisive in the enrichment of bacteria.

The 341 genera identified in all phases belong to 26 different phyla, of which Firmicutes, Actinobacteria, Proteobacteria and Chloroflexi represent 69 % of the total diversity. These 4 phyla are almost systematically pointed out as the most relatively abundant in compost, but usually represent a larger proportion of the total diversity, ranging from 72 % to 92 % (Antunes et al., 2016; Partanen et al., 2010; Wei et al., 2018; Zhou et al., 2018). This observation suggests that the amplification and annotation steps allowed for a greater diversity of microorganisms to be retrieved, which could be the result of the primers or the bioinformatics pipeline used. This high diversity, as well as the variation in temperature and oxygen that can exist at different depths, makes the analysis of the interaction of bacteria with their environment very complex as bacteria can themselves adapt to a wide gradient of temperature and oxygen conditions (López-González et al., 2015).

The different composting phases studied were characterized by the relative abundance of different phyla. Overall, Actinobacteria represented a high proportion of organisms in Litter before experiencing a significant decrease in relative abundance in Middle (Figure 4.2). Firmicutes followed the colonization by increasing their presence very markedly in Young, and then decreasing drastically in Aged. Proteobacteria accounted for almost 50 % of the individual species in Litter, alongside Actinobacteria. Their relative abundance decreased rapidly in the first thermophilic phase and then recolonized the material during the Aged and Compost phases, making up 52.9 % and 61.5 % of the diversity of these phases respectively.

These general changes can be attributed to several factors, but temperature, oxygen level, and availability of readily available carbon possibly explain a large portion of the changes (Suler & Finstein, 1977). Although oxygen level has not been monitored, it can be expected to vary with temperature, since both abiotic factors vary through aeration, which allows heat to be removed and oxygen to be incorporated, and the activity of microorganisms, which must consume oxygen in order to be able to produce heat (Bernal et al., 2009). The organic matter being mainly composed of plant biomass, the relationship between the level of organic matter and the quantity of lignocellulose is thus made.

Actinobacteria, including genera such as *Thermomonospora*, *Thermobifida* and *Thermopolyspora* represented by the species *Thermomonospora curvata*, *Thermomonospora chromogena*, *Thermobifida fusca* and *Thermopolyspora flexuosa* are widely recognized for their tolerance to high temperatures and their appetite for the initial constituents of plant biomass such as cellulose and hemicelluloses (Satyanarayana et al., 2013). On the contrary, they are not tolerant to anaerobic conditions which can reduce their chances to proliferate when oxygen becomes scarce.

On the other hand, abundant members of the Firmicutes phyla during the Young and Middle phases such as *Geobacillus, Bacillus, Ureibacillus* and *Paenibacillus,* represented by *Geobacillus stearothermophilus, Geobacillus thermodenitrificans, Bacillus coagulans, Paenibacillus lactis* and *Ureibacillus thermosphaericus*, are minimally thermotolerant or thermophilic (Ryckeboer et al., 2003). They are also less competitive than Actinobacteria in degrading lignocellulose and adapt to a wider range of carbon sources, while also being generally more tolerant to anaerobic conditions, which allows them to succeed Actinobacteria at the end of the thermophilic phase, where easily assimilable carbon sources as well as oxygen are scarcer. The conditions of high temperatures, low oxygen levels and the appearance of by-products of lignocellulose degradation also favours the proliferation of a certain group of archaea, the methanogens, of which *Methanosarcina* is very abundant during Middle (Jäckel et al., 2005).

Finally, the Proteobacteria present in high abundance in the maturation phase and in Compost are predominantly mesophilic, aerobic and are not known to specifically feed on lignocellulose, such as members of the genera *Conexibacter* and *Chryseolinea*. However, Proteobacteria are very diverse and include genera such as *Methylocaldum* (Jäckel et al., 2005), recognized as strict aerobics using only methane as a carbon source but with several species that are thermotolerant or *Steroidobacter* which includes mesophilic organisms, not adapted to degrade lignocellulose but with some members that are strict anaerobic.

These interpretations are, of course, only partial and a multitude of other organisms that do not fit the rules utilized in the analysis are present, but given the abundance of bacteria discussed, this summarizes well the trends observed in the different phases.

4.5.2.1 Mature compost community

Some species and genera observed at high relative abundance in the mature Compost (Figure 4.2 B) or that showed a significant increase in abundance in the mature Compost compared to Aged (Figure 4.2 B) are known to be plant growth-promoting bacteria (PGPB). Several bacteria are known to offer resistance to different abiotic stresses (drought, salinity, hydrocarbons, heavy metals and herbicides), such as *Pseudomonas aeruginosa*, *Bacillus* sp., *Paenibacillus yonginensis, Actinotalea ferrariae*, *Geobacter thiogenes, Leucobacter chromiireducens, Conexibacter* sp., *G. thermodenitrificans* and *G. stearothermophilus* (Aguiar et al., 2020; Marchant & Banat, 2010; Morais et al., 2004; Nevin et al., 2007; Pieterse et al., 2014; Sukweenadhi et al., 2014).

Finally, many highly abundant bacteria in Compost are thermophile able to degrade lignocellulose, such as *T. chromogena*, *Symbiobacterium thermophilum*, *Sphaerobacter thermophilus*, *Ureibacillus thermophilus*, *U. thermosphaericus*, *Geobacillus* sp., *Bacillus* sp. and *Brevibacillus* sp. These bacteria, although possibly present in vegetative form, may play an important role in the biogeochemical cycles of nitrogen, carbon and sulfur when applied to soil. Soil thermophilic bacteria (STB), ubiquitous in many regions, have been the subject of research in recent years to gain a better understanding of their role (Santana & Gonzalez, 2015; Margarida et al., 2013). They are thought to be particularly important in maintaining essential functions in soils under conditions of intense heat or drought. Compost is said to modify soil bacteria by enhancing the growth of pathogen antagonistic bacteria and by altering soil physicochemical parameters, but little information is available on the exact mechanism leading to the suppression and direct supply of PGPB from compost (Bonanomi et al., 2007; Bonilla et al., 2012; Hoitink & Fahy, 1986; Termorshuizen et al., 2006).

4.5.3 Carbon degraders dynamics

Bacteria associated with the degradation of lignocellulosic material were found in all phases of the composting process. These included specialized species, such as the cellulose degraders *Brevibacillus brevis* and *P. lactis* (Ali et al., 2019; Rawway et al., 2018), and the laccase and lignin-peroxidase producer *U. thermosphaericus* (Okuda et al., 2008) as well as

a variety of generalist species such as *T. curvata* and *G. stearothermophilus* (Satyanarayana et al., 2013) that can degrade several different carbon-based compounds.

4.5.3.1 Cellulose as extended-release carbon source

The bacteria associated with the degradation of cellulose are concentrated in the Young and Middle phases. Fourteen species of bacteria with a potential for cellulose degradation were identified as higher in relative abundance in Young than in Litter (Figure 4.5) of which over 70 % belong to the phylum Firmicutes. This group includes species such as *Bacillus borbori*, *B. coagulans*, *G. stearothermophilus*, *Thermobacillus composti*, three *Paenibacillus* species and *Clostridium isatidis* (Ali et al., 2019; Fang & Wong, 1999; Makky, 2009; Odeniyi et al., 2009; Padden et al., 1999; Togo et al., 2016; Watanabe, 2007; Zainudin et al., 2013). The bacteria presenting a lower relative abundance in Middle compared to Young are comprised of 57 % of species belonging to the phylum Actinobacteria, like *Thermobispora bispora, T. fusca, Thermocrispum municipale* and *Pseudoactinotalea terrae* suggesting that they are abundant at the very beginning of the thermophilic phase but are gradually eliminated with the further decomposition of the organic matters (Cho et al., 2017; Korn-Wendisch et al., 1995; Liolios et al., 2010; Zainudin et al., 2013). The decrease of Firmicutes followed while they represented 57 % of bacteria being relatively less abundant in the Aged phase than Middle. Putative cellulose degraders are the only ones involved in the degradation of structural carbohydrates to experience an increase in their relative abundance in the Aged and Compost phases with the increase of Actinobacteria *A. ferrariae* and *Nocardioides aestuarii* in Aged compared to Middle and subsequently in Compost compared to Aged along with the Proteobacteria *Sorangium cellulosum* and *Devosia honganensis* (Li et al., 2013; Schneiker et al., 2007; Yi & Chun, 2004; Zhang et al., 2015). These observations suggest a preferential presence of bacteria responsible for the degradation of cellulose at the beginning of the thermophilic phase and an important contribution of members from the Firmicute and Actinobacteria phyla. Although a range of biotic and abiotic interactions could underlie these microbial shifts, some changes associated with temperature were observable as reductions in the cellulolytic species with optimal temperatures of 55-60 °C from earlier phases, such as *T. composti*, *Paenibacillus barengoltzii*, *C. isatidis, T. curvata*

and *T. chromogena* (Padden et al., 1999; Satyanarayana et al., 2013; Watanabe et al., 2007; Zainudin et al., 2013) and relative increases in species with optimal temperatures <35-40 °C in later phases, such as *A. ferrariae* and *N. aestuarii* (Li et al., 2013; Yi & Chun, 2004). Cellulose is the most abundant carbohydrate in the plant and therefore represents an important source of carbon for microorganisms and its structure also makes it more resistant to degradation and requires the sequential action of enzymes (endoglucanase, cellobiohydrolase and β-glucosidase) for glucose liberation (Béguin & Aubert, 1994). These characteristics probably explain, in part, the presence of a very large number of bacteria that have either a full or partial enzymatic system, far surpassing pectin and hemicellulose degraders covering the entire composting process from start to finish. Although biological degradation of cellulose has been shown to occur more efficiently under thermophilic conditions (Tuomela et al., 2000), these results expose the major activity happening regarding cellulose degradation during thermophilic stages.

4.5.3.2 Hemicellulose, an easily accessible carbon source

The bacteria putatively responsible for the degradation of hemicelluloses are concentrated in the Young and Middle phases, like the cellulose degraders, as an increase in their relative abundance is observed in the Young phase and a decrease in the Aged phase (04.6). For both groups, a strong majority of the bacteria following this trend belongs to the phylum Firmicutes, including the thermophilic species *T. composti*, *G. stearothermophilus*, *Paenibacillus ihumii* and *B. coagulans* who were found in a higher relative abundance in Young compared to Litter (Fang & Wong, 1999; Togo et al., 2016; Watanabe et al., 2007) and *Cohnella laeviribosi*, *Cohnella fontinalis*, *G. thermodenitrificans* and *Ammonibacillus agariperforans,* amongst 13 species belonging to Firmicutes who were in a lower relative abundance in Aged compared to Middle (Anand et al., 2013; Choi et al., 2020; Sakai et al., 2015; Shiratori et al., 2010). This suggests a primary role of thermophilic Firmicutes in the degradation of hemicellulose and aligns with the 56 % decrease in hemicellulose levels during Young phase. Hemicelluloses form a ubiquitous group of several compounds. They are present in high concentration in vegetables, fruits, and crops, e.g., soluble fibers. In

addition, hemicellulose is a major component of the primary cell wall, which hinder access to the cellulose rich secondary cell wall. This could explain why a big part of its degradation happens at the beginning of the process.

4.5.3.3 Early bacterial lignin degradation

Several bacteria known to secrete one or many lignin-modifying enzymes (lignin peroxidase, manganese peroxidase, versatile peroxidase, dye decolourizing peroxidases and laccases) have been observed in the system (Figure 4.7), such as the Dye-decolourizing peroxidases (DyPs) producers *T. fusca*, *T. curvata*, *Thermobifida cellulosilytica* and *Mycobacterium thermoresistible* (Chen et al., 2015; Rahmanpour et al., 2016; Tian et al., 2016), the lignin-peroxidase producer *Ochrobactrum intermedium* (Azizi-Shotorkhoft et al., 2016), the laccase producers *Pseudomonas thermotolerans*, *T. thermophilus*, and *Brevibacillus borstelensis* (Kurian & Kumar, 2015; Miyazaki, 2005; Muhonja et al., 2018) and finally, *Comamonas testosteroni* and *U. thermosphaericus* who can produce both peroxidases and laccases (Okuda et al., 2008; Rashid et al., 2017). As for carbohydrates degraders, putative lignin degraders were concentrated in the early composting stages with an important population present in Litter, namely *C. testosteroni*, *P. thermotolerans* and *M. thermoresistible* while *O. intermedium* and *T. thermophilus* who were in higher relative abundance in Litter compared to Young. Changes also occurred between the Young and the Middle phase where 4 species were in higher relative abundance in Young than Middle, such as *O. intermedium*, *M. thermoresistible*, *T. fusca*, and *T. cellulosilytica* while *T. curvata* was in higher relative abundance in Young compared to Middle. The number of differentially abundant bacteria in the following phases is very low, with two species found at a higher relative abundance in Middle than in Aged (*T. cellulosilytica* and *U. thermosphaericus*) one more abundant in Aged than in Compost (*B. borstelensis*). A significant decrease in lignin content was measured between Litter and Young, which could be partly due to the presence of most of the putative lignin degraders in the early phases. The decline in abundance of lignin degraders corresponds to the stabilization of lignin levels from the Middle phase (Figure 4.1), which could be related, or suggest that lignolytic

activity from bacterial action would be concentrated in the Litter and early thermophilic phase.

Lignin is generally considered to be a very recalcitrant element mostly degraded by white rot fungi during the maturation phase (Ryckeboer et al., 2003). The stabilization of lignin quantities does not point to a marked degradation in the maturation phase. As the fungi communities were not studied in this study, it is difficult to stipulate on the lignolytic activity occurring in the maturation phase but these results highlight the role of bacteria in lignin degradation at the beginning of the process (Tuomela et al., 2000).

4.5.3.4 Methanogens and Methylotrophs community

Methanogens represented 95 % of the identified Archaea throughout the composting phases and belonged to the families *Methanobacteriaceae, Methanocellaceae, Methanomicrobiaceae, Methanosaetaceae* and *Methanosarcinaceae* who proliferate at the end of the thermophilic phase and during the cooling phase (Figure 4.8). The Middle phase hosted the highest relative abundance of methanogens with the prevalence of the thermophilic methanogens *Methanoculleus thermophilus, Methanoculleus hydrogenitrophicus* and two unknown putative species within the *Methanosarcina* genus, which is presumably *Methanosarcina thermophila* (Ferry, 1993). *M. thermophila* would then be the methanogens with the higher relative abundance throughout the composting phases, which could be explained by its tolerance to temperature change, nutrient availability and oxygen (Thummes et al., 2007; Yamamoto et al., 2011).

As temperatures dropped in Aged, an increase in relative abundance of mesophilic methanogens, including *Methanobacterium formicum*, as well as putative species from the genera *Methanobacterium, Methanoculleus* and from the *Methanocellaceae* family, all of which have optimal growth temperature of 40 °C or lower (Dianou et al., 2001; Krivushin, et al., 2010; Mori & Harayama, 2011; Shcherbakova et al., 2011; Zhang, 1990). This shift from thermophilic to mesophilic methanogens suggests replacement of species within the methanogen functional niche which is presumably linked to temperature, and is possibly associated with changes in substrate availability, as acetotrophic methanogens in Middle,

whose representatives have the highest relative abundance were replaced by a predominantly hydrogenotrophic community in Aged (Ferry, 1993).

Methanotrophs, and more broadly methylotrophs, were observed at a higher relative abundance in the cooling and maturation phase (Figure 4.8). Annotated ESVs belonged to 9 different families, namely the *Methylomirabilaceae*, *Methylococcaceae*, *Methylothermaceae*, *Methylocystaceae*, *Methylobacteriaceae*, *Methyloligellaceae*, *Hyphomicrobiaceae*, *Rhodobacteraceae* and *Methylophilaceae* (Hanson & Hanson, 1996). The presence of these bacteria coincided with the proliferation of methanogens during the thermophilic phase, who are providing the substrate for the subsequent proliferation of methanotrophs. The majority of methylotrophs were found at a higher relative abundance in Aged compared to Middle and in Compost compared to Aged. The dynamics of replacement of the methylotrophic population could be due to the temperature, like observed by Halet et al., (2006), but also to the transformation of the substrate which favours different organisms. Synergistic and sequential interaction can be observed in our system as *Methylocaldum tepidum* and *Methylocaldum szegediense* are found at a higher relative abundance in earlier phase, probably consuming CH_4 and producing methanol which can then allow the proliferation of methylotrophs such as *Hyphomicrobium zavarzinii* found at a higher relative abundance during the cooling and maturation phase.

Although the dynamics of methanogens (Lee et al., 2010; Thummes et al., 2007) and methanotrophs (Halet et al., 2006; Jäckel et al., 2005) have been studied in compost environment, no studies report the co-occurrence of methanogenic and methanotrophic species throughout different composting phases. This new information is important for predicting the production and oxidation of methane in compost and thus minimizing greenhouse gas emissions from composting.

4.5.4 Nitrogen Dynamics

Species capable of ammonification and denitrification were present throughout the composting process, whereas only a limited number of nitrifying bacteria were identified and were concentrated in the final stages of composting.

4.5.4.1 Nitrogen fixation and Ammonification

The free-living nitrogen fixing bacteria from the *Rhizobiales* order, *Cellvibrio diazotrophicus* (Suarez et al., 2014), was present in Litter but significantly reduced in Young. The potential for nitrogen fixation was present throughout the composting process due to the methanogen *M. formicicum* (Magingo & Stumm, 1991), which increased in relative abundance from Middle to Aged compost and the methane-oxidizing bacteria *Methylocystis echinoides* (Hanson & Hanson, 1996) which increased from Aged to Compost (Figure 4.9). The presence of bacteria capable of nitrogen fixation could impact the nitrogen balance of the composting process and their presence in mature compost has the potential to influence the long-term fertilization impact when applied to soils.

Ammonifying bacteria with the ability to lyse proteins, DNA or other forms of organic nitrogen and turn them into ammonium through the action of exogenous proteases, were diverse and present at all stages of composting in line with evidence that ammonia is the preferred nitrogen source for most microorganisms throughout the composting processes (Körner & Stegmann, 2002) (Figure 4.9). A total of 23 species were more abundant in Young compared to Litter, while the following increases were more modest with only 9 species being more abundant in Middle compared to Aged. There was a decrease in relative abundance for 27 species from Young to Middle and 40 from Middle to Aged, further supporting that ammonifiers were concentrated in the thermophilic phase, and that ammonification was underway in the early stage of decomposition. This is coherent with evidence that ammonia is the preferred nitrogen source for most microorganisms throughout the composting process.

4.5.4.2 Ammonia oxidation

Ammonia oxidation is the first and rate-limiting step of nitrification where NH_4^+ is converted to NO_2^- under aerobic conditions. Only a limited number of bacteria and archaea were associated with ammonia oxidation, the ammonia-oxidizing archaea (AOA) *N. cloacae* and potentially uncharacterised ammonia-oxidizing bacteria (AOB) from within the genus *Nitrosomonas* (Nitrosomonas_1), who were significantly higher in relative abundance in the

Aged and Compost phases when compared to earlier phases (Figure 4.9). Nitrosomonas_1 shared 100 % sequence identity to *Nitrosomonas* sp. NM 33, first isolated from a soil in Japan (Koops et al., 1991). It was later studied by Purkhold et al. (2000) who detected it in a wastewater treatment plant and placed it in the cluster 5 of Nitrosomonas. The representatives of this group are all urease negative, halosensitives and prefer agricultural soils with neutral pH (Pommerening-Röser et al., 1996). This strain has never been isolated from compost before and is not fully characterized so any phenotypic information is available. *N. cloacae* was only isolated in 2015 from a wastewater treatment plant in China (Li et al., 2016). It's a non-halophilic, neutrophilic mesophile with a temperature growth range between 25 °C and 33 °C. The analysis of its genome also pointed toward its possession of a flagellum. Besides cell mobility, archaeal flagella could facilitate the formation of biofilms which could then allow *N. cloacae* to strive in compost environment (Li et al., 2016).

The absence of well-characterized ammonia-oxidizing bacteria throughout the process and the absence of ammonia-oxidizing archaeon prior to the Aged phase are consistent with the stable level of nitrite and nitrate recorded from Litter to mature Compost. However, given the dynamism of the redox reactions of inorganic nitrogen, it is likely that nitrification occurred but did not lead to nitrate accumulation. Furthermore, as some versatile methylotrophs can also oxidize ammonia (Hanson & Hanson, 1996; Lebedeva et al., 2005), it is possible that some abundant organisms, such as *M. szegediense* and *M. tepidum,* could have driven ammonia oxidation during the thermophilic phase.

4.5.4.3 Nitrite oxidation

The nitrite oxidizing bacteria (NOB) *N. japonica* increased in relative abundance from Aged phase to mature Compost phase (being below detection in other phases). In a recent review, Cáceres et al. (Cáceres et al., 2018) highlighted some of the shortfalls in our understanding of nitrification and concluded that future research should explore uncultured *Nitrospira* bacteria in composting. Intriguingly, uncharacterised ESVs placed within the genera *Nitrospira* (Nitrospira_1 and Nitrospira_2) were differentially abundant and increased in relative abundance from Young to Middle (Figure 4.9). The ESV Nitrospira_1 shared 100 %

sequence identity with an uncultured soil bacterium (GenBank EF667461.1) and was most closely related to *Nitrospira japonica* strain NJ11 (98.01 %), while the ESV Nitrospira_2 shared 100 % sequence identity with an uncultured soil bacterium (GenBank EU012235.1) and shared most sequence similarity to *Nitrospira* sp. KM1 (97.61 %), a novel nitrite-oxidizing *Nitrospira* strain isolated from a drinking water treatment plant (Fujitani et al., 2020). While caution is necessary when speculating as to the function based on culture independent sequencing of metagenomic samples, these putative uncultured bacteria could represent new thermotolerant/thermophilic nitrite oxidizing *Nitrospira* species which could have played a role in nitrogen transformation**.**

4.5.4.4 Nitrate reduction and Denitrification

Denitrification is a complex reaction that can be considered complete, leading to the production of N_2, or incomplete, resulting in intermediate nitrogen forms, such as NO_2^-, NO and N_2O. The largest increase in nitrate reducing bacteria was found between Litter and Young, where 9 species were found to increase in relative abundance (Figure 4.9). Subsequently, relative abundance of nitrate reducers sequentially decreased from Young to Middle and from Middle to Aged indicating preferential denitrification during thermophilic phases.

Thermophilic denitrifiers able to perform complete (*G. thermodenitrificans, S. thermophilus* and *Rhodothermus marinus*) and incomplete (*Pseudomonas taiwanensis*) denitrification were present in higher relative abundance in Middle compared to Aged while mesophiles performing complete (*P. aeruginosa* and *H. zavarzinii*) and incomplete (*Ignavibacterium album*) were present at a higher relative abundance in Aged and Compost (Chen et al., 2002; Sanford et al., 2012). Additionally, *R. marinus* and *S. thermophilus* are both considered to be strictly aerobic but possesses the *NirK* genes that allow them to reduce N_2O to NO suggesting a potential for aerobic denitrification while *P. aeruginosa* and *I. album* are able to grow anaerobically with NO_3^- as the only oxygen source (Sanford et al., 2012). Despite the prevalence of nitrate reduction under anoxic conditions, denitrification in the presence of oxygen has also been observed (Gao et al., 2010).

The proliferation of methanotrophic organisms such as *M. marinum* and *Methylosarcina lacus* in the Aged and Compost phases could also have played a role in the slight decrease of NO_3^- concentrations after Middle phase (Figure 4.8). These bacteria can use NO_3^- as a source of nitrogen as well as supress nitrifiers through competition for oxygen in low oxygen conditions (Megraw & Knowles, 1987) as well as providing carbon for various denitrifiers, such as *H. zavarzinii* (Amaral et al., 1995).

This goes to show that, although the enzymatic potential of all the other identified nitrate reducers has not been studied, their high prevalence and the diversity of pathways observed on a small fraction of the community suggests potential aerobic and anaerobic as well as complete and incomplete denitrification during composting with a particularly high occurrence in the thermophilic phase. A better understanding of the dynamics of denitrifying populations and their metabolism could help evaluate the N_2O balance from composting systems and actual risk to the environment. A reduction of anoxic zone and thus an adequate turning of the composting pile might help to minimize nitrate reduction during period of high oxygen demand. While complete denitrification leading to N_2 production is not harmful to the environment, nitrogen loss during composting should be avoided as much as possible so as not to reduce the agronomic qualities of the compost.

4.6 Conclusion

Organic matter content gradually reduced as expected over the two years of a windrow-based composting process. Lignocellulose and ammonia content rapidly reduced in the first month of composting at the height of thermophilic phase, while nitrite and nitrate content remained stable throughout the process. Tracking species-level changes in bacteria and archaea across the two years composting phases revealed widespread community shifts through early thermophilic stages, aging mesophilic stages and maturation of compost. Lignocellulose degrading species, including candidate bacteria which could play a role in lignin degradation, were concentrated at the early thermophilic composting stages and corresponded to measured reductions in cellulose, hemicellulose and lignin. Methanogenic archaea and methanotrophic bacteria were present throughout the composting process but

were highly dynamic in terms of species. Similarly, species capable of ammonification and denitrification were present throughout and highly numerous, whereas only a limited number of nitrification bacteria were identified and were concentrated in the final stages of composting. Although the only nitrite-oxidizing species identified, *Nitrospira japonica*, was enriched in later maturing compost, a number of poorly characterized sequences sharing close similarity to *Nitrospira* were enriched early thermophilic stages and could represent thermotolerant nitrite-oxidisers. An improved understanding of the dynamics of these methanogenic, methanotrophic and denitrifying populations could help to better control greenhouse gas emissions, such as methane and nitrous oxide, from composting systems and their associated risks to the environment. Similarly, identification of microbial species enriched within the final mature compost included species with well-characterized nitrogen fixing, methanotrophic as well as plant growth-promoting activities which could help to inform how compost could provide soils with a rich microbial reservoir of potential benefit to agriculture.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

V. Grenier: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition **N. J. B. Brereton:** Conceptualization, Validation, Data curation, Writing - Review & Editing **E. Gonzalez:** Methodology, Software, Data Curation and Writing - Review & Editing **F. E. Pitre:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

Acknowledgements and funding

Special thanks to Jocelyne Ayotte for her help with the total carbon and nitrogen and fibers analyses and Joan Laur for her forever support. We are grateful to the Montreal Botanical Garden for giving access to their composting platform. This research was made possible by financial support from the Fonds de recherche du Québec – Nature et technologies

(FRQNT), Mitacs Acceleration program and Ramo to the first author scholarship (PhD program

Supplementary data

Supplementary tables S1-S6 can be found at:

https://www.webdepot.umontreal.ca/Usagers/p0915153/MonDepotPublic/Supplementary%20Data%20Chapitre%204

Table S4.1 – Column heading for ESV and DA Table

Table S4.2 –ESVs across all phases

Table S4.3 – Differently abundant ESVs between Litter and Young

Table S4.4 – Differently abundant ESVs between Young and Middle

Table S4.5 – Differently abundant ESVs between Middle and Aged

Table S4.6 – Differently abundant ESVs between Aged and Compost

Supplementary Figures

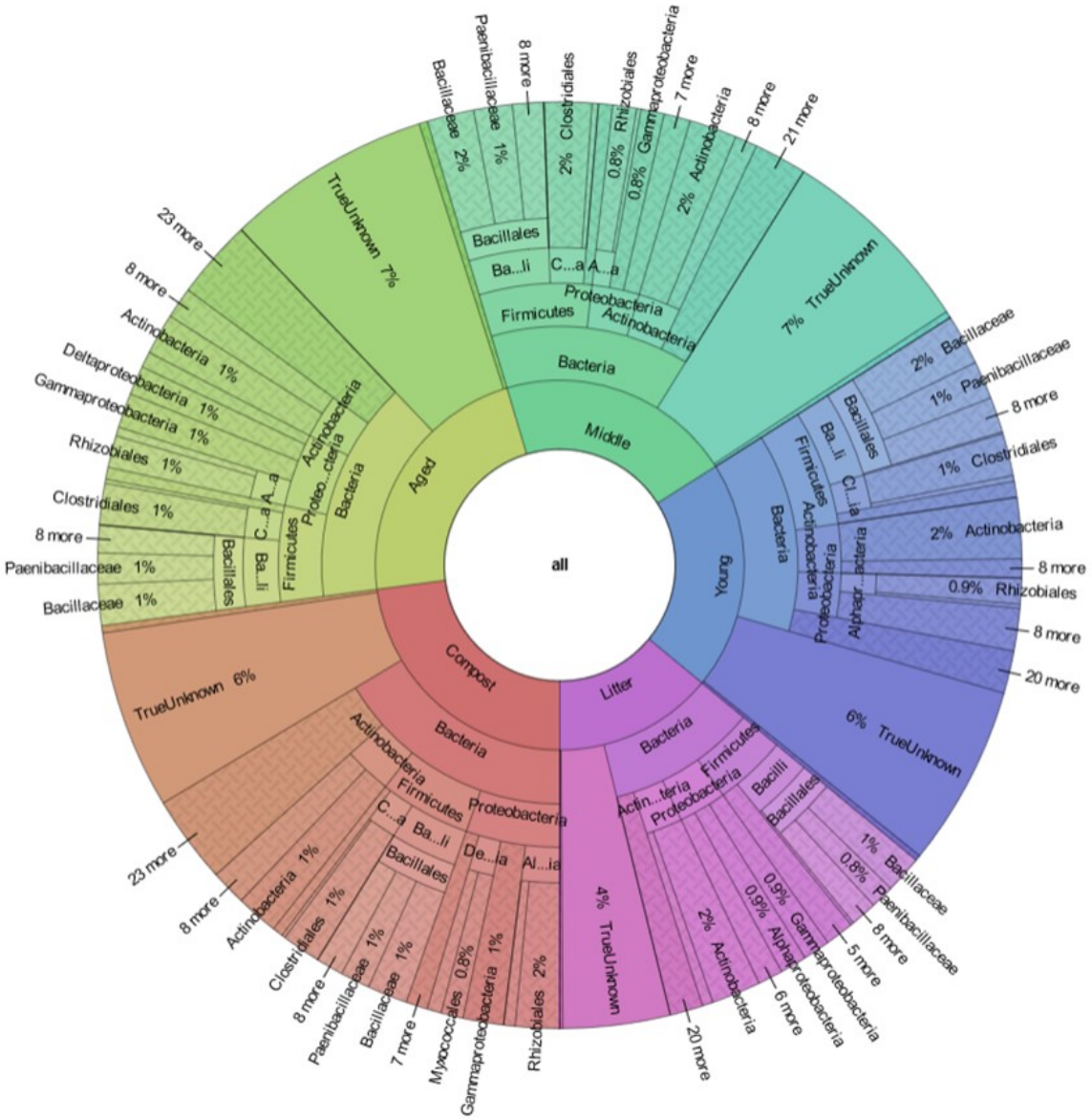

Figure S4.1 - Total amplified community makeup Krona chart (rlog abundance) presenting an overview of 2612 ESVs (Ondov et al., 2011). Interactive charts are available at: https://www.webdepot.umontreal.ca/Usagers/p0915153/MonDepotPublic/Supplementary%20Data%20Chapitre%204/rlog_abundance_Condition.html

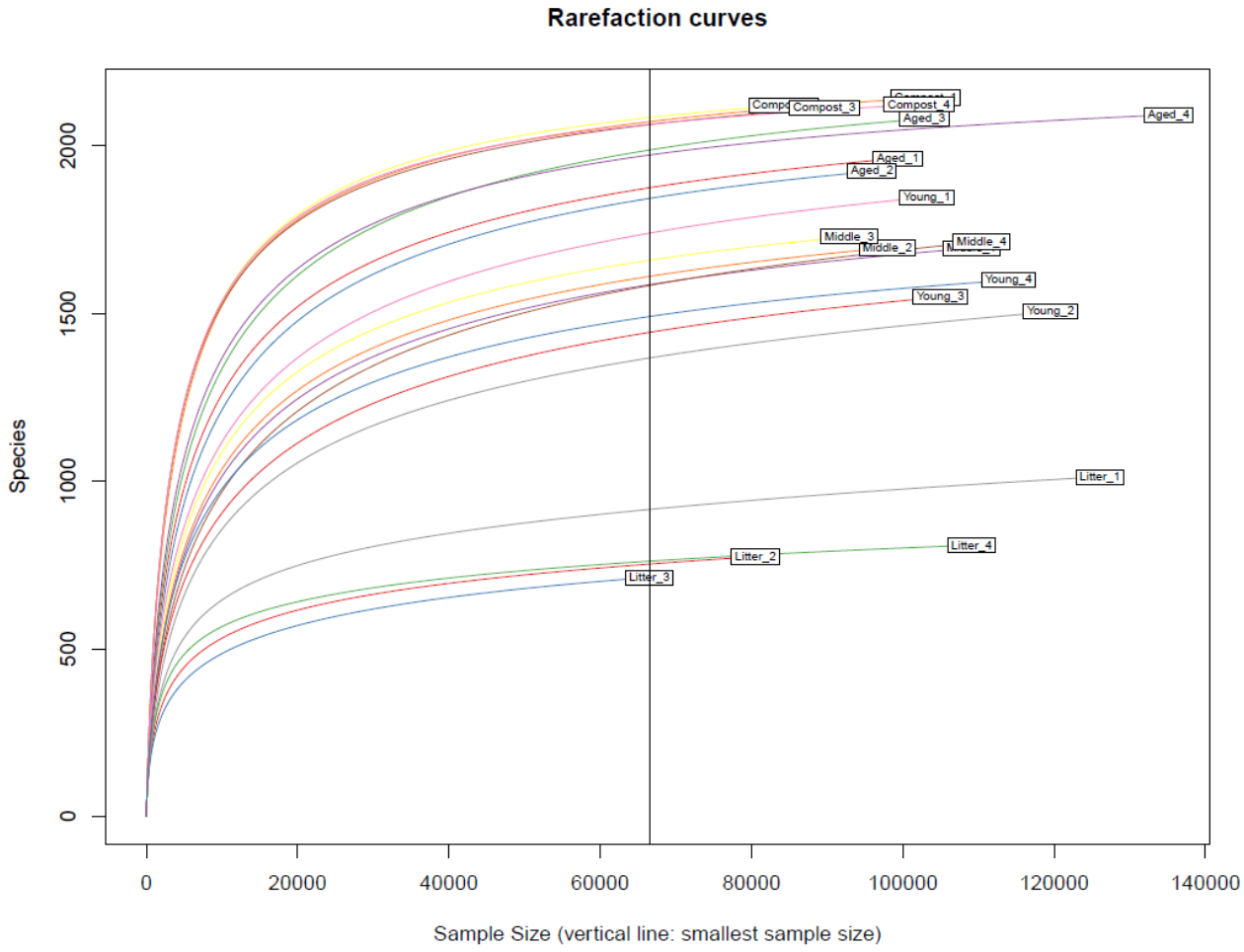

Figure S4.2 - Rarefaction curves showing describing number of ESVs detected versus number of reads sampled for the Litter, Young, Middle, Aged and Compost samples (n = 4)

Champ de maïs traité au glyphosate à La Présentation en Montérégie

Chapitre 5 – Le glyphosate a un effet négligeable sur la diversité bactérienne et la dynamique des communautés durant le compostage

Glyphosate has a negligible impact on bacterial diversity and community dynamics during composting

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In preparation

Abstract

The risks related to the introduction of contaminated organic material on composting sites are significant and, in addition to the chance of producing unsafe compost, the contaminants can also have harmful effects on the microorganisms responsible for the decomposition of the organic material during the process. Glyphosate, an herbicide that can also be harmful to bacteria, has several potential entry points to composting sites and its impact on composting processes has not yet been evaluated. In order to assess the impact of glyphosate on bacterial species diversity and abundance as well as on community composition and dynamics, we performed a mesocosm-size vessel experiment where 150 mg kg^{-1} of analytical grade glyphosate was added to a mixture of organic plant residues and horse and sheep manure. A related study showed no effect of glyphosate on the evolution of physicochemical properties during composting while glyphosate was completely dissipated at the end of the experiment. In this follow-up paper, sampling at days 0, 2, 28 and 112 of the process followed by 16S rRNA amplicon sequencing also found no effect of glyphosate on species richness and bacterial community composition. Differential abundance analyses revealed the decrease of a few different taxa in the presence of glyphosate, including the genus *Haloplasma* at T2 and *Sphaerobacter* at T28. Most differences in abundance were measured between the different sampling times, not between treatments. It was not possible to target resistant species that would have taken advantage of this toxic agent to proliferate or participate in its degradation. These results present glyphosate as a poor determinant of species recruitment during composting.

5.1 Introduction

Composting, an anthropic process based on the natural enzymatic decomposition of biomass by bacterial and fungal activity, is slowly gaining a foothold as a way to valorize organic matter of municipal origin and alleviate negative impact of landfilling on human health and green house gas emission (Alam & Ahmade, 2013; Haug, 1993; Lou & Nair, 2009). The sources of organic matter (OM) for composting are very diverse. It includes food waste,

green waste from gardens, parks, roadside and pruning, digestate from biomethanation plants, a variety of animal manures, and sewage sludge (Bernal et al., 1998; Bustamante et al., 2012; Reyes-Torres et al. 2018). Although guidelines are in place to implement the collection of organic residues and ensure a standard quality of OM at composting sites, many pollutants such as trace elements, petroleum hydrocarbons, and emerging contaminants are incidentally incorporated (Brändli et al., 2007; Epstein et al., 1992; Racke & Frink, 1989; Stevens et al., 2003; Wild et al., 1991).

One such contaminant is glyphosate N-[phosphonomethyl]glycine), the world's best-selling herbicide (Duke & Powles, 2008), which is found on plant residues of various origins and in sewage sludge (Ghanem et al., 2007; Rampoldi et al., 2011; Struger et al., 2015). Glyphosate is marketed for its herbicidal action where it disrupts the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) enzyme of the shikimate pathway, the site of aromatic amino acid biosynthesis (Duke & Powles, 2008) but the shikimate pathway is also active in bacteria and fungi. Thus, glyphosate can very well have an adverse effect on the microorganisms responsible for the decomposition of organic matter during composting.

The main degradation pathway of glyphosate is nevertheless biological and driven by bacteria (Bentley, 1990). In soils, glyphosate is primarily converted to AMPA by the action of the enzyme glyphosate oxidoreductase (GOX), while the enzyme C-P lyase, produces sarcosine and phosphate (Feng et al., 2020; Zhan et al. 2018). A number of bacteria capable of metabolizing glyphosate through the action of either of these enzymes have been isolated from contaminated soil samples, including members of the genera *Achromobacter, Agrobacterium, Alcaligenes, Aminobacter*, *Arthrobacter, Bacillus, Comamonas, Enterobacter, Flavobacterium, Geobacillus, Lysinibacillus, Ochrobactrum, Pseudomonas, Sinorhizobium* and *Streptomyces* (Firdous et al., 2020; Gorodylova et al., 2021; Hove-Jensen et al., 2014; Ouided & Abderrahmane, 2013; Singh et al., 2020; González-Valenzuela & Dussán, 2018.; Zhan et al., 2018), while most of them were also encountered in compost (Grenier et al., in preparation). Some members of *Arthrobacter, Bacillus, Sinorhizobium* and *Pseudomonas* genera have also been associated with AMPA degradation (Firdous et al.,

2020; Hove-Jensen et al., 2014; Ouided & Abderrahmane, 2013; Singh et al., 2020; Zhan et al., 2018).

No studies on the effect of glyphosate on microorganisms present during composting have been conducted so far, but most of the work done on glyphosate-treated agricultural and forest soils has not demonstrated an effect of glyphosate on bacterial diversity and community composition (Lane et al., 2012; Lupwayi et al., 2004; Newman et al., 2016; Ratcliff et al., 2006; Schafer et al., 2014; Schlatter et al., 2017; Zabaloy et al.,2012), and most studies have measured an increase in microbial activity due to the use of glyphosate as a source of nutrients (C, N or P) (Busse et al., 2001; Gomez et al., 2009; Haney et al., 2002; Haney et al., 2000; Lane et al., 2012; Lupwayi et al., 2004; Means et al., 2007; Mijangos et al., 2009a; Ratcliff et al., 2006).

A related investigation conducted in a controlled environment showed a negligible effect of glyphosate on the decomposition of OM and the evolution of physicochemical parameters at different times during composting (0, 2, 28 and 112 days) (Grenier et al., submitted). The complete dissipation of glyphosate and the absence of AMPA, at the end of 4 months of composting was also noted. The objective of the following study is therefore to assess the effect of glyphosate on bacterial diversity during the same composting experiment and to monitor potential changes in community structure and species abundance. The strong dissipation of glyphosate and the absence of AMPA as measured previously suggests an intense degradation that points to the presence of species possessing the GOX and/or C-P lyase enzymes that could take advantage of this context to proliferate at the expense of more sensitive species.

5.2 Materials and method

5.2.1 Experimental design, sampling and physicochemical analysis

Extensive detail regarding the experimental design, sampling and analysis can be found in section **3.2.1**, **3.2.2 and 3.2.3.** Briefly, mesocosm-sized vessels (19-litter) were used to perform a controlled composting experiment. Two experimental conditions were tested in

quintuplicate (n = 5).4 kg of fresh OM: untreated Control and Analytical Grade Glyphosate where analytical grade glyphosate (N-(Phosphonomethyl) glycine 96 % purity from Sigma-Aldrich was used at a final concentration of 150 ppm per vessel and diluted in DI water to a final volume of 100 mL while the same volume of DI water was added to the control. The compost was mixed weekly and watered as needed to maintain a 60 % humidity rate.

Temperature was recorded every two hours using data loggers (iButton®, Alpha Mach, Canada). Compost samples were collected from the vessels for physicochemical OM, pH and glyphosate analysis immediately after loading (T0) and after mixing at 2, 7, 28 and 112 days of composting (T2, T7, T28 and T112 respectively). Samples for glyphosate measurements were stored at -20 °C and samples for DNA extraction were stored at -80 °C until analysis while the remaining were oven-dried at 105 °C for 24 hours and milled at a particle size of < 2 mm before being stored in the dark until analysis.

The pH was analyzed in a 1:10 (w/v) water extract of oven dried samples, while OM content was assessed by determining mass loss on ignition at 600 °C to a constant weight.

Glyphosate was extracted from 2,5 g of compost using 40 mL of 0.125 M NH_4OH + 0.05 M KH_2PO_4 solution (Alferness & Iwata, 1994). The samples were then vortexed for 30 seconds, tumbled for 45 minutes at 200 rpm and centrifuged for 20 minutes at 3500 rpm. A total of 40 µl of supernatant were transferred and evaporated to dryness under nitrogen flow. Samples were derivatized by the addition of 1 ml of trifluoroacetic anhydride (TFAA) and 500 µl of trifluoroethanol (TFE), prior to heating at 100 °C for one hour. After cooling to room temperature, samples were evaporated to dryness under nitrogen flow and then resuspended with 1 ml of ethyl acetate, prior to injection (2 µl) in a Varian CP 3800 gas chromatograph coupled with an electron capture detector and equipped with a Rxi®- 5Sil MS column (Restek, Pennsylvania, USA) (30 m x 0.25mm x 0.25 µm). Initial oven temperature was set at 70 °C and held for 1 min followed by a 1 °C min^{-1} increase up to 84 °C, a 4 °C min^{-1} increase up to 120 °C and finally up to 250 °C, at an increase of 80 °C min^{-1} and held for 7 min for a total run time of 32.63 min.

5.2.2 DNA extraction, 16S rRNA gene amplification sequencing and processing

Total genomic DNA was extracted from 250 mg (wet weight) subsamples using Qiagen's DNeasy Power Soil® Pro kit according to the manufacturer's instructions. The quantity and quality of the extracted DNA was assessed with a Thermo Fisher NanoDrop 2000c. The V5-V6 region (based on Escherichia coli) of the 16S ribosomal RNA (rRNA) was selected for PCR amplification using the forward primer: P609D (5'- GGMTTAGATACCCBDGTA- 3') and reverse primer: P699R (5'- GGGTYKCGCTCGTTR-3'). The following conditions were used for the amplification: initial denaturation 94 °C for 2 min, denaturation 94 °C for 30 s, annealing 58 °C for 30 s, extension 72 °C for 30 s, final extension 72 °C for 7 min, holding at 4 °C, over 35 cycles. The resulting amplicon were sequenced on the Illumina MiSeq 2500 paired end 2 X 250 bp platform at McGill University and Genome Quebec Innovation Centre (Montreal, Canada). Reagent controls were below the limit of detection for quality assurance.

Full primer set was trimmed away from reads using Cutadapt v2.10 (Martin, 2011) with a maximum 10 % mismatch to the primer sequences. Paired-end reads without primers were discarded as were paired-end sequences where one of the reads lengths was lower than 200 bp. The surviving reads were processed using DADA2 v1.20.0 (Callahan et al., 2016). The 16S reads were filtered by allowing a maximum of 2 expected errors in each paired-end reads, and the reads were truncated at the first instance of a base with a quality score less than or equal to 2. To eliminate bad quality regions, both reads were truncated to a length of 230 bp. PhiX contaminant was also removed. During high resolution sample inference, samples were pooled (pooled parameter=TRUE) and amplicon sequence variants (ASVs) were inferred with a minimum overlap of 20 bp. Chimeras were removed with the function removeBimeraDenovo(). ASV taxonomic labels were assigned with a naive Bayesian classifier using the Silva v.138 training set.

5.2.3 Community profiling and differential abundance analysis

MicrobiomeAnalyst, a web-based pipeline (Chong et al., 2020; Dhariwal et al., 2017), was used for microbial composition, differential abundance analyses and visualization. Samples Ctrl_T0_2 and Gly_T2_1 were excluded from the analysis due to very low and very high-read counts respectively. Low count filter was set to a minimum of 1 count in 10 % of the samples and data were transformed to a relative log expression (RLE).

Shannon index was used to assess Alpha-diversity. A t-test was then conducted to compare Alpha-diversity between samples. Beta-diversity was assessed using Principal Coordinate Analysis (PCoA) and Bray-Curtis index, and evaluated using permutational multivariate analysis of variance (PERMANOVA). All P values < 0.05 were considered statistically significant.

Differential abundance (DA) analysis was performed using the DESeq2 algorithm with an adjusted p-value cut off of 0.05. Differential abundance analyses were done for 10 comparisons, where the control treatment is compared with the glyphosate treatment at each sample point, resulting in the following: Ctrl_T0 vs Gly_T0, Ctrl_T2 vs Gly_T2, Ctrl_T28 vs Gly_T28 and Ctrl_T112 vs Gly_T112. Successive phases in the Control and the Glyphosate treatment were also compared between each other, resulting in the following Ctrl_T0 vs Ctrl_T2, Ctrl_T2 vs Ctrl_T28, Ctrl_T28 vs Ctrl_T112, Gly_T0 vs Gly_T2, Gly_T2 vs Gly_T28 and Gly_T28 vs Gly_T112 where the low count filter was raised to 3 counts in 10 % of the samples. Alpha- and Beta-diversity was also measured for each comparison.

5.2.4 Statistical analysis

Statistical analyses for temperature and glyphosate measurement were carried out in GraphPad Prism 8.4.3. Results were expressed as the average of four replicates and multiple t-test with assumed homoscedasticity and correction using the Holm-Šídák method was performed, with alpha = 0.05 to compare mean between the two treatments. Each row was analyzed individually, without assuming a consistent SD.

5.3 Results

5.3.1 Physicochemical properties and glyphosate content

The complete data on the evolution of physicochemical parameters (O.M., pH, C, N, C: N, NH_3/NH_4^+, NO_2^--NO_3^- and PO_4^{3-}) during composting can be found in **section 3.3.2**. Briefly, no significant differences were measured between the control and glyphosate treatments for any of the parameters measured. The amount of organic matter decreased significantly between T0 and T112 for both treatments, going from 87.3 % ± 0.4 to 75.3 % ± 0.9 and from 87.0 % ± 1.4 to 76.6 % ± 3.1 respectively. The pH was alkaline at times T0, T2 and T28 with values above 9 for both treatments. NO_2^--NO_3^- levels increased significantly between T7 and T28 as well as between T28 and T112 for the control while the increase was only significant between T28 and T112 for the glyphosate treatment.

Temperature values at sampling times 0, 2, 7, 28 and 112 were also similar between the two treatments (0). Sampling time T2 corresponds to the thermophilic phase (45 °C), although this was very short, with a duration of 56 hours for the control treatment and a duration of 48 hours for the glyphosate treatment (**section 3.3.1**).

		Temperature (°C)		Glyphosate (mg/kg)	
Day 0	Ctrl	38.6 ± 0.4	0.882	0	< .001
	Gly	38.5 ± 0.2		147.8 ± 23.2	
Day 2	Ctrl	46.7 ± 0.3	0.743	0	< .001
	Gly	44.0 ± 2.2		104.2 ± 19.1	
Day 28	Ctrl	33.7 ± 0.3	0.882	0	0.002
	Gly	34.0 ± 0.2		11.8 ± 5.8	
Day 112	Ctrl	22.9 ± 0.5	0.927	0	NA
	Gly	23.3 ± 0.3		0	

Table 5.1 - Temperature and glyphosate values measured in the Control and Glyphosate AG treatments on days 0, 2, 28, and 112 of composting (n = 4). The first values represent the mean ± SD for the Control (Ctrl) and Glyphosate AG (Gly) treatments. The italicized value indicates the result (adjusted p-value) of the multiple t-test performed between the two treatments.

The control treatment contained no trace of glyphosate. A significant decrease was measured between T0 and T2 and between T2 and T28 as the degradation reached 28.9 % after 2 days, 76.9 % after 7 days, 92 % after 28 days and 100 % by the end of the experiment.

5.3.2 Community profile and relative abundance

A total of 3 946 043 amplicons were aligned with lengths ranging from 286 to 308 nt and 5 901 previously characterized ASVs (Amplicon sequence variant) were identified. Of these, 97.7 % could be annotated at the Phyla level, 95.9 % at the Class level, 87.7 % at the Order level, 74.8 % at the Family level, 49.6 % at the Genus level and 4.2 % at the Species level. The diversity was shared between 40 different phyla, of which Proteobacteria (30.3 %), Actinobacteria (19.5 %) and Firmicutes (16.6 %) had the highest number of total counts. There were no differences in the abundance of different phyla between the Control and Glyphosate treatments at the various sampling times. In order, Proteobacteria, Firmicutes and Actinobacteria were more abundant at T0. Proteobacteria, Actinobacteria and Firmicutes were more abundant at T2 and T28 while Proteobacteria, Actinobacteria and Bacteroidetes were more abundant in Control at T112 and Proteobacteria, Actinobacteria and Firmicutes/Bacteroidetes were more abundant in Glyphosate T112.

The 2929 ASVs identified at the genus level were distributed in 654 genera (05.1 A), including 270 that had only one member. Nine genera identified across the 8 compost samples collected corresponded to more than 2 % of the relative abundance of ASVs identified at the genus level, namely *Chryseolinea* (4.55 %), *Thermobifida* (3.59 %), *Paenibacillus* (3.14 %), *Thermobacillus* (3.03 %), *Microbacterium* (2.98 %), *Bacillus* (2.70 %), *Cellvibrio* (2.63 %), *Pseudoxanthomonas* (2.46 %) and *Acidibacter* (2.11 %).

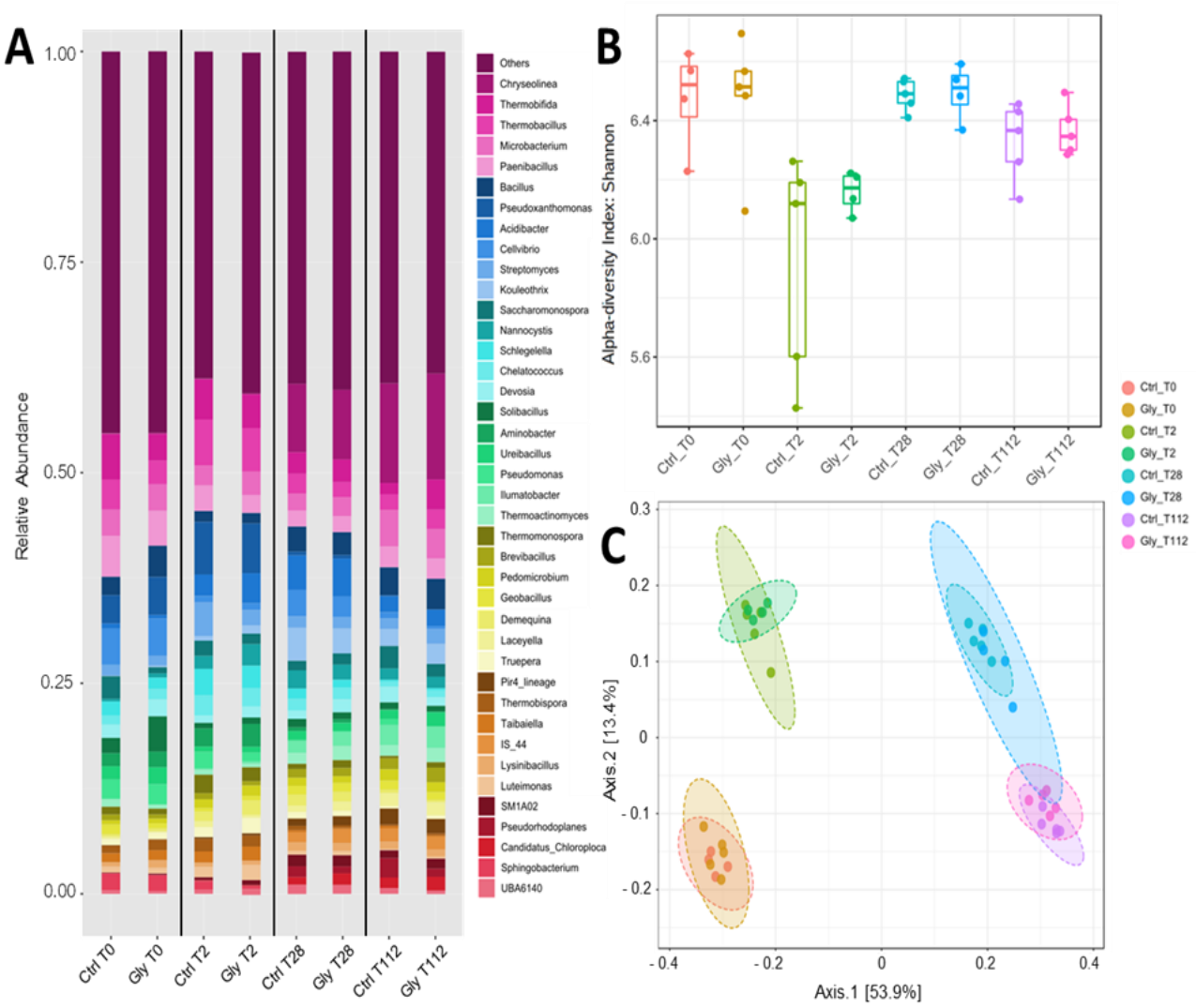

Figure 5.1 - Microbial community profiling. A) Genus-level (40 most abundant identified genus) taxonomic composition at the different sampling point, the other taxa are merged into "Others". B) Shannon index for compared alpha-diversity and C) Principal Coordinate analysis (PCoA) with Bray Curtis index

The most abundant genera are shared between the Control and Glyphosate treatments at T2 and T28. The most abundant genera at T2 are *Pseudoxanthomonas, Thermobacillus* and *Thermobifida* while the most abundant at T28 are *Chryseolinea, Acidibacter* and *Kouleothrix*. At T0, *Thermobifida*, *Paenibacillus* and *Cellvibrio* were the most abundant in the Control but not in the Glyphosate treatment in which *Pseudoxanthomonas, Cellvibrio* and *Solibacillus* were the most abundant. At Tl12, the most abundant genera in the Control

were *Chryseolinea, Microbacterium* and *Bacillus*, while *Chryseolinea, Bacillus* and *Thermobifida* were more abundant in the Glyphosate treatment.

The alpha diversity was very similar between the different samples with an average Shannon index value ranging from 5.92 for the Control treatment at T2 to 6.50 for the Glyphosate treatment at T28 (05.1 B). In general, alpha diversity was lower at T2 and higher at T28. The alpha diversity indices (Shannon) were not significantly different between the two treatments at all different sampling times. However, changes have taken place over time. The alpha diversity indices were significantly different for both the Glyphosate and Compost treatments between T0 and T2 and between T2 and T28but not between T28 and T1l2.

Principal Coordinate Analysis (PCoA) indicated that samples seemed to separate by time with the X-axis explaining 53.9 % of the variance between the samples (05.1 C). The samples do not separate according to treatment as samples of different treatments do not have a significantly different Bray Curtis index value (PERMANOVA, $p < 0.05$). Bacteria population are significantly different between T0 and T2, between T2 and T28 and between T28 and T1l2 for both treatments.

5.3.3 Differently abundant species

5.3.3.1 Differently abundant bacteria between Control and Glyphosate treatment

Differential abundance analyses performed with DESeq2 showed some variation in bacterial abundance between the Control and Glyphosate treatments. First, no ASVs were differentially abundant at the start of the experiment, i.e., at T0.

At T2, members of the order Kiloniellales, including the genera *Tagaea*, *Fodinicurvata* and *Pelagibius* were more abundant in the Glyphosate treatment than in the Control treatment, while the genus *Haloplasma*, the only representative of the family Haloplasmataceae and order Haloplasmatales was more abundant in the Control treatment.

After 28 days of composting, members of the class Myxococcia, specifically the order Myxococcales, were more abundant in the Control treatment than in the Glyphosate treatment. This order includes members of the families Anaeromyxobacteraceae, Myxococcaceae and Vulgatibacteraceae that were detected. The order Sphingomonadales was also more abundant in the Control and includes some representatives of the genera *Altererythrobacter*, *Erythrobacter*, *Sphingobium*, *Croceicoccus*, *Sphingoaurantiacus*, *Sphingomonas*, *Novosphingobium*, *Sphingopyxis* and *Qipengyuania*. Members of the family Sutterellaceae were more abundant in the Glyphosate treatment. Members of the family Thermomicrobioaceae, all identified as *Sphaerobacter* were more abundant in the Control treatment. One of these ASVs identified as *Sphaerobacter thermophilus* was also significantly more abundant in Control.

Finally, at T112, two ASVs identified at the genus level were more abundant in the Control treatment, namely *Pseudorhodoplanes* sp. and *Pedomicrobium* sp., which are the only above-mentioned ASVs identified as one of the 40 most abundant genera (05.1 A).

5.3.3.2 Differently abundant bacteria between sampling time

Nevertheless, the vast majority of the differences in abundance were measured between the different sampling times, i.e., between T0 and T2, between T2 and T28 and between T28 and T112 in the Control and Glyphosate treatments (05.2).

In the Control treatment, 648 ASVs were differentially abundant between T0 and T2, of which 332 were more abundant at T0 and 316 were more abundant at T2. Between T2 and T28, 1171 ASVs were differentially abundant, while 496 were more abundant in T2 and 675 were more abundant in T28. Finally, 394 ASVs were differentially abundant between T28 and T112, with 220 more abundant at T28 and 174 more at T112.

For the Glyphosate treatment, 629 ASVs were differentially abundant between T0 and T2, with 350 more abundant at T0 and 279 more abundant at T2. Then, 444 ASVs were more abundant in T2 compared to T28 while 603 ASVs were more abundant at T28 and finally 396 ASVs were differentially abundant between T28 and T112, of which 220 were more abundant at T28 and 174 were more abundant at T112.

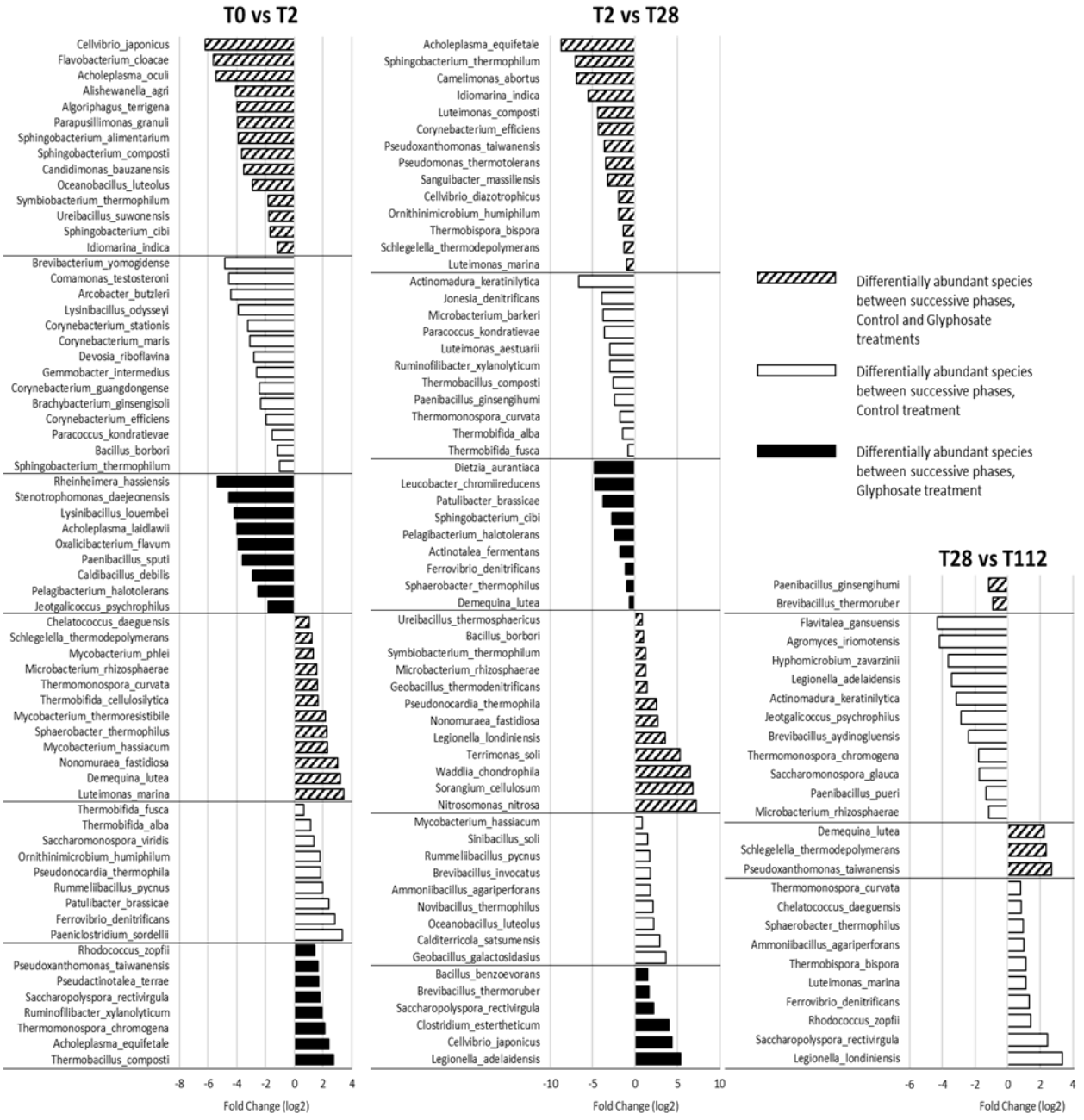

Figure 5.2 - Differentially abundant species between T0 and T2, between T2 and T28 and between T28 and T112. Fold change (FC log2) denotes relative differences in relative abundance between groups (DESeq2). The white bars represent the species that were differently abundant within the Control treatment, the black represents those that were differently abundant within the Glyphosate treatment and the hatched were differently abundant in both Control and Glyphosate treatment (the value shown is the fold change in the Control treatment).

Of these ASVs, a few could be identified at the species level (0). A total of 23 ASVs identified in the Control were differentially abundant between T0 and T2, 17 were differentially abundant in the Glyphosate treatment, and 26 ASVs varied in both the Control and Glyphosate treatments. Between T2 and T28, 20 ASVs were differentially abundant in the Control, 15 were differentially abundant in the Glyphosate and 26 ASVs varied in both the Control and Glyphosate treatments. Finally, no ASVs identified at the species level were differentially abundant in the Glyphosate treatment alone between T28 and T112, while 21 varied in the Control and 5 varied in both treatments. Whenever ASVs were found differentially abundant in both Glyphosate and Control, the shift was in the same direction.

5.4 Discussion

5.4.1 Community profile

The various analyses performed failed to reveal an effect of glyphosate on species richness and community composition (05.1). The abundant phylum and genera were the same between the Control and Glyphosate treatments. Proteobacteria were particularly dominant at T0, T2 and T28, while diversity was more evenly distributed among Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes at T112. These phyla are consistently observed in composting studies and their variation tends to vary with environmental conditions and maturation stages (Antunes et al., 2016; de Gannes et al., 2013; Grenier et al. in prep; Jurado et al., 2014; López-González et al., 2015; Neher et al., 2013; Partanen et al., 2010; Wei et al., 2018; Zainudin et al., 2014; Zhou et al., 2018). The short thermophilic phase and the relatively low temperature reached during this experiment could explain the predominance of Proteobacteria members. Some studies have indeed associated Firmicutes abundance with high temperatures (Grenier et al., in prep; Neher et al., 2013; Wei et al., 2018; Zhou et al., 2018), while Proteobacteria are rather associated with more mesophilic conditions (de Gannes et al., 2013; Grenier et al., in prep; Neher et al., 2013; Wei et al., 2018).

The great majority of the abundant genera listed include members that have known functions in the transformation of lignocellulosic material such as *Thermobifida,*

Thermobacillus, Microbacterium, Paenibacillus, Bacillus, Cellvibrio, Streptomyces, Saccharomonospora, Devosia, Solibacillus, Ureibacillus, Thermoactinomyces, Thermomonospora, Brevibacillus, Geobacillus, Demequina, Laceyella, Truepera, Thermobispora, Lysinibacillus, Luteimonas and *Sphingobacterium* which have the ability to degrade cellulose, hemicelluloses or both types of structural sugars (Albuquerque et al., 2018; Ali et al., 2019; Han et al., 2018; Huang et al., 2012; Irfan et al., 2018; Liolios et al., 2010; Lykidis et al., 2007; Meng et al., 2009; Mokrane et al., 2016; Morohoshi et al., 2012; Photphisutthiphong & Vatanyoopaisarn, 2019; Rawway et al., 2018; Roberts et al., 1990; Singh et al., 2013; Stutzenberger, 1972; Suarez et al., 2014; Touzel & Prensier, 2015; Wang et al., 2011). Some others, such as *Microbacterium, Thermobifida, Brevibacillus, Ureibacillus, Thermomonospora, Pseudomonas, Streptomyces, Sphingobacterium* and *Pedomicrobium* have the required machinery to degrade lignin (Chen et al., 2015; Hadad et al., 2005; Larsen et al., 1999; Majumdar et al., 2014; Manaia & Moore, 2002; Okuda et al., 2008; Rahmanpour et al., 2016; Stein, 2014; Taylor et al., 2012).

The hydrolysis of structural sugars and the oxidation of lignin are the main reactions that take place during composting, which in this case is composed mainly of plant residues. The transformation of lignocellulose takes place during the whole process and does not seem to be affected by the presence of glyphosate in the mixture.

The greatest difference in composition was at T0 as the dominant genera in the Control treatment were *Thermobifida* (3.69 %), *Paenibacillus* (3.31 %) and *Cellvibrio* (2.92 %), while *Pseudoxanthomonas* (3.01 %), *Cellvibrio* (2.98 %) and *Solibacillus* (2.79 %) were the most abundant in the glyphosate treatment, although 31.7 % and 33.0 % of the ASVs could not be assigned to a genus in the Control and Glyphosate treatment respectively. Sampling was done a few minutes after the addition of glyphosate, so glyphosate should not have had an effect on species abundance (no difference in DA). Since there are no abiotic effects pressuring the communities at that time (temperature) this difference may be due to slightly different initial substrate compositions and bacteria associated with.

For both treatments, diversity was lowest on day 2 of the process (T2), where it was significantly lower than T0 and T28, which could be due to the higher temperature (Ryckeboer et al., 2003). The most abundant genera at T2, *Thermobacillus* and *Thermobifida*, which are renowned thermophiles and lignocellulose degraders (Touzel & Prensier, 2015; Zhang et al., 1998) were also all different from the abundant genera at T28 and mostly different from the abundant genera at T0. Diversity was not different between T28 and T112, which could also be explained by a similar temperature (in the mesophilic range). The beta diversity analyses clearly demonstrate that the bacterial communities in the different samples associate according to the sampling time but not to the presence of glyphosate. The abiotic conditions of the environment, such as temperature, OM decomposition and pH must have been the most determining factors in the recruitment and succession of the core bacteria community at the different stages.

To date, no other studies have measured the effect of glyphosate on the diversity and dynamics of bacteria during composting. It is therefore difficult to compare the results of this study with similar work, since the specific abiotic conditions, such as high temperature and high oxygen demand in compost are very different from those in soil. When measured on soil samples, variation in community diversity and composition was shown to be primarily influenced by soil type and quality, physicochemical parameters, amount applied and number of doses received, time after application, and season (Busse et al., 2001; Lane et al., 2012; Lupwayi et al., 2004; Ratcliff et al., 2006; Schlatter et al., 2017).

Thus, the majority of studies conducted to investigate the impact of glyphosate on soil-level microorganism diversity and abundance have reached similar conclusions to those presented in this study (Lane et al., 2012; Lupwayi et al., 2004; Newman et al., 2016; Ratcliff et al., 2006; Schafer et al., 2014; Schlatter et al., 2017; Zabaloy et al.,2012). Newman et al., (2016) measured an increase in δ-Proteobacteria and a decrease in Acidobacteria in the presence of glyphosate, but these differences were not significant.

Glyphosate must therefore have been co-metabolized by a number of microorganisms that used it as a source of carbon, nitrogen or phosphorus. Some members of the genera *Bacillus*

(Castle et al., 2004; Fan et al., 2012; Singh et al., 2019; Yu et al., 2015), *Streptomyces* (Obojska et al., 1999; Singh et al., 2019), *Aminobacter* (Gorodylova et al., 2021), *Geobacillus* (Obojska et al., 2002), *Lysinibacillus* (González-Valenzuela & Dussán, 2018) and *Pseudomonas* (Jacob et al., 1988) were found to be able to degrade glyphosate and are also among the 40 most abundant genera in the several analyzed phases. Both glyphosate degradation pathways; the sarcosine pathway through the production of the enzyme C-P lyase and the AMPA pathway through the production of the enzyme GOX were potentially expressed during the experiment. Indeed, members of the genera *Streptomyces*, *Bacillus* and *Pseudomonas* are known to use both pathways to degrade glyphosate (Singh et al., 2020, 2019), while *Geobacillus* uses the AMPA pathway (Obojska et al., 2002) and *Lysinibacillus* and *Aminobacter* use the sarcosine pathway (Artuso et al., 2021; González-Valenzuela & Dussán, 2018). Moreover, this degradation of glyphosate in *Aminobacter*, *Lysinibacillus*, *Geobacillus*, *Pseudomonas* and *Bacillus* would be to acquire inorganic phosphorus molecules while *Streptomyces* uses it as a source of phosphorus and nitrogen. If these bacteria were indeed the main responsible for the dissipation of glyphosate during the experiment, the stability of phosphate contents (section 3.2.2) during the process could be explained by an important use following the degradation of glyphosate, not allowing a significant accumulation.

However, there was no difference in the abundance of these genera between the two treatments, suggesting that their ability to degrade glyphosate did not confer an advantage over other groups of bacteria. Quantification of CO_2 release or measurement of dehydrogenase activity could have revealed increased microbial activity following glyphosate degradation, but the data collected, i.e., heat production, abundance of ASVs and amount of DNA extracted, do not suggest increased activity. Given the high dissipation of glyphosate during the first month of composting, it is possible that other uncharacterized organisms have the necessary machinery to degrade the herbicide. The isolation of bacteria from glyphosate-containing mixtures could lead to the discovery of new, potentially thermophilic, strains capable of processing it.

5.4.2 Differently abundant bacteria

5.4.2.1 Differently abundant bacteria between Control and Glyphosate treatment

Some bacterial taxa varied significantly in relative abundance between the Control and Glyphosate treatments. The majority of the differences were at T2 and T28 and involved taxonomic groups that were less relatively abundant in the Glyphosate treatment compared to the Control.

At T2, members of the order Kiloniellales including an ASV identified as part of the family Fodinicurvataceae and an ASV identified with the genus *Tagaea* were more relatively abundant in the Glyphosate treatment compared to the Control. The Fodinicurvataceae family was previously observed in a compost composed of tomato stalks (Zhang et al., 2021) and in a goat manure amendment used for bioremediation of an atrazine-contaminated soil (Luo et al., 2021), perhaps suggesting a generalized tolerance towards herbicides. *Tagaea sp*, on the other hand, contains only one species (*T. marina*) isolated from a seawater sample from Taiwan (Jean et al., 2016) and reportedly observed in a compost sample made of cow manure (Sardar et al., 2021). Members of the Kiloniellales are considered heterotrophic, mesophilic, and halophilic (Imhoff & Wiese, 2013), but there is no evidence that they have a particular tolerance or interest for glyphosate. In contrast, the genus *Haloplasma* sp. was more relatively abundant in the Control treatment compared to the glyphosate treatment at T2. *Haloplasma contractil*e is the only known representative of this genus and is characterized by its lack of a cytoskeleton and its halophilic nature. It has been observed in thermophilic biogas reactors fed with agricultural waste (Sun et al., 2015), but no link exists between it and glyphosate.

The T28 phase was characterized by a higher relative abundance in the Control treatment compared to the Glyphosate treatment of an unidentified family belonging to the order Ferrovibrionales. The order Myxococcales represented by *Vulgatibacter* sp., and the family Thermomicrobiaceae and Sphingomonadaceae whose *Sphaerobacter thermophilus* and *Altererythrobacter* are respectively the representatives with the highest relative abundance

in that phase were also more abundant in the Control treatment compared to the Glyphosate one. Altogether, it is difficult to explain their lower relative abundance in the presence of glyphosate albeit the genus *Vulgatibacter* which includes only one described species, *Vulgatibacter incomptus* (Yamamoto et al., 2014), differs from its myxobacterial cousins by its inability to use macromolecules as a nutrient source. On the other hand, *Altererythrobacter* contains a dozen species, some of which have demonstrated an ability to feed on petroleum aromatics or resist high heavy metal concentration (Quan et al., 2010; Teramoto et al., 2010; Wu et al., 2014). As for *S. thermophilus,* it has been observed in compost and has already been associated with the bioconversion of cellulose through the production of endoglucanases, it also is mainly known for its resistance to various antibiotics and its thermophilic character (Shaw, 2018) but none was tested against glyphosate.

In contrast, the Sutterellaceae family was more abundant in the Glyphosate treatment compared to the Control at T28. The three ASVs present could not be annotated to the genus level, but the majority of Sutterellaceae members are recognized as inhabitants of the gastrointestinal tract and include the genus *Parasutterella, Sutterella, Mesosutterella* and *Turicimonas* (Morotomi et al., 2011). The family is phylogenetically close to the family Alcaligenaceae and Comamonadaceae which include the genera *Achromobacter* and *Alcaligenes*, and *Comamonas* respectively, some of whose strains have the potential to degrade glyphosate (Firdous et al., 2020; Shushkova et al., 2016; Talbot et al., 1984). This result could represent a lead for the identification of new species capable of degrading glyphosate.

Finally, two ASVs present at a lower relative abundance in the Glyphosate treatment than in the Control were detected at T112. Both are identified to the genus level, *Pseudorhodoplanes* and *Pedomicrobium*. The former contains only one described species, *P. sinuspersici*, that was isolated from an oil-contaminated soil (Tirandaz et al., 2015) and was never observed in compost while the latter is known for its ability to oxidize certain metals such as manganese and iron and for its laccase-like activity that could allow it to degrade lignin (Gebers & Beese, 1988; Larsen et al., 1999).

Overall, the difference in species abundance between treatments is negligible compared to the changes that occur between different sampling times. The variations observed are difficult to associate with tolerance or stress responses to glyphosate, but the greater abundance of Sutterellaceae and Fodinicurvataceae in the glyphosate-containing treatment could serve as a lead for future studies.

5.4.2.2 Differently abundant bacteria between sampling time

The number of bacteria with significantly different relative abundance was much greater between successive sampling times than between the Control and Glyphosate treatments. (05.2). This difference is expressed in thousands of organisms and suggests that abiotic changes associated with the evolution of decomposition and temperature changes are the main factors in bacterial dynamics. Comparisons are made between consecutive phases in order to follow the changes in abundance in the chronological evolution of the process.

The largest number of bacteria with a significantly different relative abundance was observed between phases T2 and T28, with phase T28 accounting for the largest number of significantly abundant bacteria. This period corresponds to a very active phase of the process in which several compounds are degraded, and by-products appear in the mixture while the temperature varies strongly between the two phases to allow the establishment of distinct organisms (0) (Ryckeboer et al., 2003). The period between T28 and T112 in the Glyphosate treatment has the lowest number of differentially abundant ASVs (394 DA ASVs), almost half of the dynamics reported in the Control treatment at the same period (728 DA ASVs). This trend is rather difficult to explain but occurs when glyphosate is almost completely degraded. Depletion of glyphosate could have slowed the strong proliferation of some microorganisms that would use glyphosate as a nutrient source, thus buffering the abundance of the species present and resulting in little changes.

Many of the ASVs identified as differentially abundant between two phases varied similarly between the two treatments (represented by the hatched bars in Figure 5.2), but others varied in abundance only in the glyphosate treatment. The increase in abundance between T0 and T2 and the decrease between T2 and T28 of some species in the Glyphosate

treatment only could suggest that these species benefit from the presence of the herbicide during this period when it is very abundant or that they participate in its degradation.

Species that increased in abundance between T0 and T2 in the Glyphosate only treatment were *Acholeplasma equifetale, Ruminofilibacter xylanolyticum, Pseudactinotalea terrae, Pseudoxanthomonas taiwanensis, Saccharopolyspora rectivirgula, Rhodococcus zopfii, Pseudoxanthomonas taiwanensis, Thermomonospora chromogena* and *Thermobacillus composti*. Subsequently, those that were significantly less abundant at T28 compared to T2 in the Glyphosate-only treatment were *Demequina lutea, Sphaerobacter thermophilus, Ferrovibrio denitrificans, Actinotalea fermentans, Pelagibacterium halotolerans, Sphingobacterium cibi, Patulibacter brassicae, Leucobacter chromiireducens* and *Dietzia aurantiaca.*

The genera *Thermomonospora, Pseudoxanthomonas, Thermobacillus, Demequina* and *Sphingobacterium* are part of the group of the 40 most abundant genera (05.1) and could thus represent an interesting avenue for further study.

5.5 Conclusion

This study reveals for the first time the bacterial community changes that occur in the presence of glyphosate during composting. The results show that glyphosate did not significantly impact the species diversity and composition of the isolated communities. However, significant changes in species abundance were recorded between sampling times, suggesting that abiotic conditions such as temperature and decomposition level are more important factors in successional dynamics. Species described as being able to secrete GOX or C-P lyase enzymes were not observed in the process, but members of relevant genera such as *Aminobacter*, *Lysinibacillus*, *Geobacillus*, *Pseudomonas*, *Bacillus*, and *Streptomyces* were present, which may offer a potential avenue for research. In addition, members of the families Sutterellaceae and Fodinicurvataceae were more abundant in the Glyphosate treatment compared to the control, while *Acholeplasma equifetale*, *Ruminofilibacter*

xylanolyticum, *Pseudactinotalea terrae*, *Pseudoxanthomonas taiwanensis*, *Saccharopolyspora rectivirgula*, *Rhodococcus zopfii*, *Pseudoxanthomonas taiwanensis*, *Thermomonospora chromogena* and *Thermobacillus composti* were specifically increased in relative abundance in the Glyphosate treatment between T0 and T2, which would also be worth further study. Compost is an extremely rich environment, whether in terms of available nutrients, the various physicochemical conditions that develop during the process or the diversity of microorganisms. Therefore, it may host species capable of degrading glyphosate that has not yet been characterized. The diversity and redundancy of the functions taken on by microorganisms could also offer a certain resilience to the microbial ecosystem that manages to complete the decomposition of organic matter despite the presence of the contaminant. This information is encouraging for composting sites likely to receive such contaminants and for the isolation of new strains capable of glyphosate degradation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

V. Grenier: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Funding acquisition; **E. Gonzalez:** Software, Data Curation **J. Laur:** Formal analysis, Writing - Original Draft, Writing - Review & Editing **F. E. Pitre:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

Acknowledgements and funding

Special thanks to Suzanne Boucher for her help in the preparation of the experiment, sampling and physicochemical analyses. We are grateful to Estelle Leca and Camille M. Chédeville for their help with sampling. Our thanks to Eszter Sas and Noël Fagoaga for their help in the preparation of the organic residue mixture. This research was made possible by financial support from the Fonds de recherche du Québec – Nature et technologies (FRQNT), Mitacs Acceleration program and Ramo® to the first author scholarship (PhD program).

Supplementary data

Supplementary tables S5.1 can be found at:

https://www.webdepot.umontreal.ca/Usagers/p0915153/MonDepotPublic/Supplementary%20Data%20Chapitre%205

Table S5.1 - ASVs across all samples

Image tirée du Guide technique pour le compostage sur site en ICI
par Alexis Fortin et Louise Héneault-Éthier (Fortin & Héneault-Éthier, 2011)

Discussion Générale

La dégradation de la matière organique est d'une importance majeure dans les cycles biogéochimiques du carbone et de l'azote, car elle permet le retour à la terre de la matière organique où elle est minéralisée par une horde de microorganismes pour offrir les éléments nécessaires à la croissance végétale. De même, les nouvelles exigences en termes de valorisation de la matière organique et les préoccupations liées à la santé des sols dans un contexte de lutte aux changements climatiques remettent au goût du jour la décomposition de la MO, aussi peu séduisante soit-elle. C'est avec l'objectif d'améliorer les connaissances sur l'impact de la composition de la biomasse et de la présence de contaminants organiques lors de la décomposition de la MO que cette thèse a été abordée. Les résultats ainsi obtenus révèlent une grande complexité, complémentarité et diversité biologique ainsi qu'une redondance fonctionnelle qui semble octroyer une certaine résilience aux communautés bactériennes responsables de la transformation de la MO face à la présence de contaminants lors du compostage.

6.1 La transformation du bois sous l'effet des contraintes intrinsèques et environnementales

La première partie de cette thèse, présentée au **Chapitre 2**, visait à rendre compte de l'impact de l'âge d'une espèce ligneuse, dans le présent cas *Salix miyabeana* 'SX67', un saule arbustif à croissance rapide et d'une période d'entreposage en conditions hivernales sur la transformation (biodégradation) de la matière lignocellulosique. Alors que la composition et la structure des tissus végétaux varient en fonction de la maturité de la plante (Gouker et al., 2021; Sennerby-forsse, 1989), l'entreposage des copeaux de bois est connu pour offrir les conditions propices à une activité biologique intense, à une augmentation des températures et à une perte de masse parfois importante (Bedane et al., 2011; Krigstin & Wetzel, 2016; Pettersson & Nordfjell, 2007). Les hypothèses initialement posées stipulaient que 1) les tiges de différents âges n'auraient pas la même composition initiale, que 2) les tiges plus jeunes subiraient une décomposition moins importante, résultant en une plus faible hausse de température en raison de l'abondance de composés phénoliques ayant des propriétés

antimicrobiennes dans l'écorce et que 3) une diminution des teneurs en sucres structuraux serait enregistrée à la fin de l'entreposage hivernal.

Les analyses préliminaires ont d'abord mis en évidence des différences dans les proportions de composés extractibles (solubles dans l'eau et l'éthanol), de glucanes et de mannanes observables entre les tiges de 2 ans et de 3 ans au moment de la récolte, confirmant l'hypothèse 1. Au cours de la période d'entreposage, qui s'est échelonnée de novembre à mai, l'augmentation de la température a été plus forte et plus rapide pour le tas de copeaux de tiges de 2 ans que pour celui de 3 ans, ce qui infirme l'hypothèse 2. Enfin, plusieurs composantes de la biomasse ont été altérées pendant le stockage, puisqu'une diminution significative des matières extractibles et une augmentation de la lignine insoluble et de la lignine totale ont été mesurées dans les deux tas, tandis qu'une augmentation des glucanes, du xylane et des mannanes a été mesurée dans le tas de copeaux de 2 ans, infirmant également l'hypothèse 3. Le stockage pendant ces 6 mois a eu un effet plus fort que l'âge des tiges sur les propriétés physicochimiques et structurelles des copeaux de saule à la fin de l'expérience.

Contrairement à ce qui était prévu, la plus grande proportion de matières extractibles dans les tiges de 2 ans n'a pas ralenti la croissance microbienne. Les extractibles auraient plutôt fourni une source de carbone et d'azote disponible pour les microorganismes afin de maintenir une activité biologique accrue conduisant à une production de chaleur significativement plus élevée. La production de chaleur a été précédemment observée par les employés responsables de l'entreposage des copeaux sur le site d'étude, quoique les tas qu'ils forment habituellement sont beaucoup plus volumineux. Il était donc intéressant de constater que la biomasse de saule à elle seule, placée en petits tas (environ 18 m^3), contenait suffisamment de carbone et d'azote pour maintenir des températures bien supérieures aux conditions ambiantes.

Une analyse détaillée des composés extractibles présents entre les deux âges de tige ainsi qu'avant et après l'entreposage aurait pu permettre d'identifier les composés solubles du bois permettant d'atteindre des conditions thermophiles dans les jeunes tiges et peut-être

de remettre en cause l'effet antibactérien de certains composés phénoliques présents dans le saule, comme l'acide chlorogénique et certains acides benzoïques (Brereton et al., 2017; Lou et al., 2011; Manuja et al., 2013) ou la tolérance des microorganismes à ces composés.

Ces informations sont également pertinentes pour informer sur les méthodes de stockage à privilégier dans le cas où cette biomasse serait destinée au biorafinnage où la dégradation d'un composé d'intérêt pourrait entraîner une perte de revenu importante (Sas et al., 2021). Parallèlement, l'étape de prétraitement de la biomasse, nécessaire pour briser la matière lignocellulosique récalcitrante avant la saccharification, est une étape cruciale et très énergivore (Lynd, 1996). Si l'entreposage pouvait agir comme un léger prétraitement, il pourrait devenir plus rentable de produire du bioéthanol à partir de la biomasse lignocellulosique, ce qui pourrait constituer une grande avancée pour l'industrie. De même, il aurait été intéressant de réaliser des tests de saccharification enzymatique ou chimique afin de déterminer l'effet du stockage sur la libération ultérieure de glucose. Les analyses de composition ont démontré qu'il n'y avait pas de diminution des quantités de glucane, une molécule associée à la cellulose (Sluiter et al., 2008). Il est cependant possible que des enzymes endoglucanases et cellobiohydrolases aient été produites par la microflore, amorçant ainsi le processus de saccharification, laissant les molécules de glucane intactes, ce qui pourrait accélérer considérablement l'hydrolyse de la cellulose lors de la production de biocarburant, par exemple.

Actuellement, les tiges de saule broyées par Ramo sont vendues comme paillis, ou plus précisément comme bois raméal fragmenté (BRF). Le BRF est le résultat du broyage de rameaux frais (non desséchés) de moins de 7 cm de diamètre, avec ou sans feuilles (Lemieux & Germain, 2000). Ces rameaux sont riches en nutriments; en sucres, en protéines, cellulose et en lignine. Selon les chercheurs qui ont développé cette technique d'amendement du sol, l'ajout du matériel lorsqu'il est fraîchement broyé permettrait une biotransformation menée par des champignons sous l'effet d'enzymes peroxydases qui protègent les noyaux benzéniques de la lignine, servant de base à la production d'humus. En laissant la matière séjourner préalablement à l'incorporation dans les sols, on favoriserait la colonisation des copeaux par des bactéries, qui, sous l'action d'enzymes laccases,

attaquent la lignine sans préserver les noyaux benzéniques indispensables à l'humification, réduisant ainsi fortement le potentiel de pédogenèse (Pettigrew, 1998). Les résultats obtenus dans cette expérience ont montré que malgré une éventuelle colonisation bactérienne, la lignine n'était pas dégradée, du moins pas au point de réduire ses proportions. Cependant, l'appauvrissement en ammonium, une forme d'azote très appréciée des microorganismes, soulève des inquiétudes quant au risque d'immobilisation de l'azote lors de l'application de BRF, puisque cette dernière est essentielle à la décomposition des copeaux de saule (Tremblay & Beauchamp, 1998). En ce sens, les copeaux âgés de 2 ans offriraient possiblement un amendement plus intéressant en raison de la plus forte teneur en azote et le pH plus élevé à l'issue de l'entreposage.

Bien que les conditions de cette expérience ne correspondent pas exactement à celles recherchées pour le compostage, et que l'entreposage puisse difficilement être qualifié de compostage, elle s'en est rapprochée par la mise en tas de la MO, l'activité biologique qui s'y est développée et l'atteinte de conditions thermophiles (analogue de la biodégradation). L'utilisation d'une source unique de résidu a permis de suivre spécifiquement la transformation du bois. La mise en place de l'expérience en extérieur, malgré les conditions particulières, a également permis de prendre en compte le réalisme des conditions environnementales, ce qui aurait été impossible en laboratoire.

Enfin, cette étude ouvre la porte à la réalisation de projets utilisant le saule en compostage, tels que l'utilisation de tiges de saule partiellement contaminées issues de la phytoremédiation comme source de carbone pour le compostage de résidus alimentaires. Cette avenue est envisagée par la Ville de Montréal, qui devra assurer un approvisionnement stable évalué à plusieurs milliers de tonnes de biomasses ligneuses par année pour aider au compostage des déchets alimentaires récoltés sur l'île dans les prochaines années.

6.2 Le rôle des bactéries dans la transformation de la matière organique

Si le **Chapitre 2** a mis en évidence un effet des propriétés initiales de la biomasse sur l'activité biologique pendant l'entreposage et une modification des composantes structurelles de la biomasse après l'entreposage, la séquence d'événements et les bactéries impliquées dans cette transformation de la MO ont été étudiées dans le **Chapitre 4**. En décortiquant cette fois-ci un processus de compostage de A à Z, l'objectif était d'identifier le maximum d'espèces de bactéries présentes durant les différentes phases, de suivre leur variation en abondance et de les associer à des fonctions dans le cycle de transformation du carbone et de l'azote. Les hypothèses initialement posées stipulaient que 1) une proportion importante de la cellulose et des hémicelluloses serait dégradée durant la phase thermophile sous l'action de plusieurs bactéries responsables de leur décomposition, 2) les bactéries responsables de la dégradation de la lignine seraient principalement présentes durant la phase de maturation, ce qui entrainerait une diminution de la lignine plus importante dans cette phase et que 3) la nitrification aurait lieu après la phase thermophile, où abonderait également les bactéries nitrifiantes. La plateforme de compostage du Jardin botanique de Montréal, avec ses trois andains correspondant à trois niveaux de maturation, la matière première utilisée et le compost produit a servi de site d'étude.

Bien que l'étude des bactéries impliquées dans le compostage ait été conduite par plusieurs chercheurs, dans différents contextes, et en utilisant une grande variété de techniques d'identification (Chandna et al., 2013; Fang & Wong, 1999; Martins et al., 2013; López-González et al., 2015; Neher et al., 2013; Ryckeboer et al., 2003), ce projet de recherche se distingue par l'utilisation du pipeline bioinformatique ANCHOR, développé spécifiquement pour les échantillons provenant d'environnements complexes (Gonzalez et al., 2019). La rétention d'amplicons de différentes tailles, l'annotation des séquences abondantes à partir de quatre bases de données avec des critères d'identité et de couverture de 99 % et une seconde ronde d'annotation des séquences moins abondantes en utilisant les séquences abondantes comme référence et des critères de couverture et d'identité de 98

% ont permis d'annoter au niveau de l'espèce près de 20 % des ESVs identifiés. Cette étude représente également la première tentative de suivi de succession des espèces à l'aide de méthodes d'analyses d'abondance différentielles, une pratique historiquement utilisée pour la comparaison d'expression génique dans l'étude de métatranscriptomes (Gonzalez et al., 2019; Love et al., 2014; Weiss et al., 2017). Ce sont donc 517 *Exact Sequence Variant* (ESVs) qui ont pu être identifiés au niveau de l'espèce à travers les différents andains échantillonnés. De plus, en comparant les phases successives entre elles, un total de 2 335 ESVs différentiellement abondants a été mesuré dans les quatre comparaisons. Le suivi des changements en abondance relative permet notamment d'observer la dynamique entourant les changements de température, alors que certaines bactéries mésophiles voient leur abondance relative diminuer drastiquement et laissent la place à plusieurs thermophiles qui augmente en abondance durant les phases plus chaudes. Ces analyses ont aussi permis de mettre en lumière l'apparition d'organismes méthanogènes en fin de phase thermophile qui ont subséquemment fait place aux méthanotrophes durant la phase de maturation. Le suivi de ces successions apporte de nouvelles informations sur les changements qui se produisent au sein des communautés qui se résument généralement à des changements de diversité.

Certaines hypothèses posées dans la mise en place du **Chapitre 4** ont été confirmées, comme celle qui supposait une abondance de bactéries capables de dégrader la cellulose et les hémicelluloses pendant la phase thermophile (hypothèse 1). L'activité lignolytique bactérienne a été observée dans le mélange de matières premières et au début de la phase thermophile plutôt que dans la phase de maturation comme attendu, ce qui infirme l'hypothèse 2. Les champignons sont possiblement responsables de la transformation de la lignine plus tard dans le processus, mais leur présence n'a pas été mesurée. La détection de NO_2^- et NO_3^- était très variable au sein des différents andains, rendant les différences mesurées non significatives. En revanche, la majorité des AOB abondantes ont été observées dans les phases Middle et Aged, tandis que les NOB étaient essentiellement présentes dans la phase Aged, suggérant ainsi qu'une partie de la nitrification a lieu durant la phase thermophile, infirmant partiellement l'hypothèse 3.

La minéralisation de la MO durant le compostage requiert une grande diversité fonctionnelle et engendre une succession des communautés bactériennes qui jouent des rôles distincts, mais redondants dans la cascade d'événements qui conduisent à la production de compost. La prolifération des bactéries ou leur appauvrissement nous renseigne sur leurs interactions et leur potentiel métabolique pendant les phases mésophiles et thermophiles et sur la façon dont cela varie en fonction des facteurs environnementaux.

Les études sur les communautés bactériennes, bien que menées dans des environnements similaires, sont difficiles à comparer. Comme souvent mentionnée, la présence d'un microorganisme à un moment donné dans un environnement donné est le résultat de plusieurs facteurs difficilement reproductibles d'une expérience ou d'un écosystème à l'autre. Outre la matière première utilisée, les conditions d'aération et d'humidité, la durée du processus, le climat, etc.; les techniques d'identification moléculaire basées sur l'extraction d'ADN comportent plusieurs étapes qui peuvent toutes influencer les résultats. Les méthodes d'échantillonnage et d'extraction, le choix des amorces et des régions génétiques à amplifier, les techniques de séquençage et les outils bioinformatiques (Engelbrektson et al., 2010; Klindworth et al., 2013; Tremblay et al., 2015).

ANCHOR a permis d'atteindre la résolution d'annotation des séquences présentées dans le **Chapitre 4**, ce qui a ensuite rendu possible une étude approfondie des espèces présentes et une estimation du potentiel métabolique lié au cycle du carbone et de l'azote dans les différentes phases. Ainsi, bien que des études descriptives des communautés microbiennes telles que celles-ci puissent sembler redondantes, elles restent pertinentes pour élargir nos connaissances sur cet écosystème complexe.

Outre sa pertinence pour améliorer les connaissances sur la dynamique des bactéries impliquées dans la décomposition de la matière lignocellulosique lors du compostage, les informations recueillies peuvent être appliquées dans une multitude de domaines. L'observation de méthanogènes pendant la phase thermophile tardive et la phase de refroidissement, ainsi que la présence de plusieurs bactéries capables de réduire les nitrates

et potentiellement de libérer du protoxyde d'azote pendant la phase thermophile, mettent en évidence des lacunes potentielles dans la gestion des andains. Sachant que ces bactéries ne se développent qu'en l'absence d'oxygène, il est possible de formuler des recommandations pour augmenter la fréquence des brassages pendant ces phases critiques où la demande en oxygène est très élevée. L'échantillonnage des gaz serait évidemment pertinent pour corroborer la présence de microorganismes avec la libération de GES. Si la production de gaz était corrélée à la prolifération de certains microorganismes, il serait possible d'utiliser certaines espèces comme biomarqueurs afin de suivre rapidement le processus, soit par culture en boîte de Pétri, soit par PCR quantitative (RT-qPCR) ou encore par le suivi en temps réel de l'expression de certains gènes. En parallèle, les méthanogènes identifiés, notamment les méthanogènes thermophiles qui semblent être très abondants dans la phase intermédiaire, pourraient être étudiés et potentiellement utilisés pour optimiser la biométhanisation de composés plus récalcitrants tels que les matières cellulosiques qui se dégradent préférentiellement à des températures plus élevées (Moset et al., 2015).

La bioprospection, une approche qui consiste à explorer le potentiel biochimique d'un organisme ou d'un écosystème afin d'isoler, par exemple, des antibiotiques ou des enzymes qui peuvent ensuite être utilisés dans différents domaines (Mateo et al., 2001) s'applique également très bien à la diversité fonctionnelle du compost. Le compost s'est déjà présenté comme une source très intéressante d'enzymes thermostables, en particulier la cellulase, les xylanases et les lignases, qui peuvent être utilisées pour la production de biocarburants (Cragg et al., 2015; Jurado et al., 2014; Sae-Lee & Boonmee, 2014; Satyanarayana et al., 2013; Yunitsyna et al., 2019) et des antifongiques s'attaquant à des phytopathogènes (Jurado et al., 2019). Des bactéries thermophiles reconnues pour sécréter de telles enzymes et appartenant aux genres *Thermobispora*, *Thermobifida* ou à l'ordre des Bacilliales ont été observées en grand nombre dans les phases Young et Middle.

De nombreuses bactéries associées à la dégradation de la lignine sont également capables de dégrader différents types d'hydrocarbures pétroliers, dans la mesure où les deux processus utilisent le même type d'enzyme (Pandey et al., 2016). Par exemple, *Gordonia*

paraffinivorans peut dégrader les hydrocarbures et la paraffine (Xue et al., 2003), *Rhodococcus zopfii* peut dégrader le toluène et les biphényles polychlorés (PCB) (Stoecker et al., 1994), *Pseudomonas thermotolerans* peut se développer sur l'hexadécane (Manaia & Moore, 2002), *Thermobifida cellulosilytica* et *Brevibacillus borstelensis* peuvent dégrader le polyéthylène téréphtalate (PET) (Hadad et al., 2005; Herrero Acero et al., 2013) et *Ochrobactrum intermedium* possède les enzymes nécessaires pour dégrader le glyphosate (Firdous et al., 2020). L'utilisation de compost pour la bioremédiation de sols contaminés a déjà fait ses preuves (Semple et al., 2001), mais repose généralement sur le principe que les champignons sont les principaux responsables de cette activité. Il est donc possible de supposer que l'incorporation de sol contaminé à un mélange de résidus organiques pourrait accélérer la dégradation des contaminants puisque l'activité lignolytique bactérienne était principalement concentrée dans le début de la phase thermophile. Il serait intéressant dans ce cas de comparer l'efficacité de la bioremédiation réalisée avec du compost mature avec celle obtenue en utilisant un mélange en décomposition active (Zhang et al., 2020).

Finalement, l'identification d'une importante population de méthanotrophes dans le compost mature confirme le potentiel de capture du méthane qui lui est souvent attribué. Ce type de compost pourrait être utilisé pour couvrir les LET qui atteindront leur capacité dans les années à venir, en conjonction avec les systèmes traditionnels de capture des gaz (Tanthachoon et al., 2008).

6.3 La dégradation du glyphosate et son impact sur les bactéries

Les informations recueillies et l'expertise développée sur la transformation du matériel végétal lors du compostage (**Chapitre 2**) et sur le type de bactéries responsables de sa dégradation (**Chapitre 4**) ont ouvert la voie à des essais contrôlés où l'on s'intéresserait à la présence d'un contaminant et à son effet sur le processus. Le glyphosate s'est présenté comme un bon sujet en raison de sa capacité à être dégradé par plusieurs microorganismes, de son omniprésence dans l'environnement, de la forte probabilité qu'il soit présent sur des matières destinées au compostage et de sa couverture médiatique en lien avec les risques

potentiels sur la santé humaine (Agostini et al., 2020). L'objectif était donc de combiner du glyphosate de grade analytique (AG) et un herbicide à base de glyphosate (HBG) à un mélange de matière organique et de mesurer l'impact de la présence de contaminants sur l'évolution des paramètres physicochimiques durant le compostage (**Chapitre 3**) et sur la diversité, l'abondance et la succession des bactéries durant les différentes phases (**Chapitre 5**). Le projet visait également à mesurer le potentiel de dégradation du glyphosate au cours du processus et à mesurer la production d'AMPA et son éventuelle accumulation dans le compost mature (**Chapitre 3**).

Les résultats obtenus au **Chapitre 3** n'ont pas confirmé les hypothèses selon lesquelles le glyphosate aurait un effet sur la minéralisation du carbone présent dans la biomasse initiale et que l'augmentation de la température serait plus faible en sa présence. Aucune différence n'a été mesurée dans l'évolution des propriétés physicochimiques entre le traitement témoin et ceux contenant le glyphosate AG et le HBG. L'augmentation de la température et son maintien étaient comparables entre les différents traitements. Par ailleurs, la dissipation du glyphosate a été beaucoup plus importante et rapide que prévu. La dissipation était significativement plus rapide dans le traitement HBG où près de la moitié de la dose appliquée était dissipée après seulement deux jours de compostage. À la fin des 112 jours de compostage (4 mois), tout le glyphosate présent dans le traitement AG s'est dissipé, tandis qu'environ 2 % des niveaux initiaux appliqués subsistaient dans le traitement GBH. Surprenamment, aucun AMPA n'a été mesuré, à aucun moment de l'échantillonnage pour les deux traitements. Sans trop de surprise cette fois-ci, étant donné les résultats recueillis précédemment, les observations compilées au **Chapitre 5** montrent que le glyphosate n'a eu aucun effet sur la diversité bactérienne par rapport au traitement témoin, ce qui infirme également l'hypothèse initialement posée qui prédisait une diminution de la diversité et un impact sur la succession bactérienne. Il n'y avait également pratiquement aucune différence dans l'abondance des différents organismes entre le traitement témoin et le traitement contenant le glyphosate AG, à l'exception de quelques taxons, qui pour la plupart était moins abondants en présence de glyphosate. Les membres présents de l'ordre des Kiloniellales et de la famille des Sutterellaceae étaient plus abondants dans le traitement

glyphosate à T2 et T28 respectivement, mais aucun lien n'a pu être fait entre leur présence et une possible capacité à tolérer ou dégrader l'herbicide.

Selon toute vraisemblance, le glyphosate et les agents présents dans le HBG ne posent pas de problème aux bactéries responsables de décomposer la MO. Le glyphosate s'y trouve également dissipé plus rapidement et extensivement que dans les sols, ce qui pourrait être le résultat de plusieurs facteurs. Par exemple, 1) la concentration assez élevée de phosphate dans le substrat utilisé pour l'étude peut avoir créé une compétition avec le glyphosate pour les sites d'adsorption en rendant le glyphosate plus disponible pour la dégradation (De Jonge et al., 2001). 2) Puisque la charge nette du glyphosate dépend du pH, le pH élevé mesuré pendant le processus pourrait avoir favorisé la solubilisation d'une grande partie du glyphosate, favorisant ainsi sa dégradation (De Jonge et al., 2001). 3) L'adsorption du glyphosate sur les résidus végétaux est plus faible que sur les particules du sol, favorisant une dégradation plus importante lors du compostage (Aslam et al., 2014; Cassigneul et al., 2016). 4) L'abondance d'azote organique comparativement à ce qui est trouvé dans le sol aurait pu offrir une source d'acides aminés aromatiques substantielle qui peuvent alléger l'impact du glyphosate sur la voie du shikimate des bactéries (Forlani et al., 1997; Gresshoff, 1979). Enfin, 5) l'intense activité microbiologique résultant de la décomposition active de la biomasse végétale ainsi que la température chaude aurait favorisé le cométabolisme du glyphosate (Bento et al., 2016; Muskus et al., 2019; Muskus et al., 2020; Zabaloy et al., 2012). Ainsi, le glyphosate aurait servi de source de C, de N ou de P pour les microorganismes colonisant le compost au début du processus. Il pourrait être pertinent de recréer le même genre d'expérience en utilisant de la biomasse qui aurait internalisé le glyphosate après une application, comme des plantes génétiquement modifiées pour être résistante à l'herbicide. Cela permettrait d'évaluer si la dégradation se déroule à un rythme semblable dans un contexte où le glyphosate est moins facilement accessible puisque piégé dans les tissus de la plante.

Les mécanismes qui ont conduit à la dissipation presque complète du glyphosate ne sont pas très clairs pour le moment. L'absence d'AMPA complique l'interprétation de ce résultat, suggérant que soit 1) la voie de la sarcosine était la voie préférée, soit 2) l'AMPA était

dégradé au même rythme qu'il était produit. La première option est peu probable puisque le phosphate a un effet répressif sur l'opéron qui régule les gènes impliqués dans la voie de la sarcosine (Hove-Jensen et al., 2014). Ce dernier, qui était présent en quantité relativement importante aurait ainsi détourné le glyphosate vers la voie de l'AMPA. L'hypothèse la plus probable est donc que l'AMPA a effectivement été produit, mais qu'il a été rapidement dégradé. L'AMPA, bien que souvent décrit comme une molécule récalcitrante dans l'environnement, peut être dégradé de plusieurs façons. Sa récalcitrance dans les sols est en fait due à sa capacité à se lier plus fortement aux particules du sol que le glyphosate (Grandcoin et al., 2017). Comme le compost ne contient pas les mêmes minéraux argileux et les mêmes ions métalliques (Al et Fe) que le sol, il est possible que l'AMPA soit demeuré accessible après sa formation et ait subi une dégradation très rapide, soit en quelques minutes ou quelques heures.

Un traitement expérimental supplémentaire contenant une quantité d'AMPA équivalente à la quantité de glyphosate utilisée aurait permis d'observer la dégradation de l'AMPA au cours du processus. L'extraction d'ARN du mélange de matière organique aurait également permis de cibler les gènes impliqués dans l'activation des enzymes GOX et C-P lyase et de mesurer leur expression à différents temps d'échantillonnage par RT-qPCR. Cela aurait permis de statuer sur la voie métabolique privilégiée au cours de l'expérience.

Les résultats combinés des **Chapitres 3** et **5** suggèrent qu'il y a beaucoup plus de microorganismes capables de dégrader le glyphosate et l'AMPA que ce qui est rapporté dans la littérature. Aucune des espèces présentées dans **la section 1.2.4.4** n'a été observée pendant l'expérience. Quelques genres d'intérêt comme *Achromobacter, Bacillus, Comamonas, Enterobacter, Flavobacterium, Geobacillus, Ochrobactrum, Pseudomonas* et *Streptomyces* ont été identifiés, mais le faible taux d'identification au niveau de l'espèce complique l'interprétation de ces résultats. En effet, seulement 4,2 % des ASV annotés par le pipeline bioinformatique DADA2 utilisé au **Chapitre 5** ont pu être identifiés au niveau de l'espèce, contre près de 20 % des séquences annotées par ANCHOR au **Chapitre 4**. Le nombre d'ASV annoté au niveau du genre, toutefois, atteint 49,6 %, ce qui dépasse légèrement les 46,4 % de séquences annotées au niveau du genre par ANCHOR. Cette

information n'est cependant pas très utile lorsqu'on a affaire à un genre contenant des centaines d'espèces comme *Bacillus* ou *Pseudomonas*. Diverses raisons ont conduit à l'utilisation de DADA2 pour le traitement des données de séquençage dans le **Chapitre 5**, mais ANCHOR aurait pu permettre une annotation plus précise et l'identification des espèces différentiellement exprimées en raison de la non-modification des séquences avant l'annotation. Quoi qu'il en soit, l'activité microbienne intense nécessaire à la décomposition de la matière organique lors du compostage semble avoir également eu raison du glyphosate. Cette information permet de mieux évaluer les risques potentiels associés au compostage de biomasse contaminée et pourrait servir d'entrée en matière pour le traitement d'autres types de résidus.

6.4 Conclusion

La portée des résultats collectés dans cette thèse dépasse le cadre compostage. Que ce soit pour la biométhanisation, la production de biocarburant, la mitigation et la réduction des GES, la bioremédiation, la fertilisation des sols et même la production de bière, de vin et de spiritueux, tous les bioprocédés utilisant de la matière végétale ou des bactéries capables de la transformer peuvent bénéficier des informations contenues dans ce document. Le potentiel enzymatique du compost a déjà été exploité pour le domaine des biotechnologies, mais la caractérisation effectuée dans cette thèse ouvre la porte à une bioprospection beaucoup plus ciblée. La biostimulation ou bioaugmentation à partir de bactéries d'intérêt présentées dans cette thèse pourraient permettre d'accélérer le processus de compostage tandis que la gestion des facteurs abiotiques pourrait limiter la libération de GES causée par certains microorganismes comme les méthanogènes ou les bactéries dénitrifiantes. L'optimisation des procédés de compostage basé sur la biologie pourrait aussi ouvrir la porte à une production de compost de spécialité destiné à différents emplois comme la bioremédiation, la biofertilisation ou la bioséquestration. Dans le présent contexte de crise climatique, les écosystèmes microbiens peuvent représenter une source d'information sous-estimée qui pourrait soutenir les efforts collectifs de transition énergétique et écologique par leur richesse et leur diversité.

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