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L'effet de l'utilisation du thé de compost sur la diversité et la structure bactérienne du sol et les rendements de soja dans les champs

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L'effet de l'utilisation du thé de compost sur la diversité et la structure bactérienne du sol et les rendements de soja dans les champs

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Résumé :

La fertilité de terres agricoles dépend en large partie du recyclage des nutriments dans le sol. Généralement, ce recyclage est effectué en grande partie par les communautés bactériennes du sol. On assume donc souvent que la diversité bactérienne du sol peut constituer un indicateur de sa santé/fertilité. Cependant, certaines pratiques agricoles conventionnelles nuisent à la diversité bactérienne du sol. Parmi ces pratiques, le labourage et les applications d'intrants chimiques tels que les pesticides, les antibiotiques et les engrais influencent négativement la diversité microbienne. Par conséquent, des recherches actives sont menées pour développer des façons de rétablir la diversité microbienne dans les sols en agriculture conventionnelle.

Plusieurs alternatives biologiques ont été développées au fil des ans, aboutissant à des produits commerciaux en tant que des biostimulants incluant des substances d'origines biologiques, des microorganismes ou la combinaison des deux. Entre autres, le thé de compost a été développé et suggéré comme étant un produit riche en microorganismes bénéfiques, ayant les capacités d'améliorer les cultures et la durabilité des systèmes agricoles biologiques. Cependant, sa performance et son application à grande échelle dans les systèmes de production conventionnelle demeurent peu étudiées. L'objectif de ce mémoire et d'évaluer l'effet du thé de compost sur l'abondance, la diversité et la structure des communautés bactériennes du sol et les rendements, dans un essai en champs de la production du soja dans un système conventionnel de monoculture. Dans un champ d'environ trois hectares est subdivisé en six blocs, chacun contenait deux parcelles : l'une a été traitée par le thé de compost frais et l'autre a été utilisé comme témoin avec thé de compost stérilisé à la chaleur pour tuer les microorganismes. Notre hypothèse est que le thé de compost frais améliore la croissance du soja et son rendement avec l'apport de microorganismes bénéfiques et l'enrichissement des communautés bactériennes des sols.

Le séquençage à haut débit de l'ADN ribosomique 16S bactérien extrait de différents échantillons (thé de compost, sol traité et sol témoin), associé aux analyses bio-informatiques et statistiques, a démontré que le traitement du thé de compost frais n'a pas influencé de manière significative les communautés bactériennes, ni par des changements dans la diversité alpha, ni dans la structure de la communauté de celles-ci. De plus, les résultats des analyses de croissance des plantes et de rendement ont eu aucun effet significatif du thé de compost frais sur la biomasse végétative des plantes ou le poids des graines de soja.

Nos résultats de recherche indiquent que le thé de compost frais utilisé dans notre expérience n'a pas modifié les communautés bactériennes des sols traités et n'a pas influencé la croissance des plantes ni le rendement en grain. Notre hypothèse n'est pas supportée par ces résultats qui suggèrent que les bénéfices relatifs à l'application du thé de compost frais ne sont pas dus aux microorganismes vivants mais plutôt à un apport potentiel des nutriments. L'absence d'effets positifs dans notre étude pourrait être attribué spécifiquement à notre conception expérimentale, au thé de compost utilisé, ou à la dose ou la fréquence d'application de celui-ci. D'autres expériences sont nécessaires afin de tirer des conclusions robustes quant à l'effet et la performance du thé de compost sur des cultures conventionnelles.

Mots clés

Fertilité des sols, diversité bactérienne, structure bactérienne, agriculture conventionnelles, thé de compost, séquençage à haut débit, croissance, rendement, soja.

Abstract:

The fertility of agricultural lands largely depends on the recycling of nutrients in the soil. Usually, this recycling is carried out largely by bacterial communities in the soil, that their diversity is an important indicator of the health and fertility of agricultural soils. However, some agricultural practices, especially in conventional production systems, harm the essential functions of these soil bacterial communities. Among these practices, tillage and the applications of chemical inputs such as pesticides, antibiotics and fertilizers negatively influence the diversity and structures of microbial communities. As a result, the abundance and diversity of these beneficial microorganisms and the potential services they provide decrease in these soils.

Several biological alternatives have been developed over the years, resulting in commercial products as biostimulants including substances of biological origin, microorganisms or a combination of the two. Among others, compost tea has been developed and suggested as a product rich in beneficial microorganisms, with the capacity to improve crops and the sustainability of organic farming systems. However, its performance and large-scale application in conventional production systems remains little studied. The objective of this master's thesis is to assess the effect of fresh compost tea on the abundance, diversity and structure of soil bacterial communities and yields, in a field trial of soybean production in a conventional system of monoculture. In a field of about three hectares is subdivided into six blocks, each one contained two plots: one was treated with fresh compost tea and the other was used as a control with heat sterilized compost tea to kill microorganisms. Our hypothesis is that fresh compost tea improves soybean growth and yield with the addition of beneficial microorganisms and the enrichment of bacterial communities in soils.

High throughput sequencing of bacterial 16S ribosomal DNA extracted from different samples (compost tea, treated soil and control soil), combined with bioinformatics and statistical analyzes, demonstrated that processing of compost tea did not significantly influenced bacterial communities, neither by changes in alpha diversity nor in their community structure. In addition, the results of plant growth and yield analyzes had no significant effect of fresh compost tea on plant vegetative biomass or soybean weight.

Our research results indicate that the fresh compost tea used in our experiment did not change the bacterial population in the treated soils and it did not show a significant effect on either plant growth or yield. Our hypothesis is not supported by these results which suggest that the relative benefits of the application of compost tea are not due to living microorganisms but rather to a potential supply of nutrients. The lack of positive effects in our study could be attributed specifically to our experimental design, the compost tea used, or the dose or frequency of its application. More experiments are needed in order to draw robust conclusions about the effect and performance of compost tea on conventional crops.

Keywords

Soil fertility, bacterial diversity, bacterial structure, conventional agriculture, compost tea, high-throughput sequencing, growth, yield, soybean.

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ASV: Amplicon sequence variant

α: Level of statistical significance

α-diversity: Alpha diversity

β-diversity: Beta diversity

bp: Base pair

CS1, CS2: Adapters serve as the sequencing primer site

DNA: Deoxyribonucleic acid

dNTP: Deoxyribonucleotide triphosphate

GLMM: Generalized linear mixed models

ha : Hectare

Illumina MiSeq: Next Generation Sequencer

IRBV: Institut de recherche en biologie végétale

ISA: Indicator species analysis

KCl: Potassium chloride

kg: Kilogram

L: Litre

LMMs: linear mixed models

mg: Milligram

Min: Minutes

mL Milliliter

N: Azote

NH_4^+: Ammonium

NO_3^-: Nitrate

NSERC: Natural Sciences and Engineering Research Council of Canada

O_2: Oxygen

P: probability

PCA: Principal component analysis

PCR: Polymerase chain reaction

perMANOVA: Permutational Multivariate Analysis of Variance

pH: Scale used to specify the acidity or basicity of an aqueous solution

PO_4^{3-}: Phosphate ion

rDNA : Ribosomal DNA

RNA: Ribonucleic Acid

rRNA ribosomal RNA

s: Second

Taq: Thermostable DNA polymerase named after the thermophilic bacterium Thermus aquaticus

V3-V4: A specific region of the 16S rRNA gene that can identify the bacteria

%: Percentage

µl: Microlitre

µM: Micrometre

ºC: Degree Celsius

Dédicace

À mon point faible et force dans la vie...

À mon ange, qui a commencé m'accompagner depuis le début de ce projet…

À mon héros, qui prouvera au monde entier que la volonté rend l'impossible...

*Mon petit garçon, **Victor**.*

x

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Chapitre 1

2.1 Introduction générale:

Les bactéries du sol remplissent plusieurs fonctions écosystémiques essentielles dans les sols. Elles participent au cyclage des nutriments, à la protection des plantes contre les pathogènes, et contribuent globalement à la fertilité des sols (Davison 1988; Altieri, 1999; Nannipieri et al., 2003; van der Heijden et al., 2008; Lladó et al., 2017). Les communautés bactériennes des sols sont donc considérées comme une « ressource » importante pour la gestion durable des systèmes agricoles (Altieri, 1999; Kennedy, 1999; Barrios, 2007; Hayat et al., 2010; Mohammadi & Sohrabi, 2012). Il est donc primordial de comprendre comment nos pratiques agricoles modifient les communautés bactériennes, avec des effets rétroactifs potentiels sur la performance des cultures, et plus largement, la durabilité de nos régies culturales (McLaughlin & Mineau, 1995; Ponge et al., 2013; Ortiz-Cornejo et al., 2017).

L'agriculture conventionnelle des grandes cultures de céréales et de légumineuses, domine le paysage agricole mondial, et répond à une demande croissante en nourriture (FAO, 2017). Toutefois, les pratiques de l'agriculture conventionnelle peuvent réduire l'abondance et la diversité des communautés bactériennes de plusieurs manières, via entre autres les intrants chimiques épandus (engrais et pesticides) ou les travaux mécaniques des sols (Liang et al., 2020 ; AL-Ani et al., 2019; Janušauskaite et al., 2013). Par exemple, des études ont largement signalé les effets négatifs des pratiques de travail du sol sur la diversité bactérienne (Silva et al., 2013; Sun et al., 2018; Liu et al., 2020). D'autres études ont démontré que les engrais chimiques nuisent d'une part aux fonctions des communautés bactériennes du sol (Tsiafouli et al., 2015) et d'autre part, aux

relations trophiques entre les organismes du sol (par exemple, Ma et al., 2018; Bai et al., 2020; Ji et al., 2018).

Étant donné l'importance de la diversité des bactéries bénéfiques dans les sols et leur capacité à améliorer la croissance et la productivité des plantes, en utilisant différents mécanismes associés et en appliquant des changements dans les communautés bactériennes rhizosphériques, comme le démontrent de nombreuses études (Kennedy, 1999; Nannipieri et al., 2003; Barrios, 2007; van der Heijden et al., 2008; Hayat et al., 2010; Lladó et al., 2017). En plus des effets néfastes des pratiques conventionnelles sur ces micro-organismes et leurs services, il est primordial de revoir et corriger certaines pratiques agricoles afin de maintenir et préserver la richesse biologique et la fertilité des sols pour assurer une production alimentaire durable. Par conséquent, il faut donc développer des méthodes agricoles intégrées respectueuses de l'environnement (Ghany et al., 2014; Bhardwaj et al., 2014; Souza et al., 2015; Boraste et al., 2009; O'Callaghan, 2016; Swami, 2020; Raina et al., 2020; etc.).

L'application du thé de compost est un exemple d'une méthode d'inoculation microbienne qui a été proposée et elle a gagné en popularité aux États-Unis, et son application a été répandue dans le reste du monde au cours des dernières décennies (Ingham, 2003; Ingham, 2005; Scheuerell & Mahaffee, 2002;Naidu et al., 2010). L'approche de cette méthode consiste en production d'une suspension riche en microorganismes et microfaunes du sol à partir d'un compost infusé dans une solution nutritive bien oxygénée. Le thé de compost est produit par le trempage des mélanges de composts finement calibrés dans l'eau en aérobie avec des sources de carbone simples (par exemple : la mélasse et la farine de céréales) pour favoriser la multiplication de divers microorganismes dont les bactéries copiotrophes (Fierer et al., 2007) et les protozoaires (leurs prédateurs) (Naidu et al., 2010; Ingham, 2005; Kannangara et al., 2006).

Des milliards de microorganismes bénéfiques contenus dans seulement quelques litres de thé et non quelques tonnes de composts peuvent être atteints dans une courte période (généralement en 48 heures), et ainsi le thé de compost peut être filtré pour inoculer ces biotas vivants sous forme liquide dans de grandes surfaces (Naidu et al., 2010; Scheuerell & Mahaffee, 2002).

L'utilisation du thé de compost aéré pourrait donc améliorer la performance des cultures en augmentant l'abondance et la diversité des organismes bénéfiques du sol (y compris des bactéries) qui pourraient contribuer au cycle des nutriments dans le sol (Scheuerell & Mahaffee, 2002; Kannangara et al., 2006; Ingham, 2005).

Plusieurs études ont confirmé l'efficacité de l'utilisation du thé de compost sur les différentes cultures en tant qu'inoculant biologique bénéfique, ce qui contient une grande quantité et diversité des communautés microbiennes bénéfiques, lesquelles offrent à leur tour des services environnementaux essentiels à la fertilité du sol et à la bonne croissance des plantes (Hargreaves et al., 2009; Islam et al., 2016; Kim et al., 2015). Par exemple, Pant et al. (2012) ont constaté que l'application de thé de compost augmentait la croissance et la teneur en nutriments minéraux du pak choi. De même, Abdrabbo & Desoky. (2014) ont montré que l'utilisation de thé de compost augmentait de manière significative la croissance végétative, le rendement et le contenu nutritionnel du concombre.

Considérant les résultats de ces travaux sur des avantages associés au thé de compost aéré, il a été suggéré que son utilisation comme une solution environnementale prometteuse pour améliorer l'agriculture conventionnelle de grande surface (Scheuerell & Mahaffee, 2002; Ingham, 2005). Cependant, ces études n'ont pas démontré hors de tout doute que l'effet positif du thé de compost venait réellement de son apport en microorganismes bénéfiques. Plusieurs questions peuvent donc être posées :

Est-ce que l'application de thé de compost agit-elle par un apport de bactériens bénéfiques aux sols? Est-ce que celles-ci s'établissent dans les sols après l'application du thé de compost? Plus d'efforts sont encore nécessaires pour étudier les changements qui peuvent se produire dans les communautés bactériennes après l'inoculation par le thé de compost et vérifier les effets positifs du thé sur la performance de la croissance et le rendement des cultures. Le but principal de la présente étude est d'évaluer l'impact de l'application de thé de compost sur l'abondance et la diversité des communautés bactériennes du sol, et les performances des cultures dans un système conventionnel de monoculture de soja en champ.

Nous avons émis l'hypothèse selon laquelle l'application du thé de compost modifierait la structure et la diversité des communautés bactérienne du sol dans notre champs d'étude. Nous avons basé notre hypothèse sur le fait que le thé de compost utilisé devrait contenir une grande quantité de sucres, ce qui résulterait en une dominance de certaines souches de protéobactéries qui sont parfois reconnues comme des copiotrophes opportunistes (Fierer et al., 2007) et qui devraient se multiplier rapidement en présence de source de carbone comme nutriment dans la préparation du thé de compost. Nous prévoyions que ce changement dans les communautés bactériennes améliorerait la croissance et le rendement du soja.

Chapitre 2

Chapitre 2 est présenté comme un article de recherche publié en journal scientifique (Manuscript ID: plants-1302135; doi: 10.3390/plants10081638).

J'ai participé à l'échantillonnage et au traitement de tous les échantillons. J'ai réalisé les extractions des ADNs et j'ai fait toutes les réactions PCRs et la préparation de l'envoie des PCR pour le séquençage. J'ai également fait les dosages chimiques et déterminé les paramètres agronomiques (croissance végétative et rendement du soya). J'ai aussi réalisé des analyses bio-informatiques et statistiques, et rédigé l'ébauche l'article.

Article

Fresh compost tea application does not change rhizosphere soil bacterial community structure, and has no effects on soybean growth or yield

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Abstract:

Soil bacteria drive key ecosystem functions, including nutrient mobilization, soil aggregation and crop bioprotection against pathogens. Bacterial diversity is thus considered a key component of soil health. Conventional agriculture reduces bacterial diversity in many ways. Compost tea has been suggested as a bioinoculant that may restore bacterial community diversity and promote crop performance under conventional agriculture. Here, we conducted a field experiment to test this hypothesis in a soybean-maize rotation. Compost tea application had no influence on bacterial diversity or community structure. Plant growth and yield were also unresponsive to compost tea application. Combined, our results suggest that our compost tea bacteria did not thrive in the soil, and that the positive impacts of compost tea applications reported elsewhere may be caused by different microbial groups (e.g., fungi, protists, nematodes) or by abiotic effects on soil (e.g.,

contribution of nutrients and dissolved organic matter). Further investigations are needed to elucidate the mechanisms through which compost tea influences crop performance.

Keywords: Conventional agriculture; sustainable agriculture; compost tea; bacteria; biodiversity; Illumina MiSeq sequencing; plant growth; yield; soybean

2.1 Introduction

Soil bacteria drive key ecosystem functions, including litter decomposition, nutrient mobilization, crop protection against pathogens, and soil aggregation [1-4]. Bacterial species are not functionally redundant, which translates into positive correlations between ecosystem functioning and bacterial diversity [5-7]. As a result, bacterial diversity is now recognized as a component of soil health and a central issue in the development of sustainable agriculture practices [2,8,9].

Conventional agriculture can negatively influence bacterial diversity, community stricture and biomass in many ways. Tillage has been widely reported to negatively affect bacterial diversity in croplands [10-13]. The same is true for chemical fertilizers that have been shown to reduce soil functional diversity [14], and lead to community dominance by a few taxa [15-18]. Pesticides also reduce soil microbial diversity and enzymatic activity, which may compromise soil health and plant performance in the long run [19-21]. Various strategies have been suggested to alleviate the negative effects of these conventional practices. Among these, plant biostimulants such as microbial-based inoculants are deemed a promising solution for improvement of plant performance and ecosystem functioning [22-27].

Numerous microbial inoculants have been developed for organic and conventional farming [28-31]. Mycorrhizal fungi and nitrogen-fixing bacteria, for example, are widely used to promote plant growth and soil fertility in harsh conditions [32]. Likewise, various rhizobacteria have been found to promote plant growth and vigor [33]. However, the positive impacts of these microbial inoculants are likely to be context-dependent [34], and unlikely to restore microbial diversity in agricultural soils. Alternative solutions that better promote microbial diversity, and thus ecosystem function [7], and resilience [35], should therefore be explored. Compost tea has been suggested as one such solution [36,37].

Compost tea is an inoculant prepared through aerobic, liquid-based incubation of compost amended with carbon sources. This promotes microbial proliferation [36-38]. Over a short incubation time (typically 48 hours), high cell densities can be achieved, allowing the application of a diluted suspension over large surfaces. Aerated compost tea is presumed to be an environmentally safe product that could enhance crop performance, in part, through the reintroduction of diverse soil bacteria contributing to nutrient cycling [37,38].

Positive yield responses to compost tea have been reported for a variety of crops [39-41]. Based on such findings, many authors have concluded that compost tea treatment could be used as a plant growth-promoting technique in organic cultivation of crops. However, we still lack basic information on the mechanisms through which compost tea influences indigenous microbial communities and crop yields. Specifically, we still do not know whether bacteria inoculated through compost tea can survive, successfully establish themselves and compete against indigenous bacterial communities in the soil. Further studies are required to trace and track changes in microbial communities following compost tea application, with appropriate experimental controls, in order to verify the potential impacts of the tea on crop growth and yield. We also need

to better distinguish the biotic effects of applying compost tea to soil (i.e., its contribution of beneficial biotas) from its abiotic effects (i.e., its addition of nutrients to the soil in mineral and dissolved organic forms). In this study, we conducted a field experiment to evaluate the impact of compost tea application on bacterial community structure and crop performance in a conventional soybean monoculture system. We hypothesized that compost tea application would promote bacterial diversity, and more specifically, Proteobacteria, which are commonly regarded as opportunistic copiotrophs [42], that should capitalize on simple sugars included as amendments during compost tea preparation. We anticipated that this shift in bacterial communities would, in turn, improve soybean growth and yield, given the wide range of known plant growth-promoting taxa among Proteobacteria.

2.2 Results

2.2.1. Bacterial community composition

From our total of 119 samples (108 soil samples and 11 compost tea samples), we retrieved 737 bacterial ASVs belonging to 13 phyla. To determine patterns of bacterial richness, we performed ASV rarefaction analysis for all samples, which showed that our sequencing depth was appropriate since all curves reached an asymptote (Fig. S1). Microbial communities were dominated by Planctobacteria (63%), Verrucomicrobia (18%), Chloroflexi (7%), and Patescibacteria (6%) (Fig. 1.a).

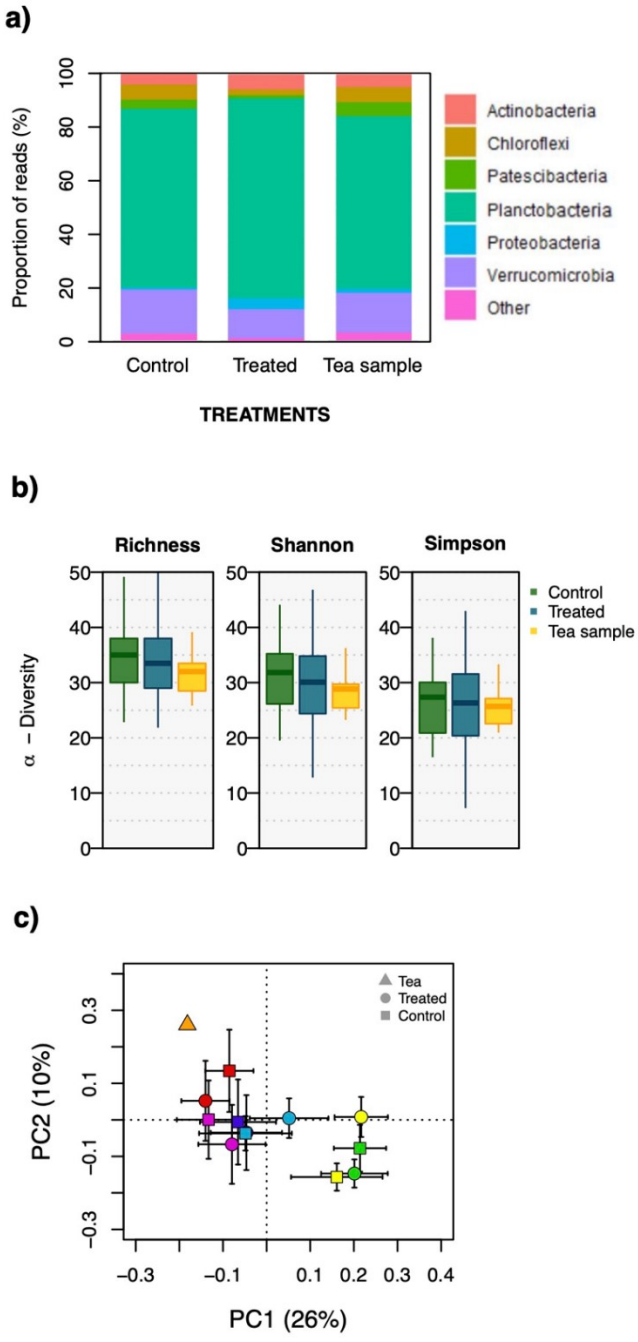

Figure 1. a) Relative abundance of ASVs belong to the different bacterial phyla present in soils treated with living compost tea ("Treated"), with sterilized compost tea ("Control"), or from tea samples taken prior to application ("Tea sample"). b) Alpha-diversity of bacterial communities (exponential Shannon (eH) and inverse Simpson indices). c) Principal component analysis (PCA) of Hellinger-transformed bacterial relative abundances. Bacterial communities tend to cluster according to experimental blocks (yellow, green, dark-blue, light-blue, pink and red, represent six experimental blocks; orange triangle represents compost tea). Shapes (circle and square) represent

treatments. Symbols represent the mean scores of samples from a given plot, and error bars represent 95% confidence intervals.

When comparing plots treated with living vs sterilized compost tea, there was no significant effect of fresh compost tea application on bacterial communities, neither through shifts in α-diversity (ASV richness: P = 0.64 / Shannon's diversity: P = 0.26 / Inverse Simpson's diversity: P = 0.56), nor shifts in community structure (β-diversity; perMANOVA pseudo-F = 1.17, d.f. = 2, P = 0.216; Fig. 1.b,c).

Indicator species analysis revealed that: (1) there were many indicator bacterial ASVs of compost tea (in fact, the majority of our indicator taxa belonged to compost tea); (2) only one ASV belonging Planctobacteria, was found to be an indicator species of both compost tea and treated soil samples; (3) several ASVs were indicators species of both treated and control plots; and (4) control plots or only treated plots didn't share any common indicator species. (Fig. 2).

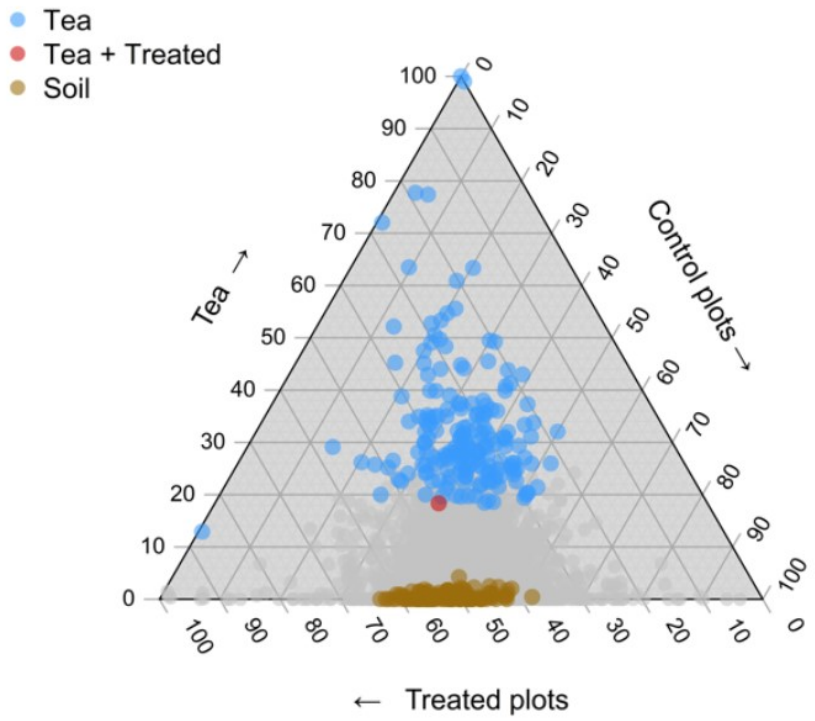

Figure 2. Ternary triangle presenting the relative distribution of reads from each ASV in treated plots, control plots or tea samples. Each symbol represents an ASV. Blue symbols are ASV indicator for tea samples; brown symbols are ASV indicator for control plots; the red symbol is the only ASV indicator for both tea and treated plots; grey symbols are those ASVs that are not indicator for any category.

2.2.2. Soybean growth and productivity

Compost tea application did not improve plant growth (shoot dry mass, P = 0.36) or grain yield (grain dry weight, P = 0.14; Fig. 3). Statistical power analyses indicated that compost tea application had small effect sizes (power = 23% and 30%, respectively, for growth and yield). We estimated that minimal sample size to detect an effect would have been 28 blocks for plant growth, and 20 blocks for plant yield, confirming the small effect size of our compost tea treatment.

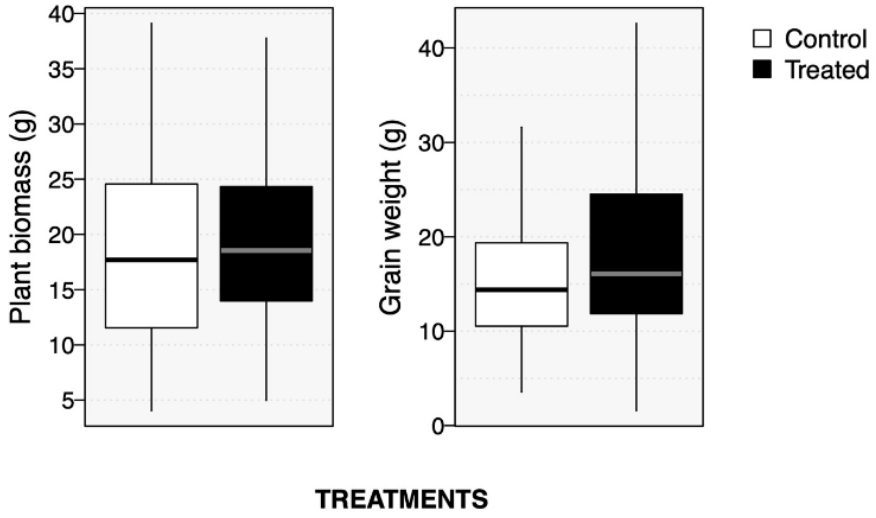

Figure 3. Boxplots showing plant growth (left) or yield (right) in control plots (red) or treated plots (cyan).

2.3 Discussion

Surprisingly, both the control and treated soil samples were largely dominated by Planctobacteria (Fig. 1.a), a result contrasting with several studies identifying Proteobacteria as the dominant bacterial phylum in soils, followed by Chloroflexi, Bacteroidetes, Actinobacteria, and Acidobacteria [43-47]. Planctobacteria are a unique divergent phylum of aquatic bacteria [48-53], that can be isolated from nonaquatic environments such as soil [52,54]. These bacteria are assumed to prefer anaerobic soil micropores [55-57], as they can tolerate low O2 pressures, which allows them to displace obligately aerobic taxa in low-O2 microsites/horizons [55,56]. Here, we hypothesize that soil dominance by Planctobacteria could be explained by the recent installation of drainage infrastructures in the subsoil horizon of our study site. This caused the mixing of topsoil with deep subsoil (2 meters deep), which was presumably (1) less aerated and (2) less colonized by roots, which accordingly would account for the low abundance of copiotrophic

rhizosphere specialists belonging to the Proteobacteria phylum [42]. This would be in line with Kepel et al. [58], who recently found that the only soil in their dataset dominated by Planctobacteria was from a rice field, which are typically characterized by low soil O2 pressures.

Because Planctobacteria were also abundant in the compost tea preparations (Fig. 1.a), we could also hypothesize that the Planctobacteria DNA retrieved in our soil samples (both from treated and control plots) belonged to dead bacterial cells, and this DNA had not fully degraded at the time of soil sampling. However, considering the total volume of liquid applied per surface in our treatments, we would find it surprising if the non-degraded portion of this dead DNA constituted the majority of the DNA we extracted afterwards from our soil samples. Moreover, by looking specifically at Planctobacteria communities in our soil and tea samples (i.e., by filtering our ASV table so that only Planctobacteria remain), we find that distinct Planctobacteria taxa dominated tea samples vs treated soil samples (Fig. S3).

Our principal component analysis (Fig. 1c) revealed a clustering of bacterial communities according to their plot origin rather than their treatment (i.e., living vs sterilized tea), which further shows that bacterial populations were spatially heterogeneous at our site, but not influenced by the treatment. This could be explained, in part, by contrasting soil properties across blocks (e.g., N availability; see Table 1).

The molecular analysis of bacterial community structure overall suggests a poor establishment of microbial taxa from the tea in the soil. This is supported by the fact that only one out of 737 ASVs was commonly found in both compost tea samples and treated soil samples (Fig. 2). As our sensitivity analysis revealed that type I errors could represent around 3-4% of the dataset, we cannot rule out the possibility that the single indicator ASV for tea and for treated plots resulted from a type I error and thus was not truly an indicator for tea and treated plots. In fact, of our 737

ASVs, 322 were identified as indicator taxa (44%). This is well above the random expectation of 3-4%, but still, this means that roughly 10% of our indicator taxa may have arisen in the analysis by chance alone. However, this does not affect our conclusions, as most indicator taxa were indicators of either tea (probably taxa from tea that failed to thrive in the soil) or soil alone (resident soil taxa present prior to application). In both cases, this would suggest a poor establishment of tea bacterial taxa in our plots. Overall, this offers compelling evidence for the hypothesis that in our study, the tea bacteria failed to establish themselves in the soil, either because of low application density (and thus low initial population sizes) and/or because of a poor competitive ability against resident soil bacteria.

Compost tea application did not improve plant growth or yield in this experiment. Statistical power analyses confirmed the small effect size of our compost tea treatment, thus any impact of the compost tea on the living soil community (bacterial or not) would have been modest and would not have translated to large shifts in crop performance.

2.4 Materials and methods

2.4.1. Site Description

Our study was conducted in a field of approximately 3 hectares, located in Sainte-Christine, Quebec, Canada (see Fig. S4; 72.434353 W, 45.613667 N). This field has a several-decade history of conventional soybean-maize monocrop rotations and conventional agricultural practices. In spring 2018, installation of a drainage system in the field resulted in a severe soil disturbance in which the plow zone was mixed with the less biologically active, deeper horizons [12]. On June 6th, soybean was sown at a density of 382,850 seeds/ha.

2.4.2. Experimental design

We conducted our compost tea application using a randomized block design. We divided the field (344m x 82.5m) into six experimental blocks (172m x 27.5m). Each block was then divided into two plots, with one receiving the treatment (living compost tea) and the other receiving the control (compost tea sterilized by boiling). Before application, we characterized initial soil properties by collecting composite soil samples from each block (Table 1).

Table 1. Soil properties were measured on composite samples taken from each experimental block. Mehlich III – PO43- = orthophosphates extractible with Mehlich-III solution; KCl-NH4+ and KCl-NO3- = respectively ammonium and nitrates extractible using 2N KCl.

Block	pH	Organic matter content (%)	Gravimetric moisture (%)	Melich III -PO_4^{3-} (mg/kg)	KCl-NH_4^+ (mg/kg)	KCl-NO_3^- (mg/kg)
A	6.20	12.75	25.26	75.61	68.91	5.84
B	6.25	9.30	20.79	26.28	62.74	8.44
C	6.16	14.02	22.53	26.60	81.77	9.38
D	6.36	10.27	24.46	32.73	91.31	20.43
E	6.64	6.88	21.42	32.06	28.38	14.04
F	6.56	12.21	25.16	24.26	44.48	19.89
Mean	**6.36**	**10.91**	**23.27**	**36.26**	**62.93**	**13.01**

2.4.3. Compost tea preparation and application

Aerated compost tea was prepared in two phases: a pre-activation phase, aiming to increase microbial population densities in the compost, and a dilution phase, producing a liquid suspension from the compost (i.e., tea) for inoculation.

In the pre-activation phase, two different kinds of compost were mixed in equal quantities: the first, an especially carbon-rich vermicompost, consisting of up to 50% ramial wood chips and leaf litter and matured through the activity of earthworms; and the second, a thermal compost, consisting of 10% chicken manure, 15% horse manure, 30% fresh green plants, 20% ramial wood chips and 25% leaf litter, mixed at high temperatures (60-70° C) for 30 days. In a 75L container, we combined 20L of this compost mixture with 300 mL oatmeal, 150 mL alfalfa flour, 150 mL fish hydrolysate, 100mL seaweed flour, 30mL molasses, 5 ml humic acid solution and non-chlorinated water (to reach 50% humidity). This blend was incubated for 72 hours and mixed every 12 hours to maintain aerobic conditions. Compost tea was prepared by washing this aerated mixture at room temperature in 20L washing bags (mesh size = 400 μm), and then combining 10 L of the aerated mixture with 0.8 L water, 3 L oatmeal, 2.5 L fish hydrolysate, 1.5 L humic acid solution and 0.5 L soluble algae. Air was pumped into the mixture for two days to avoid anaerobic fermentation. Half of this compost tea preparation was then sterilized by heating at 95°C for 90 min, in order to be used as an experimental control (i.e., to distinguish the effects of the compost tea's living organisms from the abiotic effects of its minerals and dissolved organic nutrients).

The compost tea and sterilized control solution were prepared and applied to the field 4 times during the summer of 2018, on June 9th, June 22nd, July 5th and July 19th. Dilutions and dosages were adapted to weather conditions during the growing season, thus the compost tea dilution ratios for the specified dates were 1:1, 1:4, 1:4 and 1:3, respectively, with dosage densities of 121.57 l/ha, 486.26 l/ha, 486.26 l/ha and 364.7 l/ha, respectively. In addition, subsamples of each of the compost tea preparations (i.e., concentrated, applied, and sterilized) were kept at -20°C for molecular characterization of the bacterial communities.

2.4.4. Field samplings

Two field samplings were conducted. A first sampling campaign was done during the vegetative growing stage, on August 14th, in order to (1) measure the aboveground dry biomass as an indicator of vegetative plant growth, and (2) characterize the bacterial communities present in the soils. In each plot, nine individual soybean plants were excavated and their rhizospheric soil collected (by shaking the root system in a plastic bag) and kept frozen at -20°C for DNA extraction. Aboveground biomass was dried (at 65°C for 1wk) and weighed. The second field sampling was conducted a day before crop harvest, on October 3rd, when 30 individual soybean pods per plot were randomly collected and transferred to the laboratory to measure grain weight as an indicator of yield.

2.4.5. Molecular analyses

DNA was extracted from 250 mg of rhizospheric soil and compost tea samples using a Power Soil DNA kit (Qiagen Inc., Canada) according to the manufacturer's instructions. Double-stranded DNA was quantified using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific Inc., Canada). DNA extracts were PCR-amplified using 16S rDNA primers with CS1 and CS2 adapters show in italics (forward CS1-341F: 5′ ACACTGACGACATGGTTCTACACCTA-

CGGGNGGCWGCAG-3′; reverse CS2-806R: 5′ TACGGTAGCAGAGACTTGGTCTGA-

CTACHVGGGTATCTAATCC-3′; [59]), targeting the hypervariable V3-V4 region of the 16 rRNA gene. PCR reactions were performed in a total volume of 50 µl containing 1X PCR buffer, 0.5 µM of each primer, 5.0 µL of dNTPs (10 mM), 0.4 µL of Taq DNA polymerase and 2 µL of template DNA. PCR conditions were as follows: 4 min denaturation at 94°C, followed by 35 cycles of denaturation (94°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 60 s),

and a final 10 min extension at 72°C. PCR reactions that gave a visible amplification band on agarose gel were sent for Illumina MiSeq sequencing (300 bp paired-end library) at the Génome Québec Innovation Center.

2.4.6. Bioinformatics

Analysis of the sequence data was coded in R (v4.0.1; R Development Core Team, 2014) using the DADA2 R package (v1.1.2; [60]). Sequences were quality filtered and primers were removed. We removed sequences with less than 290 bp and 260 bp (forward and reverse, respectively), as the base quality of the sequences showed a clear drop below these thresholds. For this we used the DADA2 command filterAndTrim with a maxEE score of 2 and trunQ score of 6. We then calculated the error rates using the learnErrors command and merged the forward and reverse sequences. Next, chimeras were removed and the amplicon sequence variants (ASV) table was built, and finally taxonomy was assigned to the ASVs using the SILVA reference database [61].

A total of 2,171,433 raw reads were generated from 119 individual samples (108 soil samples and 11 compost tea samples). Sequences classified as chloroplasts or mitochondria were removed from the ASV table, as were any sequences classified as Eukarya or Archaea. Samples were then rarified to 1749 reads per sample using the function rrarefy from the R package vegan [62]. To avoid focusing on potential sequencing artefacts or on especially rare bacterial taxa, we filtered our dataset to remove: (1) any occurrences with 5 reads; and (2) any ASVs that only appeared in 1 or 2 samples.

2.4.7. Statistical analysis

To determine the effect of compost tea and sterilized compost tea (control) treatments on plant growth and yield, we used linear mixed models (LMMs) as implemented in the R package lme4, including plot identity as a random effect [63]. We used the pwr R package to estimate the power of our analysis comparing the plant growth or yield of treated plots versus control plots [64].

We evaluated the impact of compost tea application on both bacterial α-diversity and community structure (β-diversity). Alpha diversity was assessed using ASV richness, the exponential form of Shannon diversity, and inverse Simpson diversity [65]. Alpha diversity was compared between treatments using Poisson regression (generalized LMM) for ASV richness and Gaussian LMMs for Shannon and Simpson diversities.

Shifts in community structure following treatments were assessed with permutational multivariate analysis of variance (perMANOVA; [66]) using the function adonis of the R package vegan [62]. The Hellinger distance [67] was used to evaluate pairwise β-diversity across samples, which was visualized using principal component analysis (PCA).

In order to identify bacterial ASVs that were specifically associated either with soil samples treated with compost tea or control samples treated with sterilized compost tea, we conducted an indicator species analysis (ISA) using the function multipatt of the R package indicspecies [68]. We used a threshold of $\alpha = 0.01$ because this analysis implied a high number of taxa in permutation-based statistical tests (i.e., 1 per bacterial taxon), which may inflate type I errors. However, traditional P-value correction methods (e.g., Bonferroni) would have resulted in overly conservative tests given the very high number of bacterial ASVs. This would have resulted in high type II error rates, which is why we decided to manually set α at 0.01. To evaluate how

prone, we were to detecting false positives (i.e., indicator taxa that would be associated with one treatment or another simply by chance), we conducted ISA on randomized metacommunities generated using the null model vaznull in the R package bipartite [69]. These simulations indicated that roughly 3-4% of indicator taxa may arise as false positives (Fig. S2).

2.5 Conclusions

Our results showed that aerated compost tea application had no influence on bacterial diversity or community structure. Accordingly, plant growth and yield were unresponsive to compost tea application. We note that our results do not undermine the potential role of compost tea in increasing crop yield or contributing to sustainable agriculture. Our design did not include plots where compost tea was not applied. Our study thus reveals that the positive effects of compost tea found in other studies could be due to: (1) the nutritional effects of compost tea (i.e., its contribution of minerals to the soil through dissolved organic nutrients); or (2) its alteration of other physicochemical properties of the soil (e.g., increased cation exchange capacity due to dissolved organic matter in compost). Alternatively, the absence of effects in our study could be ascribed specifically to the compost we used or to the dose or frequency of tea application. Much remains to be studied regarding the mechanistic nature of the impact of compost tea on crop performance. Our only conclusion here is that in this field trial on a severely disturbed soybean monoculture field, living compost tea application did not influence bacterial communities or crop yield.

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Author Contributions: † These authors contributed equally to this work.

Data Availability Statement: The datasets generated and analyzed during the current study are available in the Sequence Read Archive (SRA).
[http://www.ncbi.nlm.nih.gov/bioproject/728448].

Conflicts of Interest: (Not applicable).

Code Availability SubmissionID: SUB9541804\ BioProject ID: PRJNA728448.

2.7 Appendix.1 Supplementary figures

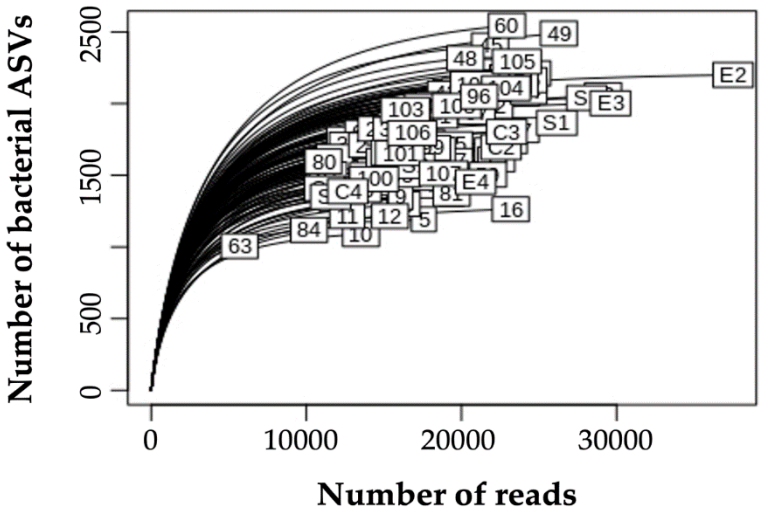

Figure S1. Rarefaction curves of ASVs for individual samples across the different samples.

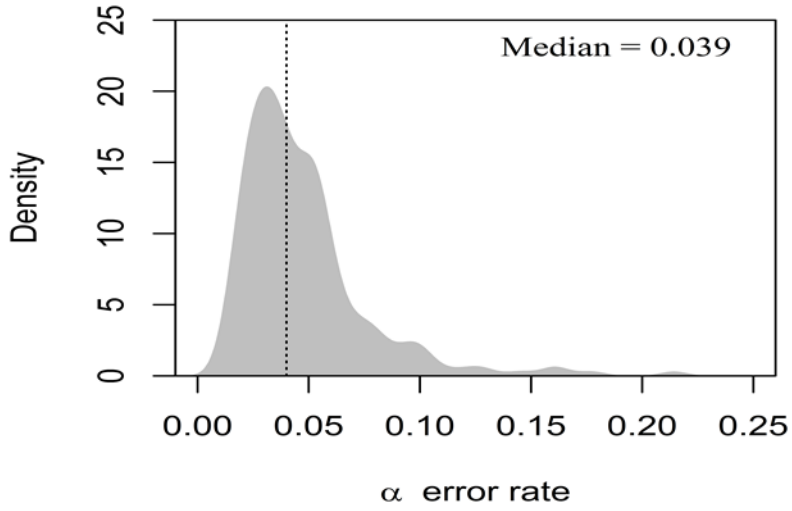

Figure S2. Evaluation of type I error rates in our indicator species analysis. For 1000 random trials, our bacterial metacommunity (i.e., 118 sites [samples] as rows and bacterials ASVs as columns) was randomized using a null model (vaznull in the R package bipartite) that constrains connectance and marginal totals, which is a conservative approach to metacommunity randomization. Then, we ran an indicator species analysis, to evaluate the frequency of false

positives that would arise in these trials (i.e., the frequency with which ASVs would be flagged as indicative of a given "treatment" even though the metacommunity had been randomized). For each run, we calculated the proportion of ASVs considered "indicator taxa" as the a-error rate. We report the density plot of this a-error rate for our 1000 random trials. The vertical line report the median value of our 1000 trials, i.e. 3.9%.

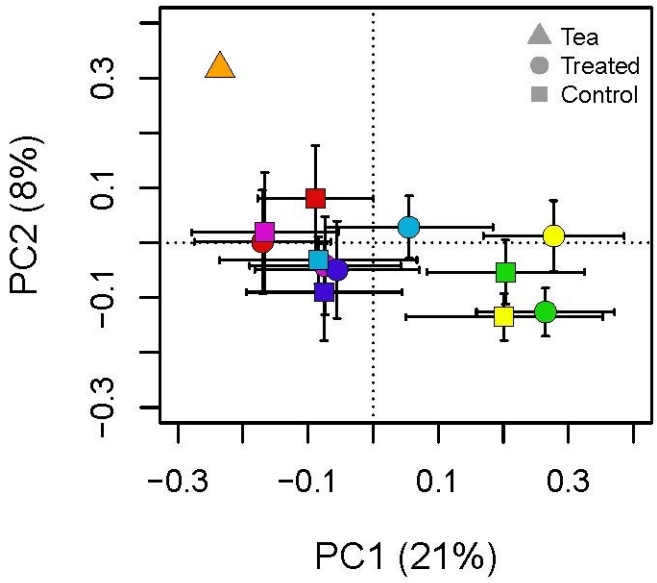

Figure S3. Principal component analysis of Hellinger-transformed relative abundances of Planctobacteria which was the dominant phylum in our dataset. Bacterial communities tend to cluster according to experimental blocks (yellow, green, dark-blue, light-blue, pink and red, represent six experimental blocks; orange color, represents compost tea). Shapes represent treatments.

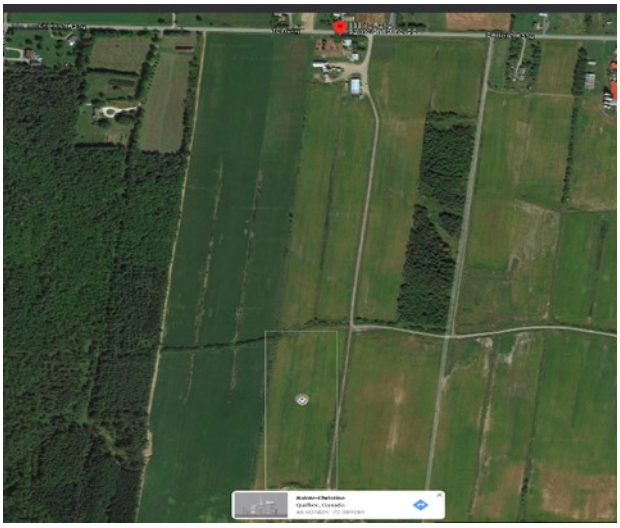

Figure S4. Aerial view of the site, located in Ste-Christine, Montérégie (QC, Canada). The region is dominated by intensive cropping systems, predominantly soybean-maize rotations.

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Chapitre 3

Conclusion générale :

Dans cette étude, nous avons évalué les effets que le traitement du thé de compost pourrait apporter aux communautés bactériennes dans le sol et, en conséquence, sur la croissance et la productivité des plantes. Les micro-organismes bénéfiques du sol se composent principalement de bactéries (ex. : PGPR, solubilisateurs de P, etc.) ; ils devraient être très abondants dans le thé de compost. Un extrait liquide de ce compost (i.e., thé de compost) devrait constituer un inoculum potentiellement très enrichi en ces organismes, qui à leur tour devrait fournir des services environnementaux représentés en augmentant la croissance et le rendement de la culture dans un système conventionnel de monoculture de soja. Cela se produit si le thé est fabriqué et appliqué de manière appropriée et dans les bonnes conditions environnementales. Dans d'autres circonstances (ex. : conditions anaérobies), le thé de compost pourrait contenir des micro-organismes pathogènes et avoir des effets négatifs sur la croissance et la production des plantes.

Les résultats que nous avons obtenus n'ont pas montré d'évidences probantes que le thé de compost améliore la croissance et la productivité des cultures grâce à l'ajout de bactéries bénéfiques uniques. Cela pourrait entre autres être dû à un faible établissement des populations microbiennes des dilutions de thé dans le sol. Comme l'un des plus grands défis auxquels est confronté l'efficacité de l'inoculum microbien est la capacité des microorganismes inoculés de s'établir dans le sol contre les microorganismes initiaux déjà présents avec une densité élevée.

Nos résultats sont quelque peu surprenants. En fait, l'absence d'effet de notre traitement de thé de compost sur les communautés bactériennes présents dans le sol, et en conséquence sur la croissance et la productivité des plantes, pourrait être due à plusieurs facteurs liés à notre étude spécifiquement et non au thé de compost en général. Par exemple, cela pourrait être attribué à la perturbation grave qu'avait subi notre champ d'étude avant le début de notre expérience, ce qui pourrait endommager les populations microbiennes du sol et nous empêcher de trouver un effet positif clair. Encore, cela pourrait être attribué à notre conception expérimentale qui n'incluait pas de parcelles sans application de thé de compost du tout.

En plus, d'autres facteurs liés aux étapes de fabrication du thé (par exemple, la qualité du thé utilisé, les ingrédients ajoutés, le temps de lavage, etc.) ou de l'application du thé (comme l'intensité de la dose d'application, la fréquence d'application, le temps entre chaque application, etc.) pourraient être des raisons pour lesquelles nous n'avons pas obtenu une différence claire entre les sols traités par le thé de compost et les sols contrôles, traités par le thé de compost stérile. Finalement, étant donné que notre expérience était dans un réel champ d'étude qui pourrait contenir un paquet de paramètres biotiques et abiotiques qu'on ne peut pas contrôler, il est possible qu'il y ait eu un paramètre inconnu lié à notre champ. Par contre, il est très difficile de savoir lequel exactement dans l'expérience réaliste.

Nous voulons rapporter que dans notre essai sur le terrain, sur un champ de monoculture de soja gravement perturbé, l'application de thé de compost vivant n'a pas entraîné d'altérations des communautés bactériennes et du rendement des cultures. Par contre, cela ne fausse pas les hypothèses de toutes les études identifiant le thé de compost comme un produit biologique efficace pour la restauration de la diversité des communautés bactériennes et pour promouvoir les performances des cultures dans le cadre de l'agriculture conventionnelle. La plupart de ces études était des études comparatives, qui avaient comparé l'effet de l'utilisation de compost et du thé de compost dans des pots expérimentaux et sous des conditions environnementales contrôlées.

À l'avenir, des investigations supplémentaires sont nécessaires pour mieux faire la comparaison avec d'autres études intéressées à ce sujet et mettre en opposition les études en labo versus celles en milieu réel. Par exemple, la comparaison de la dose appliquée au champ versus la dose en milieu expérimental. En plus, on a besoin de plus d'investigations sur la nature mécaniste de l'impact du thé de compost sur les communautés microbiennes et bien comprendre comment le thé de compost pourrait affecter les communautés microbiennes présentes au sol. Celles-ci vont contribuer à une meilleure compréhension du thé de compost performances dans les cultures conventionnelles.

Annexe.2 Photos supplémentaires du projet

Photo 1. Une photo prise de notre terrain d'étude avant l'application de thé de compost

Photo 2. La conception de parcelles aléatoires de notre champ d'étude: notre champ de (344 x 82,5 m) a été divisé en 6 blocs expérimentaux (172 mx 27,5 m), et chaque bloc a été divisé en deux parcelles.

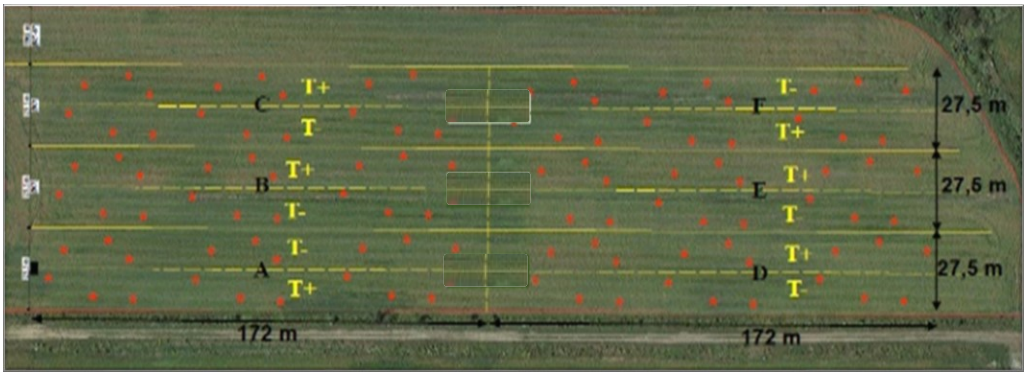

Photo 3. Lors du premier échantillonnage, le sol de rhizosphères a été collecté en secouant le système racinaire dans un sac en plastique, puis il a été placé dans des tubes de 15 ml et stocké à -20 ° C pour l'extraction d'ADN afin de caractériser les communautés bactériennes.

Photo 4. La mesure du poids des grains récoltés lors de dernier échantillonnage comme indicateur de rendement.

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