THE EFFECT OF CHEMICAL FUMIGATION AND BIOFUMIGATION ON THE SOIL NITROGEN CYCLE, SOIL RESPIRATION, AND THE SOIL MICROBIAL COMMUNITY

by

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
January 2022

Dalhousie University is located in Mi'kma'ki, the Ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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ABSTRACT

Chemical fumigation and biofumigation are used to reduce soil-borne diseases in potato crop production systems. However, soil fumigation may impact non-targeted soil microorganisms, consequently altering essential soil processes. In a first microcosm experiment, chemical fumigation with chloropicrin (CP) or metam sodium (MS) either used alone or combined with an organic amendment (OA) and biofumigation with mustard plant residues were assessed for their effects on key soil nitrogen (N) processes, the abundance of N-related microorganisms, and soil bacterial diversity. Biofumigation did not affect net N mineralization, nitrification, or nitrifier and denitrifier abundance, but increased denitrification. Chemical fumigation used alone did not affect net N mineralization. All chemical fumigant treatments inhibited nitrification and decreased nitrifier abundance compared to biofumigation. Only CP fumigation decreased denitrifier abundance. Biofumigation transiently decreased bacterial evenness at early time points. Conversely, CP fumigation had an enduring impact on bacterial diversity, decreasing species evenness and yielding the most dissimilar community. Unexpectedly, MS fumigation combined with an OA delayed the flux of soil respiration compared to amended non-fumigated soil, indicating chemical fumigation may impact plant residue decomposition. This was further investigated in a second microcosm experiment, in which chemical fumigation (with CP or MS) combined with an OA of contrasting carbon (C) availability were assessed for their effects on soil respiration, soil inorganic N concentrations, and soil bacterial and fungal diversity. When combined with an OA, chemical fumigation decreased soil respiration, regardless of C availability, and apparent plant residue decomposition compared to amended non-fumigated soil. Chemical fumigation with MS only affected bacterial diversity when combined with a labile OA. MS fumigation did not affect fungal diversity. Chemical fumigation with CP used alone or combined with an OA decreased bacterial and fungal species richness and evenness and yielded the most dissimilar bacterial and fungal community compared to all other treatments. Overall, the results of this thesis suggest that 1) chemical fumigation had a greater impact on the soil N cycle, N-related microorganisms, and bacterial diversity than biofumigation, and 2) chemical fumigation reduced soil respiration, indicative of reduced OA decomposition, and altered microbial diversity regardless of soil OA or C availability.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ADF	Acid detergent fibre
amoA	Ammonia monooxygenase
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
ASV	Amplicon sequence variant
bp	Base pair
BR	Fresh young barley residues
CP	Chloropicrin
CP-M	Chloropicrin and amendment with mature barley residues
CP-U	Chloropicrin with no organic amendment
CP-Y	Chloropicrin and amendment with young barley residue
CT	Untreated control; no chemical fumigation or biofumigation
CT-M	Untreated control and with mature barley residues
CT-U	Untreated control with no organic amendment
CT-Y	Untreated control and amendment with young barley residues
HSD	Honest significant difference
ITS2	Fungal ribosomal internal transcribed spacer region 2
MR	Fresh mustard residues
MS	Metam sodium

MSBR	Metam sodium and amendment with fresh young barley residues
MS-M	Metam sodium and amendment with mature barley residues
MS-U	Metam sodium with no organic amendment
MS-Y	Metam sodium and amendment with young barley residues
NDF	Neutral detergent fibre
nirK	Copper-containing nitrite reductase
nirS	Cytochrome cd_1-containing nitrite reductase
nMDS	Nonmetric multidimensional scaling
nosZ I	Nitrous oxide reductase clade I
nosZ II	Nitrous oxide reductase clade II
OTU	Operational taxonomic unit
PEI	Prince Edward Island
SOC	Soil organic carbon
WFPS	Water filled pore space
16S rRNA	Bacterial 16S ribosomal RNA gene

ACKNOWLEDGEMENTS

Throughout the writing of this dissertation, I have received a great deal of support and assistance from my committee members. Dave, Bernie, Claudia, and Derek, thank you for all of your invaluable insight and guidance over the past four years.

I extend my sincere thanks to Dave for providing me with the opportunity to study at Dalhousie University and for fostering my interest in agricultural research through your recommendation of pursuing the fast-tracked PhD program. Thank you for always being cool, calm, and collected, pulling me out of the rabbit hole of perpetual and distractive detail, and encouraging me always to see the bigger picture.

I am also grateful to Bernie, whose expertise was invaluable in formulating research questions and methodology. Who knew that a student job spent harvesting potatoes, soil sampling, and hand-picking potato beetles on hot summer days would have led me here. Before embarking on my studies, you told me that completing a PhD takes average intelligence but above-average determination, dedication, and insightfulness. I now understand the gravity of this statement. Thank you for bringing me to AAFC, introducing me to Dave and Claudia, and instilling the importance of agricultural research.

I express my deepest appreciation to Claudia. Thank you for your patient support and for all of the opportunities I was given to further (and nurture!) my interest in soil microbiology. Your careful attention to detail, insightful feedback, and time spent explaining concepts have elevated my research and my confidence as a young woman in STEM. Thank you for standing by me through the ups and downs (I can finally

laugh about losing chloropicrin, not once, but twice in the mail!). I am forever indebted to the Goyer Lab and AAFC.

A special thanks to Ginette and Karen for their technical assistance throughout my studies and for sharing their cute pets with me! And to Sean, thank you for teaching me about the dynamic world of omics, being a listening ear, and keeping me on my toes in the lab (although I don't think my mental math skills have improved).

To my parents, thank you for your unconditional support, wise counsel, and sympathetic ear. Thank you to my grandparents for being my biggest fans and encouraging me to learn about research avenues beyond the world of human health (although now I see that soil health is human health!). And finally, to Lincoln, my partner in crime. I don't know who was crazier. Me, the one who pursued a doctoral degree, or you, the one who agreed to live under the same roof as someone writing their dissertation (and during a global pandemic, nonetheless!). Thank you for your unconditional understanding and the happy distractions to rest my mind outside of my research.

CHAPTER 1: INTRODUCTION

Potato (*Solanum tuberosum* L.) is the largest vegetable crop in Canada, accounting for 27% of all vegetable receipts and totaling $1.3 billion in receipts in 2019 (Agriculture and Agri-Food Canada, 2020). However, potato production faces numerous constraints to profitability and productivity, including a high susceptibility to disease (Larkin et al., 2017; Larkin & Lynch, 2018; Hills et al., 2020).

Chemical fumigation is an agronomic practice used to mitigate soil-borne diseases and pathogens in high-value crop systems, including potato production systems. The practice of soil fumigation involves the application of volatile biocides to the soil that subsequently diffuse throughout the soil profile, generating a fumigated environment. The two most commonly used chemical fumigants in Canadian potato production systems are chloropicrin and metam sodium. Chemical fumigation has been found to reduce soil-borne diseases effectively, including those common to potato production systems such as *Verticillium* wilt and *Fusarium* dry rot (De Cal et al., 2005; Collins et al., 2006; Larkin et al., 2011; Triky-Dotan et al., 2007). However, chemical fumigants are non-specific, and therefore, may inadvertently impact non-targeted microorganisms.

Soil microorganisms are vital to the soil ecosystem and agricultural production, as they play an important role in soil nutrient transformations and soil organic matter decomposition. The soil nitrogen (N) and soil carbon (C) cycles are two essential soil processes that are controlled by a diverse network of soil microorganisms (Song et al., 2020: van Groenigen et al., 2015). Several studies have demonstrated that changes to

soil microbial community diversity and composition have led to changes in soil N and C dynamics (Baumann et al., 2013; Jaurez et al., 2013; Louis et al., 2016; Philippot et al., 2013). For example, reduced microbial diversity resulted in reduced soil organic matter decomposition (Baumann et al., 2013; Juarez et al., 2013). Consequently, these alterations may have further implications on soil nutrient availability, biological productivity, and environmental sustainability (Bender et al., 2016; Berthrong et al., 2013; Denk et al., 2017; Fierer et al., 2012).

Previous studies have demonstrated that chemical fumigation with chloropicrin or metam sodium altered the soil microbial community by significantly decreasing soil nitrifier and denitrifier abundance, microbial species richness, and microbial diversity (Dangi et al., 2017; Ibekwe et al., 2001; Li et al., 2017ab; Sederholm et al., 2018; Zhu et al., 2021). In addition, chemical fumigation has been found to alter soil N and C dynamics by significantly promoting net N mineralization (De Neve et al., 2004; Yan et al., 2013a), inhibiting nitrification (Yan et al., 2013ab; Yan et al., 2015), increasing emissions of the greenhouse gas nitrous oxide (Fang et al., 2018b; Spokas & Wang, 2003; Yan et al., 2015;), and decreasing soil respiration (Omirou et al., 2011; Li et al., 2017a).

Given its broad biocidal activity and potential to alter essential soil processes, interest exists in alternatives to chemical fumigation. Biofumigation has been proposed as a plant-based option for disease management in potato production (Hills et al., 2020; Larkin & Lynch, 2018) and refers to the incorporation of fresh biomass from *Brassica* plants into the soil (Sarwar et al., 1998). Specifically in Canadian potato crop production systems, biofumigation is performed using a mustard (*B. juncea*) crop

rotation. The biofumigation concept is based on high tissue glucosinolate (GSL) concentrations, which are secondary metabolites found in plants of the *Brassicaceae* family (Fahey et al., 2001; Sarwar et al., 1998). After tissue disruption and incorporation into the soil, GSLs are hydrolyzed by the endogenous thioglucosidase myrosinase to release natural volatile fumigants known as isothiocyanates (ITCs) (Gimsing & Kirkegaard, 2009; Sarwar et al., 1998).

Few studies have investigated the effects of biofumigation on the soil N cycle, soil respiration, and soil microbial communities. With respect to soil N cycling and respiration, the addition of high-GSL *Brassica* tissues compared to low-GSL or non-*Brassica* tissues has been found to yield lower quantities of net N mineralization (Marchetti et al., 2015; Ryan et al., 2006), transiently inhibit nitrification (Brown & Morra, 2009; Snyder et al. 2010), and delay the timing of the maximum flux of soil respiration (Snyder et al., 2010) under controlled conditions. With respect to the soil microbial community, biofumigation has been shown to significantly affect soil bacterial and fungal communities (Friberg et al., 2009; Hollister et al., 2013; Mazzola et al., 2001; Wang et al., 2014b); however, the observed effects are not consistent among studies. For example, in a field study, Wang et al. (2014b) reported that biofumigation with *Brassica* seed meal significantly increased bacterial diversity and decreased fungal diversity, whereas under controlled conditions, Hollister et al. (2013) reported biofumigation with *Brassica* seed meal significantly decreased both bacterial and fungal diversity and species richness.

Additionally, although biofumigation has been proposed as an alternative to chemical fumigation for the management of soil-borne diseases in potato production

systems, there are currently no studies that have directly compared the effects of chemical fumigation and biofumigation on the soil N cycle, soil respiration, or the abundance of N cycling genes. Moreover, only three studies have directly compared the effects of chemical fumigation and biofumigation on soil bacterial communities; however, these studies either used microbial assessment tools that are not as reliable or powerful as high throughput amplicon-based next-generation sequencing (NGS) (Omirou et al., 2011; Wang et al., 2014b) or used biofumigant-derived products, such as liquid *Brassica* seed meal pellets (Wei et al., 2016), which may not aptly reflect the current practice of biofumigation in agricultural production systems. Therefore, a further comparative investigation is needed regarding the effects of chemical fumigation and biofumigation on the soil N cycle, soil respiration, and soil microbial communities.

The aim of this thesis was to provide further insight into the inadvertent ecological impact of chemical fumigation, either used alone or combined with an organic amendment, and biofumigation on essential soil processes and the soil microbiome. The specific objectives of this thesis were to: 1) compare the effects of chemical fumigation with either chloropicrin or metam sodium and biofumigation using high GSL-mustard plant residues on soil net N mineralization, nitrification, denitrification, soil respiration, and soil nitrifier and denitrifier abundance (Study 1 objective); 2) compare the effects of chemical fumigation and biofumigation on soil bacterial community diversity and composition using amplicon-based NGS (Study 2 objective); 3) investigate the effects of chemically fumigated soil compared to non-fumigated soil amended with substrates of contrasting C availability on soil respiration,

N_2O emissions, and soil inorganic N concentrations (Study 3 objective); and 4) investigate the effects of chemically fumigated soil compared to non-fumigated soil amended with substrates of contrasting C availability on soil bacterial and fungal community diversity and composition using amplicon-based NGS (Study 4 objective).

To meet these objectives, two soil microcosm experiments were performed. The first soil microcosm experiment (Study objectives 1 and 2) focused on the soil N cycle, the abundance of N-related microorganisms, and soil bacterial diversity. Based on observations from the first microcosm experiment, there was evidence that chemical fumigation reduced plant residue decomposition. Therefore, the second soil microcosm experiment was designed to assess the effect of chemical fumigation and substrate C availability on soil respiration, soil N cycling, and soil bacterial and fungal diversity (Study objectives 3 and 4).

This thesis is structured in a manuscript format. Studies 1, 2, 3, and 4 are presented under Chapters 2, 3, 4, and 5, respectively. Chapter 6 presents overall conclusions from the preceding chapters and reflects on the experimental limitations and contributions of the thesis.

CHAPTER 2: EFFECT OF CHEMICAL FUMIGATION AND BIOFUMIGATION ON SOIL NITROGEN CYCLING AND THE ABUNDANCE OF NITROGEN CYCLING MICROORGANISMS

This chapter presents a version of the manuscript titled 'Influence of chemical fumigation and biofumigation on soil nitrogen cycling processes and nitrifier and denitrifier abundance' that was previously published online in Soil Biology & Biochemistry on September 11, 2021. This was a multi-authored publication, in which the PhD Candidate contributed to the research design, data collection, data analysis, and writing. The publication can be retrieved using the following citation:

Sennett, L., Burton, D. L., Goyer, C., & Zebarth, B. Z. (2021). Influence of chemical fumigation and biofumigation on soil nitrogen cycling processes and nitrifier and denitrifier abundance. *Soil Biology & Biochemistry*, *162*, 108421.
doi: https://doi.org/10.1016/j.soilbio.2021.108421

2.1 Abstract

Chemical fumigation and biofumigation are used to reduce soil-borne diseases in agricultural production systems; however, other essential soil processes such as the soil nitrogen (N) cycle may also be affected. This study compared the effects of chemical fumigation and biofumigation on soil net N mineralization, nitrification, denitrification, and soil nitrifier and denitrifier abundance. Six treatments were compared using soil microcosms over a 160-day incubation period: fumigation with chloropicrin; fumigation with metam sodium used alone (MS) or combined with barley plant residues (MSBR); biofumigation with mustard residues; addition of non-biofumigant barley residues; and an untreated control. Biofumigation did not inhibit soil nitrification, whereas chemical fumigation with MS (with or without barley) and chloropicrin inhibited nitrification for 16 and 64 days, respectively. Biofumigation, barley residues, and MSBR increased soil respiration, N_2O emission, and denitrification rates. However, biofumigation had lower denitrification and N_2O cumulative emissions and rates compared to barley residues, suggesting that biofumigation may reduce N_2O production pathways. All chemical fumigation treatments significantly decreased nitrifier gene abundance compared to biofumigation; however, only chloropicrin decreased the abundance of denitrifying genes. After 160 days, 22% of the added plant residue dry matter remained in MSBR-treated soil, which was ~2-fold greater than barley-treated soil (7%), indicating chemical fumigation may also affect the carbon cycle. Overall, these results suggest that chemical fumigation, especially with chloropicrin, has a greater impact on nitrification and nitrifier and denitrifier gene abundance than biofumigation with mustard residues.

2.2 Introduction

Chemical fumigation is an agronomic practice used to protect various crops from soil-borne diseases. Chemical fumigation involves the application of volatile biocides to the soil that diffuse throughout the soil profile, generating a fumigated environment. The two most commonly used chemical fumigants in Canadian potato (*Solanum tuberosum* L.) crop production systems are chloropicrin and metam sodium. Although chemical fumigation has been shown to effectively reduce soil pathogens (Collins et al., 2006; De Cal et al., 2005; Larkin et al., 2011), fumigants are non-specific and may impact non-targeted microorganisms (Li et al., 2017ab; Sederholm et al., 2018). As a result, other essential soil processes mediated by soil microorganisms, such as the soil nitrogen (N) cycle, may also be affected.

Previous studies have reported that chemical fumigation with chloropicrin and metam sodium altered the soil N cycle by increasing N mineralization (De Neve et al., 2004; Yan et al., 2013a), inhibiting nitrification (Yan et al., 2013b; Yan et al., 2015), and promoting soil nitrous oxide (N_2O) greenhouse gas emissions (Fang et al., 2018b; Spokas and Wang, 2003). Additionally, previous studies have reported that chloropicrin and metam sodium decreased the abundance of several N cycling microorganisms (Fang et al., 2018a; Li et al., 2017ab). Given these ecological risks, there is a need to better understand the impact of chemical fumigation on soil N cycling and N cycling microorganisms in agricultural potato production systems.

In view of its broad biocidal activity and potential to alter the soil N cycle, interest exists in chemical fumigation alternatives. Biofumigation has been proposed as a plant-based option for disease management in potato production (Hills et al., 2020;

Larkin & Lynch, 2018) and refers to the incorporation of fresh biomass from *Brassica* plants into the soil (Sarwar et al., 1998). The biofumigation concept is based on high tissue glucosinolate (GSL) concentrations, which are secondary metabolites found in plants of the *Brassicaceae* family (Fahey et al., 2001; Sarwar et al., 1998). After tissue disruption and incorporation into the soil, GSLs are hydrolyzed by the endogenous thioglucosidase myrosinase to release natural volatile fumigants known as isothiocyanates (ITCs) (Gimsing & Kirkegaard, 2009; Sarwar et al., 1998).

While the nature of the ITC products depends on the parent GSL molecule, ITCs are non-specific (Hollister et al., 2013; Hu et al., 2015), indicating that biofumigation may also impact non-target microorganisms and essential soil processes. There is currently a lack of information regarding the effect of biofumigation on the N cycle and nitrifier and denitrifier abundance. Furthermore, chemical fumigation and biofumigation have not previously been investigated for their impact on essential soil N processes and soil N cycling microorganisms. Therefore, the first objective of this study was to compare the effects of chemical fumigation, either used alone or combined with an organic amendment, and biofumigation on essential soil N processes, including soil net N mineralization, nitrification, and denitrification, and soil nitrifier and denitrifier abundance in an eastern Canadian agricultural soil under controlled conditions in a 160-day incubation experiment.

Previous studies reported a substantial flux of carbon dioxide (CO_2) and N_2O to occur within the first 24-hours following labile C additions (Adviento-Borbe et al., 2006; Bore et al., 2017; Senbayrum et al., 2012), thus important trends that explain differences between biofumigation and the addition of non-biofumigant organic

amendments might be observed rapidly after the start of the incubation. Therefore, the second objective of this study was to compare the immediate effects of biofumigation and the addition of non-biofumigant organic amendments on soil respiration, N_2O emission, and denitrification rates, and soil inorganic N concentrations under controlled conditions in a separate 2-day incubation experiment.

It was hypothesized that: 1) both biofumigation and chemical fumigation would increase net N mineralization due to labile N released from fresh plant residues and microbial debris; 2) chemical fumigation would inhibit soil nitrification, decrease denitrification, and reduce soil nitrifier and denitrifier abundance compared to biofumigation; 3) biofumigation would increase soil respiration, denitrification, N_2O emission rates, and denitrifier abundance due to an increase in available C and N supply from plant residue addition; and 4) the presence of labile plant residues in chemically fumigated soil would result in early recovery of nitrification compared to chemical fumigation used alone due to an increase of available labile C and N released from plant residues promoting the growth of soil microbes and soil heterotrophic nitrifiers.

2.3 Material and Methods

2.3.1 Experimental design

2.3.1.1 160-day incubation experiment

This microcosm experiment consisted of six treatments in a randomized complete block design (RCBD) with five replicates that were blocked in time due to the large number of soil microcosms, the fact that biofumigation is a time-sensitive process, and the limited space within the pesticide fume hood. The six treatments

included chemical fumigation with 1) chloropicrin (CP) and 2) metam sodium either used alone (MS) or 3) combined with barley plant residues (MSBR), 4) biofumigation with high GSL mustard plant residues (MR) (*Brassica juncea*), 5) the addition of barley plant residues (BR) (*Hordeum vulgare* L.), and 6) an untreated control (CT).

Treatments CP and MS were selected because they are the most commonly used soil fumigants in Canadian potato crop production systems. The active component of MS is methyl-ITC, similar to the active components released from mustard residues; therefore, MSBR was selected to determine if any observed changes to the soil N cycle or N cycling microorganisms were due to the addition of organic C or the fumigant. Mustard cultivar Caliente 199 produces high concentrations of ITC-liberating GSLs (Rudolph et al., 2015; Waisen et al., 2020), and therefore was selected to serve as a high potential biofumigation treatment. BR was selected to serve as a non-biofumigant organic amendment control.

To ensure that the entire duration of nitrification inhibition was captured in this study, the incubation length of 160 days was selected based on previous studies that investigated the effects of chemical fumigation on soil N dynamics (i.e., the duration of nitrification inhibition based on soil NH_4^+-N concentrations) (De Neve et al., 2004; Yan et al., 2013a; Yan et al., 2015).

2.3.1.2 2-day incubation experiment

A second microcosm experiment was used to measure the immediate effects of biofumigation and the addition of barley plant residues on soil respiration, N_2O emissions, and total denitrification rates and soil inorganic N concentrations during 2 days of incubation. For safety reasons, the chemical fumigant treatments were not

included in this experiment due to the presence of toxic products at high concentrations. This incubation consisted of three treatments in an RCBD with five replicates blocked in time to manage a large number of microcosms and to effectively perform the time-sensitive process of biofumigation. The three treatments included 1) MR, 2) BR, and 3) CT. The 2-day incubation was performed independently of the 160-day incubation.

2.3.2 Soil sampling

Soil (0–20 cm) for both the 160 and 2-day incubation experiments was collected from a field under potato and barley crop rotation at the Fredericton Research and Development Centre for Agriculture and Agri-Food Canada located in Fredericton, New Brunswick, Canada (45˚55'30"N, 66˚37'W). The field had no previous fumigation or biofumigation history. The soil was loam-textured (410 g sand kg^{-1}, 480 g silt kg^{-1}, 110 g clay kg^{-1}) (pipette method with organic matter removal, Kroetsch & Wang, 2007). Soil organic C and total N concentrations were 21.3 g C kg^{-1} and 2.21 g N kg^{-1}, respectively, as determined by dry combustion (Elementar varioMACRO, Skjemstad & Baldock, 2007). Soil pH (1:1 water dilution) was 5.7.

The soil was collected on five dates between late August and mid-September 2018. At the time of sampling, the field was planted with a barley crop. Each sampling date was used for an experimental replicate for the preparation of soil microcosms. At each sampling date, the field moist soil was passed through a 4.75 mm sieve and stored at room temperature (~20°C) for five days before implementing the experimental treatments to avoid a potential flush of microbial activity following a change in soil

temperature or the sieving of soil. The gravimetric water content at the time of soil collection for each experimental replicate ranged between 0.24 – 0.27 g g^{-1} dry soil.

2.3.3 Microcosm preparation

A 500 mL glass canning jar served as an individual soil microcosm. Soil (275 g equivalent of oven-dry soil) was loosely added to the microcosms, and the water content was adjusted such that after the soil was packed to the target bulk density, a water-filled pore space (WFPS) of 55% would be achieved. The soil remained loosely packed for the first 48-hours of incubation following treatment implementation to ensure that: (1) a fumigation effect was effectively achieved by promoting the diffusion of gaseous fumigant products throughout the soil during a 24-hour sealed period; and (2) any remaining toxic fumigant products were able to escape the soil during a 24-hour ventilated period to proceed safely with the experiment.

The mustard and barley plants were grown in a greenhouse with a daytime temperature of 20-24°C, a nighttime temperature of 18-22°C, and a 16 hr photoperiod. The mustard plants were grown until the 50% flowering growth stage, where GSL tissue concentration is at its highest (Doheny et al., 2018). The barley plants were grown to a growth stage in which they should have a similar C/N ratio to the mustard plants (Zadoks growth stage 37).

Once reaching the appropriate growth stage, the mustard and barley plants were harvested 5 cm above the soil, immediately chopped in a food processor so residues were 2-3 cm in length, and immediately incorporated into the soil. The practice of biofumigation and chemical fumigation in the field involves soil compaction to reduce fumigant loss; therefore, the microcosms were sealed immediately with screw top lids

to simulate this practice. Biofumigation was performed under 10 minutes, as 87% of the volatile ITCs are produced within 30 minutes of tissue disruption (Gimsing & Kirkegaard, 2006). The residues (6.5 g per microcosm) were added based on the typical above-ground mustard biomass harvested at the end of a field season in New Brunswick, Canada (4000 kg dry matter ha^{-1}) (unpublished). The mustard and barley C/N ratios, as determined by dry combustion (Elementar varioMACRO, Skjemstad & Baldock, 2007), were 13.1 and 13.2, respectively. Mustard and barley plant residue tissue C content and total tissue N content are listed in Table 2.1.

Chloropicrin (19.5 µL of PIC100 per microcosm, Trinity Manufacturing Inc.) and metam sodium (87.5 µL of VAPAM per microcosm, AMVAC Chemical Corporation) were added to the microcosms based on field recommended application rates specific to eastern Canadian potato production (235 L ha^{-1} and 700 L ha^{-1}, respectively) (Trinity Manufacturing Inc.; AMVAC Chemical Corporation). The chemical fumigants were applied using a micropipette to the top of the soil to avoid blocking the pipette tip, and the microcosms were sealed immediately.

Table 2. 1 Characteristics of mustard (*B. juncea*) and barley (*H. vulgare* L.) plant residues.

Plant Residue	**C** ($g\ kg^{-1}$)	**N** ($g\ kg^{-1}$)	**C/N**	**Tissue dry matter added** (g per microcosm)	**Tissue C added** (g per microcosm)	**Tissue N added** (g per microcosm)
Mustard	393	30.0	13.1	0.77	0.30	0.02
Barley	397	30.0	13.2	0.81	0.32	0.02

The sealed microcosms were incubated in a pesticide fume hood at room temperature (~20°C) for a 24-hour period. After 24 hours, the lids were removed, and the microcosms were ventilated for a 24-hour period.

For the 160-day incubation, the soil in the microcosms was then packed to a pre-drawn line on the microcosm to establish a bulk density of 1.1 Mg m^{-3}. The microcosms were covered with Parafilm pierced with holes to reduce water evaporation and allow for gas exchange and then placed in incubators at 20°C for the remainder of the experiment (model MIR-553, Sanyo Scientific, Japan). The microcosms were weighed weekly, and deionized distilled water was added to maintain the desired water content. The microcosms were destructively sampled i.e., were discarded after sampling at nine different incubation lengths, where an individual treatment, time, and replication combination served as an individual destructive sample. In total, there were 540 microcosms with 108 microcosms per block. The microcosms were destructively sampled after 3, 4, 8, 16, 32, 64, 96, 128, and 160 days of incubation.

For the 2-day incubation, the microcosms were destructively sampled at six incubation lengths, where an individual treatment, time, and replicate combination served as an individual destructive sample that was discarded after sampling. In total, there were 180 microcosms with 36 microcosms per block. The microcosms were destructively sampled throughout the 24-hour sealed period and 24-hour ventilated period after 0.25, 0.5, 1.0, 1.25, 1.5, and 2.0 days of incubation.

2.3.4 Soil respiration, nitrous oxide emissions, and denitrification rates

2.3.4.1 160-day incubation experiment

Soil respiration (i.e., emissions of carbon dioxide, CO_2), N_2O emissions, and total denitrification (N_2O+N_2) rates were measured from two sets of microcosms sealed with a screw-top lid fitted with a rubber septum at the time of sampling. One set of microcosms had 10% of headspace volume replaced with compressed air to quantify

N_2O and CO_2 emission rates, whereas the second set of microcosms had 10% of headspace volume replaced with acetylene (C_2H_2) to quantify denitrification rate. The presence of C_2H_2 inhibits N_2O reductase activity; consequently, N_2O emissions quantified in the presence of C_2H_2 reflect total denitrification as specified by Groffman et al. (2006). A 60-min C_2H_2 diffusion period occurred before the removal of the initial gas sample. In both sets of microcosms, 20 mL of headspace gas was collected at 0, 30, 60, and 90 min and transferred to a pre-evacuated 12 mL glass vial (Exetainers; Labco, High Wycombe, U.K.). Headspace gas samples were analyzed for N_2O and CO_2 concentrations using a Varian Star 3800 gas chromatograph (Varian, Mississauga, ON), as described by Burton et al. (2008). N_2O emissions, denitrification, and respiration rates were calculated as the change in the mass of N_2O-N or CO_2-C in the microcosm headspace over 90 min as described by Zebarth et al. (2012). Cumulative gas emissions over the 160-day incubation were calculated using the linear trapezoidal method of integration, as described by Burton et al. (2008).

2.3.4.2 2-day incubation experiment

At each sampling point, CO_2, N_2O emission, and denitrification rates were measured from two sets of microcosms following the same methods described in the 160-day incubation. Compressed air or C_2H_2 was added to the sealed microcosms for the sampling points 0.25, 0.5, and 1.0 days of incubation. For the sampling points 1.25, 1.5, and 2.0 days of incubation that were under oxic conditions, the microcosms were first sealed with a screw lid fitted with a rubber septum, then compressed air or C_2H_2 was added. Headspace gas samples were collected and analyzed for N_2O and CO_2 as described for the 160-day incubation.

2.3.5 Soil inorganic N concentrations

After completing gas sampling, sub-samples of 20 g moist soil were taken from the set of microcosms used for CO_2 and N_2O emission measurements. Concentrations of NH_4^+ and NO_3^- were determined by shaking 20 g of moist soil for 30 min with 100 mL 2 M KCl. Extracts were filtered and stored at -20°C pending analysis. Extracts were analyzed for NH_4^+-N and NO_3^--N concentrations on a QuikChem 8500 Series 2 Flow Injection Auto-Analyzer (Lachate Instruments) following QuikChem Method 90-107-06-3-A and Method 90-107-04-2-A, respectively.

Soil net N mineralization was inferred from total mineral N (NH_4^+-N + NO_3^--N) concentrations. The potential rate at which NH_4^+-N can be depleted in soil by nitrification is commonly greater than the potential for soil to produce NH_4^+-N through net N mineralization (Roberston & Groffman, 2007); subsequently, soil NH_4^+-N concentrations do not commonly accumulate in agricultural soils. In this study, the incubation conditions (i.e., WFPS adjusted to 55% and temperature of 20°C) were favourable for nitrification (Norton & Ouyang, 2019). Therefore, nitrification inhibition was inferred by an accumulation of soil NH_4^+-N over time, which has been used as evidence in previous studies (Chaves et al., 2006; Chen et al., 2010; Yamamoto et al., 2008; Yan et al., 2013a).

2.3.6 DNA extraction

After 3, 8, 32, 96, and 160 days of incubation in the 160-day incubation experiment, soil sub-samples (~10 g) were taken from the microcosms used for measurements of CO_2 and N_2O emissions after completing gas sampling. The soil samples were stored at -80°C until processed. Genomic DNA was extracted from 0.25

g of freeze-dried soil using the MagAttract PowerSoil DNA isolation kit (Qiagen, Germantown, MD) and Thermo Scientific KingFisher Flex magnetic particle processor according to manufacturer protocols. The extracted DNA was quantified with the PicoGreen Reagent using Invitrogen Quant-iT PicoGreen dsDNA assay kit according to manufacturer protocol.

2.3.7 Soil nitrifier and denitrifier abundance

Quantitative PCR (qPCR) was performed using an Applied Biosystems (Streetsville, Canada) ABI StepOnePlus thermal cycler and SYBRGreen detection. Copy numbers of archaeal *amoA* (AOA-*amoA*) and bacterial *amoA* (AOB-*amoA*) nitrifier genes were assessed as described by Wertz et al. (2011). Copy numbers of *nirS*, *nirK*, and *nosZ* clade I denitrifier genes were assessed as described by Dandie et al. (2011). Gene copy number of *nosZ* clade II was assessed as described by Jones et al. (2013). No template controls (NTC) were included for each primer set. Standard curves were obtained using three replicates of serial dilutions of linearized plasmids containing cloned nitrifier, denitrifier, and *nosZ* clade II sequences. For nitrifiers, the AOB-*amoA* and AOA-*amoA* sequences from *Nitrosospira multiformis* and a fosmid clone 54d9 were used, respectively (Nicol *et al*., 2008). For denitrifiers, the *nirS*, *nirK*, and *nosZ* clade I, sequences from *Pseudomonas stutzeri* (Thröback et al., 2004), *Alicaligenes faecalis* (Henry et al., 2004), and *Pseudomonas brassicacearum* (Miller et al., 2008) were used, respectively. For *nosZ* clade II, a sequence from *Pseudomonas denitrificans* strain PD1222 was used (Jones et al., 2013). The standard curve descriptors were: for AOA-*amoA*: slope -3.36 to -3.57, *E* (efficiency) =90.6%-98.5%, R^2 =0.970-0.981 *y*(intercept) =26.7-31.6; for AOB-*amoA*: slope -3.27 to -3.68, *E*

=87.1%-102.1%, R^2 =0.916-0.971 *y* =38.4-40.2; for *nirS*: slope -3.38 to -3.58, *E* =90.6%-97.8%, R^2 =0.994-0.996 *y* =41.6-43.1; for *nirK*: slope -3.44 to -3.79, *E* =83.6%-95.4%, R^2 =0.995-0.997 *y* =34.7-35.9; for *nosZ clade I*: slope -3.29 to -3.56, *E* =90.8%-102%, R^2 =0.991-0.978 *y* =46.6-47.1 for *nosZ clade II*: slope -3.27 to -3.67, *E* =87.1%-102%, R^2 =0.958-0.996 *y* =53.4-57.6.

2.4.8 Plant residue dry matter

The percentage of added plant residue dry matter remaining after 160 days of incubation was determined for microcosms treated with plant residues, including the treatments MSBR, BR, and MR. At the end of the incubation from the set of microcosms used for CO_2 and N_2O emission measurements after completing gas sampling and collecting sub-samples for inorganic N and DNA extractions, the remaining soil was gently sieved through a 2 mm mesh. The remaining plant residues were collected, washed to remove excess soil, dried, and weighed.

2.3.9 Statistical analyses

The RStudio software base stats package was used to conduct statistical analyses (v.4.0.5) (RStudio Team, 2020). Data were assessed for normality (i.e., normal and independent distribution) using homoscedasticity diagnostic plots and Shapiro-Wilks tests. Non-normal data were transformed using Box-Cox transformations with the optimal lambda (λ) value using the MASS package in RStudio (Venables, 2002). A General Linear Model analysis of variance (ANOVA) was performed based on an RCBD with treatment, time, treatment x time interactions, and blocks as fixed factors. Treatment, time, and interaction means were tested using post-hoc Tukey Honest Significant Difference (HSD) tests. Spearman correlations (rho [ρ] rank coefficient)

were used to determine possible relationships between soil properties and gas emission rates and between nitrifier or denitrifier abundance and soil properties or gas emission rates using the Hmisc package in RStudio (Harrell, 2021). The treatment means and standard errors presented in the figures and tables were calculated from untransformed data. Significance was accepted at $P \leq 0.05$.

2.4 Results

2.4.1 Soil inorganic N concentrations

Soil mineral N (NH_4-N + NO_3-N) concentrations before treatment implementation (Time = 0) were relatively similar between blocks, ranging from 20.7-24.5 and 27.6-32.5 mg N kg^{-1} dry soil for the 2-day and 160-day incubation, respectively (data not shown). There was no significant treatment x time interaction on soil mineral N concentrations in the 2-day or the 160-day incubation ($P = 0.074$ and $P = 0.623$, respectively); however, there was a significant treatment effect in both incubations (both $P < 0.001$), as well as a significant time effect (both $P < 0.001$), where mineral N concentrations increased over time (data not shown).

In the 2-day incubation, when averaged over time, MR and BR-treated soil had significantly greater mineral N concentrations (25.5 mg N kg^{-1} dry soil, respectively) compared to the CT-soil (22 mg N kg^{-1} dry soil) ($P < 0.001$) (Figure 2.1a). In the 160-day incubation, when averaged over time, BR and MSBR-treated soil had significantly greater mineral N concentrations (average of 79 and 69 mg N kg dry soil, respectively) compared to MS-treated soil and the CT-soil (average of 39.5 and 31 mg N kg dry soil, respectively) (all $P < 0.01$) (Figure 2.1b). MR, CP, and MS-treated soil exhibited a numeric increase in mineral N concentrations (average over time of 60, 50, and 40 mg

N kg^{-1} dry soil, respectively) compared to CT-soil; however, these treatments were not significantly different from one another or CT-soil (Figure 2.1b).

There was a significant treatment x time interaction on soil NH_4^+-N concentrations in both the 2-day and 160-day incubation (both $P < 0.001$). In the 2-day incubation, MR and BR-treated soil had significantly greater NH_4^+-N concentrations (ranging 0.7-3.0 and 0.7-4.0 mg N kg^{-1} dry soil, respectively) compared to CT-soil (ranging 0.2-0.3 mg N kg^{-1} dry soil) between 1.0 and 2.0 days ($P < 0.001$) (Figure 2.1c).

Throughout the entire 160-day incubation, the CT-soil had low NH_4^+-N concentrations (average over time of 0.58 mg N kg^{-1}). MR and BR-treated soil reached maximum NH_4^+-N concentrations after 4 days (~17 to 24-fold increase compared to CT-soil, respectively) and decreased to similar concentrations of the CT-soil after 16 and 32 days, respectively (both $P < 0.001$) (Figure 2.1d). MS and MSBR-treated soil reached maximum NH_4^+-N concentrations after 16 days (~14 to 27-fold increase compared to CT-soil, respectively) and decreased to similar concentrations of the CT-soil after 64 days (both $P < 0.001$) (Figure 2.1d). In contrast, CP-treated soil reached maximum NH_4^+-N concentrations after 64 days (~62-fold increase compared to CT-soil) and remained significantly different from all treatments at the end of the incubation (all $P < 0.05$) (Figure 2.1d).

There was no significant treatment x time interaction on soil NO_3^--N concentrations in both the 2-day and 160-day incubation ($P = 0.073$ and $P = 0.284$, respectively); however, there was a significant treatment effect in both incubations (both $P < 0.001$). There was also a significant time effect in the 160-day incubation (P

< 0.001), where soil NO_3^--N concentrations increased over time; however, there was no significant time effect on soil NO_3^--N concentrations in the 2-day incubation (P = 0.067).

In the 2-day incubation, MR-treated soil had similar NO_3^--N concentrations (average over time of 23.6 mg N kg^{-1} dry soil) compared to both CT and BR-treated, whereas BR-treated soil had significantly greater NO_3^--N concentrations (average over time of 24.4 mg N kg^{-1} dry soil) compared to CT-soil (average over time of 21.9 mg N kg^{-1} dry soil) (P = 0.001) (Figure 2.1e). In the 160-day incubation, MSBR and BR-treated soil had similar soil NO_3^--N concentrations compared to each other (average over time of 62.0 and 65.7 mg N kg^{-1} dry soil, respectively) and MR-treated soil, as well as significantly greater NO_3^--N concentrations compared to CP, MS, and CT-soil (all P < 0.001) (Figure 2.1f). In contrast, MR, MS, and CP-treated soil had similar NO_3^--N concentrations compared to each other (average over time of 50.3, 45.4, and 50.1 mg N kg^{-1} dry soil, respectively) that did not significantly differ from the CT-soil (average over time of 41.8 mg N kg^{-1} dry soil) (Figure 2.1f).

2.4.2 Soil respiration, N_2O emission, and denitrification rates

There were significant treatment x time interactions on soil respiration, N_2O emission, and denitrification rates in both the 2-day and 160-day incubation (all P < 0.01). Throughout the 2-day incubation, the CT-soil had low respiration rates (average over time of 5 mg CO_2-C kg^{-1} dry soil day^{-1}), indicating low amounts of available organic C (Figure 2.2a). Both MR and BR-treated soil had significantly greater respiration rates throughout the 2-day incubation compared to CT-soil, ranging from 56-108 and 28-81 mg CO_2-C kg^{-1} dry soil day^{-1}, respectively (P < 0.01) (Figure 2.2a).

MR and BR-treated soil were significantly greater than the CT-soil on all sampling points; however, MR-treated soil had significantly greater respiration rates after 0.5 days compared to BR-treated soils (~1-fold increase) (Figure 2.2a).

Throughout the 160-day incubation, the CT-soil had low soil respiration rates (average over time of 3.2 mg CO_2-C kg^{-1} dry soil day^{-1}) (Figure 2.2b). Both the MR and BR-treated soil had significantly greater respiration rates between 3 and 8 days of incubation compared to the CT-soil (~3 to 15 and ~4 to 18-fold increase, respectively) (both $P < 0.001$) and decreased to similar rates of the CT-soil after 16 days (Figure 2.2b). MSBR-treated soil had significantly greater respiration rates between 4 and 16 days compared to the CT-soil (~5 to 7-fold increase) ($P < 0.01$) and decreased to similar rates of the control treatment after 32 days (Figure 2.2b). Respiration rates in CP and MS-treated soil were not significantly different from the CT-soil throughout the 160-day incubation. After 32 days of incubation, all treatments had respiration rates that were not significantly different from the CT-soil for the remainder of the incubation (Figure 2.2b).

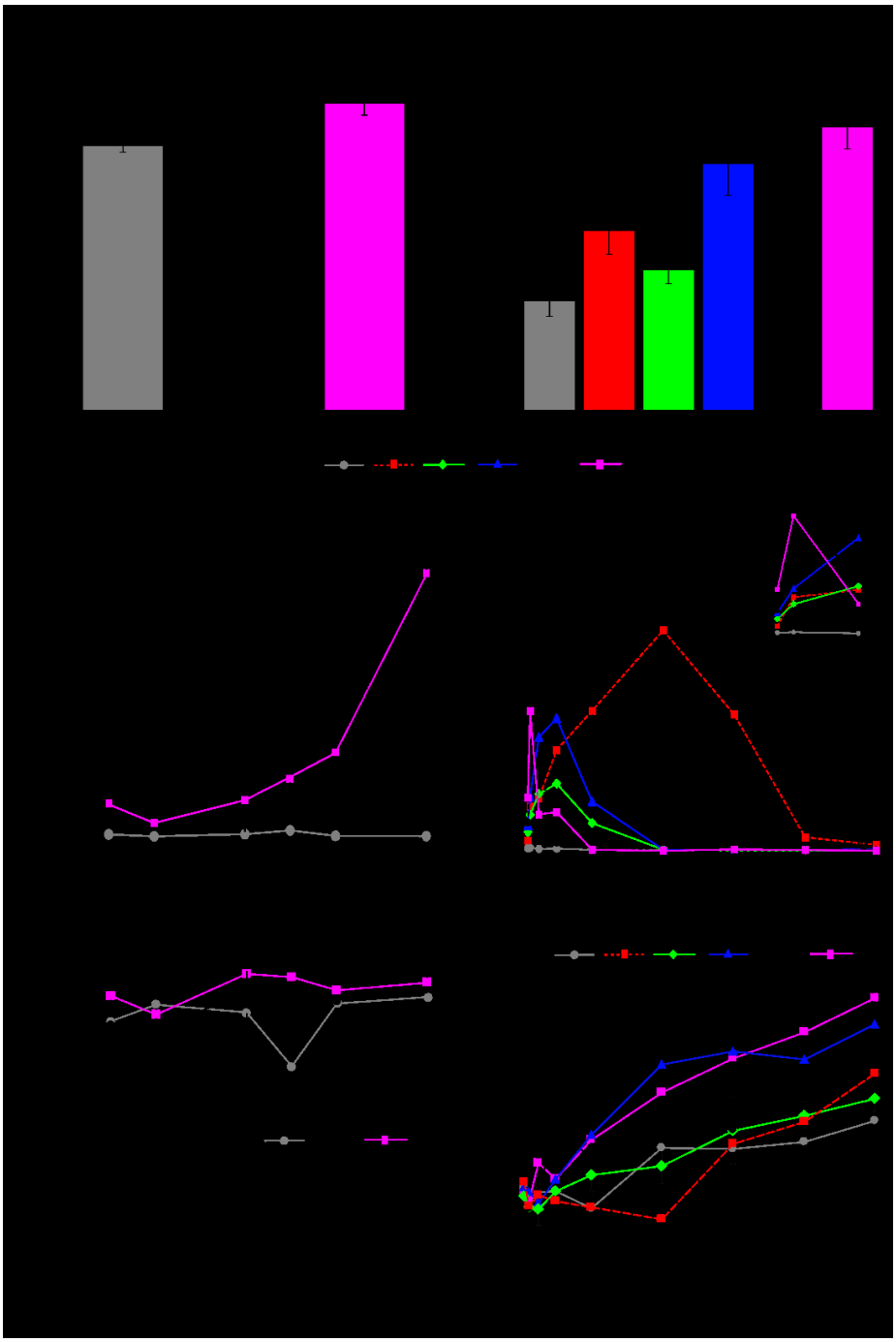

Figure 2. 1 Soil (a, b) total mineral N (NH_4^+-N + NO_3^--N), (c, d) NH_4^+-N, and (e, f) NO_3^--N concentrations in the 2-day and 160-day incubation. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Soil microcosms for both the 2-day and 160-day incubation experiments were sealed for the first 24 hours and ventilated for 24 hours in a fume hood. For the 160-day incubation, microcosms were then incubated under oxic conditions for the remainder of the experiment. Values for

total mineral N concentrations are means over time ($n = 30$ and $n = 45$ for the 2-day and 160-day incubation, respectively), whereas values for NH_4^+-N and NO_3^--N concentrations are means at each time point ($n = 5$). Error bars are the standard errors. Significant differences (HSD, $P \leq 0.05$) among treatment means are represented three ways: (1) by a letter adjacent to an individual time point to indicate significant differences between treatments at each sampling time point (c, d), (2) by a letter above bars to indicate significant differences between overall treatment means (a, b), and (3) by a letter in the legend to indicate significant differences between overall treatment means (e, f).

The CT-soil had low N_2O emission and denitrification rates throughout the 2-day (average over time of 0.98 and 0.95 μg N_2O-N kg^{-1} dry soil day^{-1}, respectively) and 160-day incubation (average over time of 0.92 and 1.2 μg N_2O-N kg^{-1} dry soil day^{-1}, respectively) (Figure 2.2c-f). Both MR and BR-treated soil had significantly greater N_2O emission rates within the 2-day incubation compared to the CT-soil ($P < 0.001$), ranging from 4-28 and 7.0-40 μg N_2O-N kg^{-1} dry soil day^{-1}, respectively. MR-treated soil reached maximum N_2O emission rates after 1.0 day of incubation and decreased to similar rates of the CT-soil after 1.5 days (Figure 2.2c). In contrast, BR-treated soil reached maximum N_2O emission rates after 1.25 days and remained significantly greater than MR-treated soil and the CT-soil after 2.0 days of incubation (Figure 2.2c). Similarly, MR and BR-treated soil had significantly greater denitrification rates within the first 2 days of incubation compared to the CT-soil ($P < 0.001$), ranging from 3.0-28 and 8.0-62 μg N_2O-N kg^{-1} dry soil day^{-1}, respectively (Figure 2.2e). Denitrification rates in MR-treated soil increased between 0.25 and 1.25 days and decreased to rates similar to the CT-soil after 1.5 days of incubation (Figure 2.2e). In contrast, denitrification rates in BR-treated soil increased between 0.25 and 1.5 days of incubation and remained significantly greater than the MR-treated soil and the CT-soil after 2.0 days of incubation (Figure 2.2e).

In the 160-day incubation, MR and BR-treated soil had significantly greater N_2O emission rates between 3 and 4 days of incubation compared to the CT-soil (~18 to 51 and 67 to 119-fold increase, respectively) (both $P < 0.001$) and decreased to rates similar to the CT-soil after 8 and 16 days of incubation, respectively (Figure 2.2d). MR and BR-treated soil also had significantly greater denitrification rates between 3 and 4 days of incubation compared to the CT-soil (~17 to 29 and 79 to 90-fold increase, respectively) (both $P < 0.001$) and decreased to rates similar to the CT-soil after 8 days of incubation (Figure 2.2f). MSBR-treated soil had significantly greater N_2O emission rates between 3 and 4 days of incubation compared to the CT-soil (~3 to 7-fold increase) ($P < 0.001$) and decreased to rates similar to the CT-soil after 8 days (Figure 2.2d). MSBR-treated soil had significantly greater denitrification rates only after 8 days of incubation compared to the CT-soil (~6-fold increase) ($P < 0.05$) and decreased to similar rates of the CT-soil after 16 days (Figure 2.2f). CP-treated soil had significantly greater N_2O emission rates between 3 and 4 days of incubation compared to the CT-soil (~3 to 6-fold increase) ($P < 0.001$) and decreased to similar rates of the CT-soil after 8 days of incubation (Figure 2.2d). CP did not affect soil denitrification rates compared to the CT-soil. MS did not significantly affect soil N_2O emission rates or denitrification rates over time compared to the control (Figure 2.2d, f). After 16 days, soil N_2O emission and denitrification rates in all treatments were not significantly different from the CT-soil for the remainder of the incubation (Figure 2.2d, f).

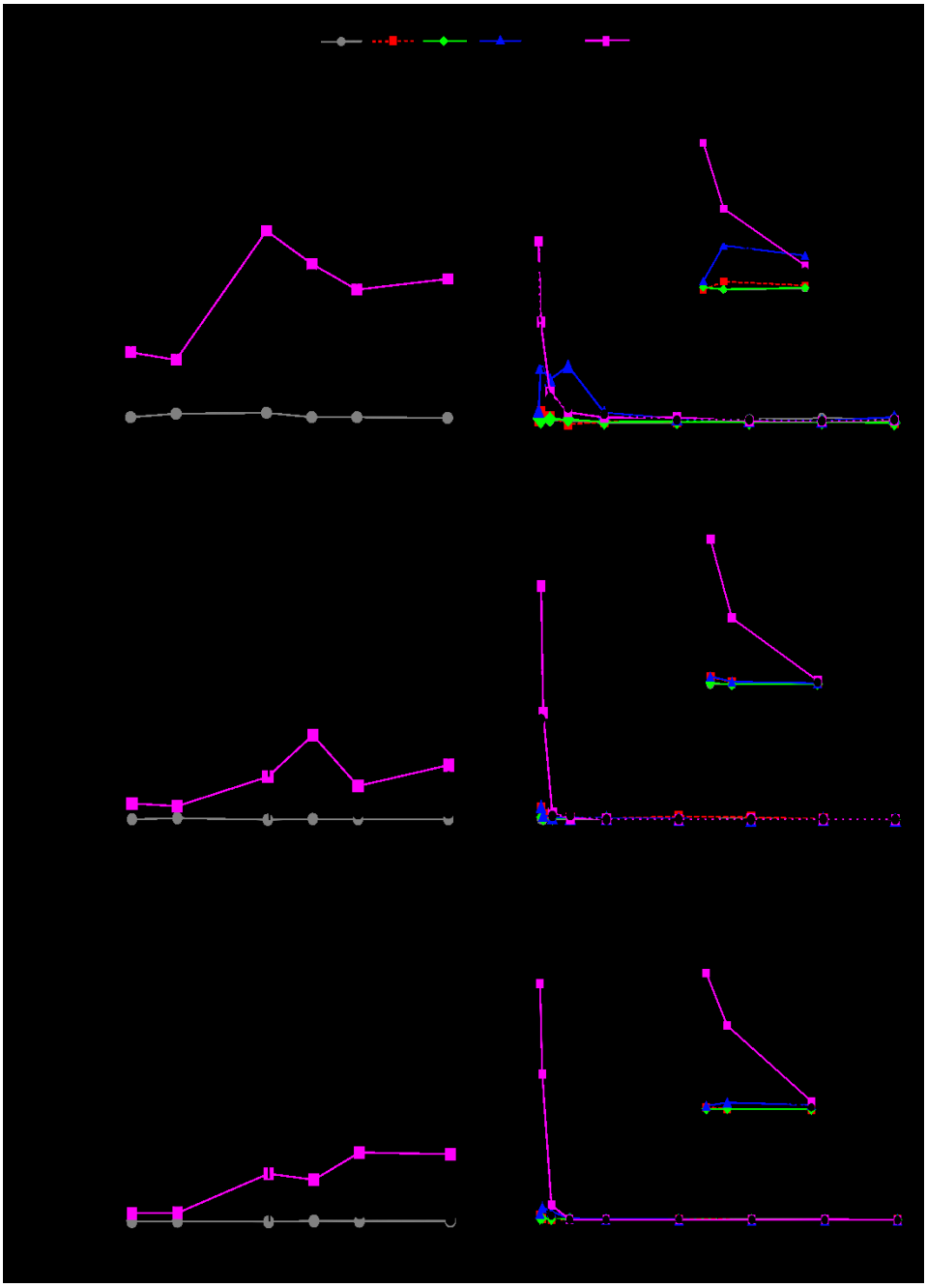

Figure 2. 2 Soil (a, b) respiration rate, (c, d) N_2O emission rate, and (e, f) total denitrification rate in the 2-day and 160-day incubation. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Soil microcosms for both the 2-day and 160-day incubation experiments were sealed for the first 24 hours and ventilated for 24 hours in a fume hood. For the 160-day incubation, microcosms were then incubated under oxic conditions for the remainder of the experiment. Soil microcosms were treated with C_2H_2 to measure denitrification rates or without C_2H_2 to measure soil respiration and N_2O emission rates. Values are means (n = 5), and error bars are the standard errors.

Significant differences (HSD, $P \leq 0.05$) among treatment means for individual time points are represented by letters adjacent to the time points.

Throughout the 160-day incubation, CP, MS, MSBR, and CT-soil had similar denitrification and N_2O emission rates, suggesting that most of the denitrification emission rates were emitted as N_2O. In contrast, in the BR and MR-treated soil, the ratio of N_2O emission rates to denitrification rates ranged between 0.3-0.7, suggesting that half of the gaseous N was emitted in the form of N_2O in these treatments.

2.4.3 Cumulative soil respiration, N_2O, and denitrification emissions

Net cumulative gas emissions over the 160-day incubation are listed in Table 2.2. BR, MR, and MSBR-treated soil had similar cumulative respiration compared to each other that were significantly greater than the cumulative respiration in MS, CP, and CT-soil (all $P < 0.001$). MS and CP-treated soil also had similar cumulative respiration compared to each other that were significantly greater than the cumulative emissions in CT-soil (both $P < 0.001$). Both cumulative N_2O and denitrification emissions in BR-treated soil were significantly greater than all other treatments (all $P < 0.01$). MR-treated had significantly greater cumulative N_2O emissions compared to MSBR, MS, and CT-soil (all $P < 0.01$), but had similar N_2O emissions compared to CP-treated soil. MR-treated soil also had significantly greater cumulative denitrification emissions compared to CT, CP, MS, and MSBR-treated soil. MSBR-treated soil had significantly greater cumulative N_2O emissions compared to CT-soil ($P = 0.019$). MSBR-treated soil had significantly greater cumulative denitrification emissions compared to MS-treated soil ($P = 0.026$); however, MSBR-treated soil had significantly lower cumulative N_2O and denitrification emissions compared to MR and

BR-treated soil (both $P < 0.01$). CP-treated soil had significantly greater cumulative N_2O emissions compared to MS and CT-soil (both $P < 0.01$), but had similar cumulative denitrification emissions compared to MS and CT-soil. MS-treated soil had similar cumulative N_2O and denitrification emissions compared to CT-soil.

Table 2. 2 Cumulative soil respiration (mg CO_2-C kg-1 dry soil), nitrous oxide (µg N_2O-N kg-1 dry soil), and denitrification (µg N_2O-N kg-1 dry soil) emissions from 0-160 days of incubation. Soil microcosms were treated with C_2H_2 to measure denitrification or without C_2H_2 to measure soil respiration and N_2O emissions. Values represent the mean (n=5) and standard error (± SE). Significant differences among means are based on Tukey's HSD tests ($P \leq 0.05$) and indicated by a letter adjacent to mean value.

Cumulative emissions	CT	CP	MS	MSBR	MR	BR
Soil respiration (mg CO_2-C kg^{-1} dry soil)	21.2 ± 0.57**c**	425 ± 28.2**b**	428 ± 22.1**b**	1064 ± 84.2**a**	962 ± 92.2**a**	1006 ± 44.3**a**
Nitrous oxide (µg N_2O-N kg^{-1} dry soil)	149 ± 6.87**e**	257 ± 24.1**bc**	161 ± 9.03**de**	206 ± 15.9**cd**	347 ± 38.3**b**	642 ± 135**a**
Denitrification (µg N_2O-N kg^{-1} dry soil)	180 ± 19.1**cd**	186 ± 21.6**cd**	171 ± 14.9**d**	267 ± 38.6**c**	462 ± 32.1**b**	1246 ± 301**a**

Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), mustard residues (MR), barley residues (BR), and the untreated control (CT).

2.4.4 Soil nitrifier and denitrifier abundance

There were no significant treatment x time interactions on soil nitrifier and denitrifier gene abundance. When averaged over time, nitrifier abundance in MS, MSBR, MR, and BR-treated soil did not differ from the CT-soil. However, CP-treated soil had significantly lower nitrifier AOA and AOB-*amoA* abundance compared to the CT-soil (~0.5-fold decrease each) (both $P < 0.001$) (Figure 2.3a, b). Soil treated with chemical fumigants (CP, MS, and MSBR) had significantly lower AOA and AOB-*amoA* abundance compared to MR-treated soil (all $P < 0.05$) (Figure 2.3a, b). AOB was numerically greater in BR-treated soil compared to MSBR, but this was not statistically

significant, likely due to the high variability within the data. Similarly, CP-treated soil had significantly lower denitrifier *nirK*, *nirS*, and *nosZ* clade I abundance compared to all treatments (~0.5 to 0.6-fold decrease compared to the control) (all $P < 0.01$) (Figure 2.3c-e). MS, MSBR, BR, and MR did not significantly affect denitrifier gene abundance. There was no significant treatment effect on *nosZ* clade II gene abundance (Figure 2.3f).

There were also significant time effects on the abundance of all genes examined except for AOB. AOA abundance was significantly greater after 96 and 160 days of incubation compared to after 8 days of incubation (~1.4 and 1.2-fold increase, respectively) (both $P < 0.05$) (data not shown). The *nirK* and *nirS* abundances were significantly lower after 96 and 160 days of incubation compared to after 3 and 8 days of incubation (~0.4 to 0.5-fold decrease) (all $P < 0.05$) (data not shown). The *nosZ* clade I abundance was significantly lower after 160 days of incubation compared to after 8 and 32 days of incubation (~0.5 and 0.4-fold decrease, respectively) (both $P < 0.05$), while *nosZ* clade II abundance was significantly lower after 160 days compared to after 32 days of incubation (~0.2-fold decrease) ($P = 0.05$) (data not shown).

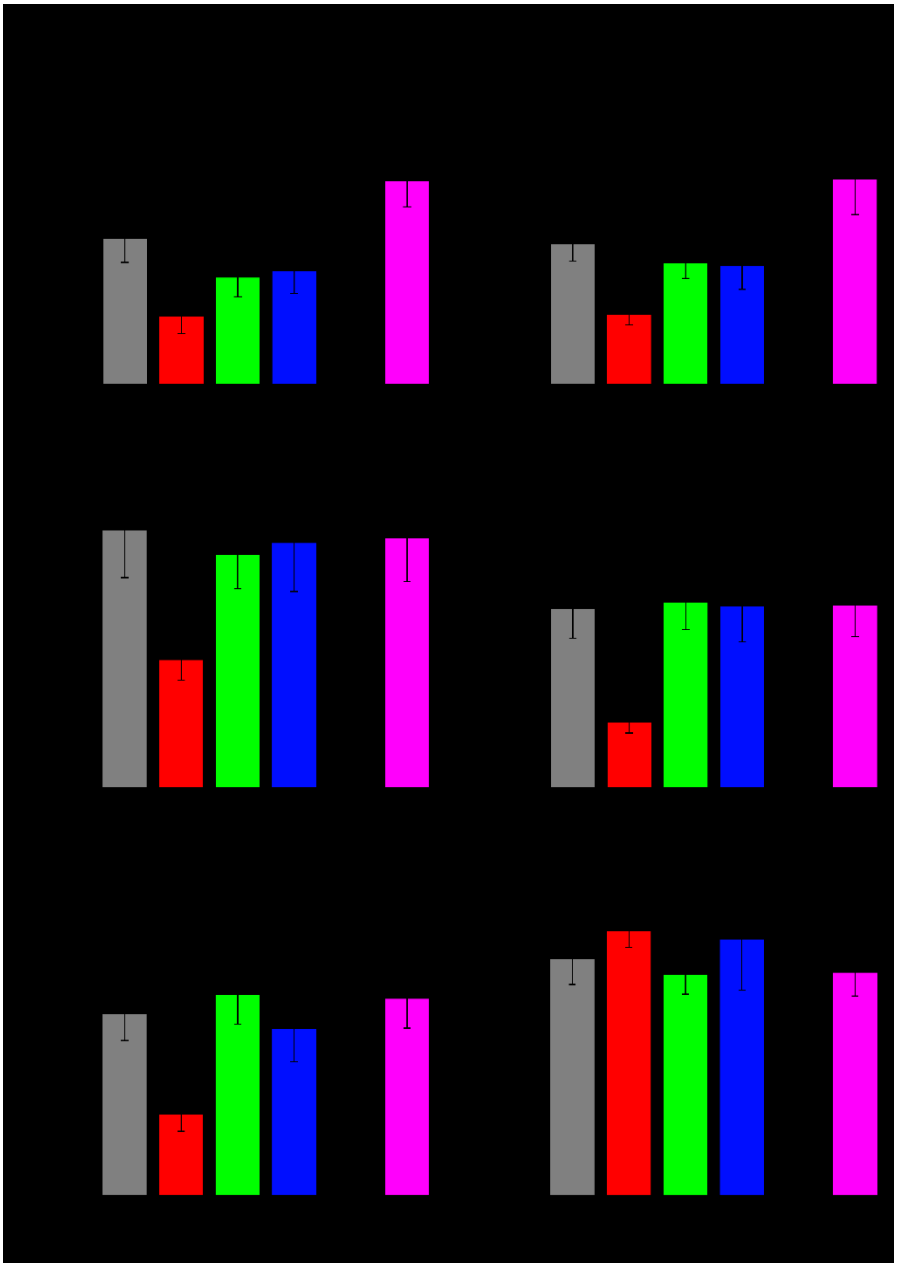

Figure 2. 3 Abundance of nitrifying AOA-*amoA* and AOB-*amoA* genes (a, b) and denitrifying *nirK*, *nirS*, *nosZ* Clade I genes, and *nosZ* Clade II gene (c, d, e, f) in the 160-day incubation. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Values are averaged across sampling dates ($n = 25$), and error bars are the standard errors. Treatment means with the same, or no letter are not significantly different (HSD, $P \leq 0.05$).

There were significant correlations between AOA-*amoA* abundance and soil NH_4^+-N concentrations ($\rho = -0.37$, $P = 0.04$), NO_3^--N concentrations ($\rho = 0.41$, $P = 0.02$), N_2O emission rates ($\rho = 0.41$, $P = 0.02$), and denitrification rates ($\rho = 0.46$, $P = 0.01$), between AOB-*amoA* abundance and soil denitrification rates ($\rho = 0.38$, $P = 0.04$), between soil respiration rates and N_2O emission rates ($\rho = 0.73$, $P < 0.001$) and denitrification rates ($\rho = 0.82$, $P < 0.001$), and between denitrification rates and N_2O emission rates ($\rho = 0.89$, $P < 0.001$) (Figure 2.5).

2.4.5 Remaining plant residue dry matter

After 160 days of incubation, the percentage of plant residue dry matter remaining in the MR-treated soil compared to the BR-treated soil was not significantly different ($P = 0.86$), where on average, 4.6% and 7.1% of the added mustard and barley plant residue dry matter remained, respectively (Figure 2.4). In contrast, on average, 21.9% of the added barley plant residue dry matter remained in the MSBR-treated soil after 160 days of incubation, which was significantly greater than the percentage of plant residue dry matter remaining in the MR and BR-treated soil (both $P < 0.05$) (Figure 2.4).

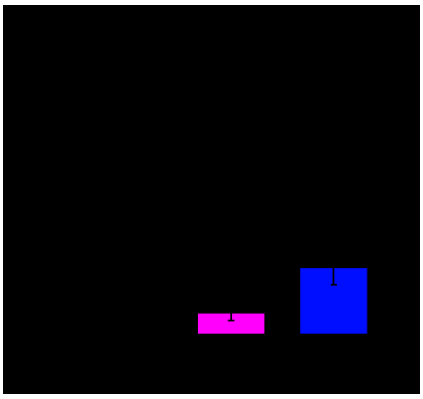

Figure 2. 4 The percentage of plant residue dry matter that remained after 160 days of incubation in soil microcosms treated with organic amendments, including biofumigation with mustard residues (MR), barley residues (BR), and chemical

fumigation with metam sodium combined with barley residues (MSBR). Soil was gently sieved through a 2 mm mesh, and the remaining plant residues were collected, washed to remove excess soil, dried, and weighed. Values are means (n = 4), and error bars are the standard errors. Treatment means with the same letter are not significantly different (HSD, $P \leq 0.05$).

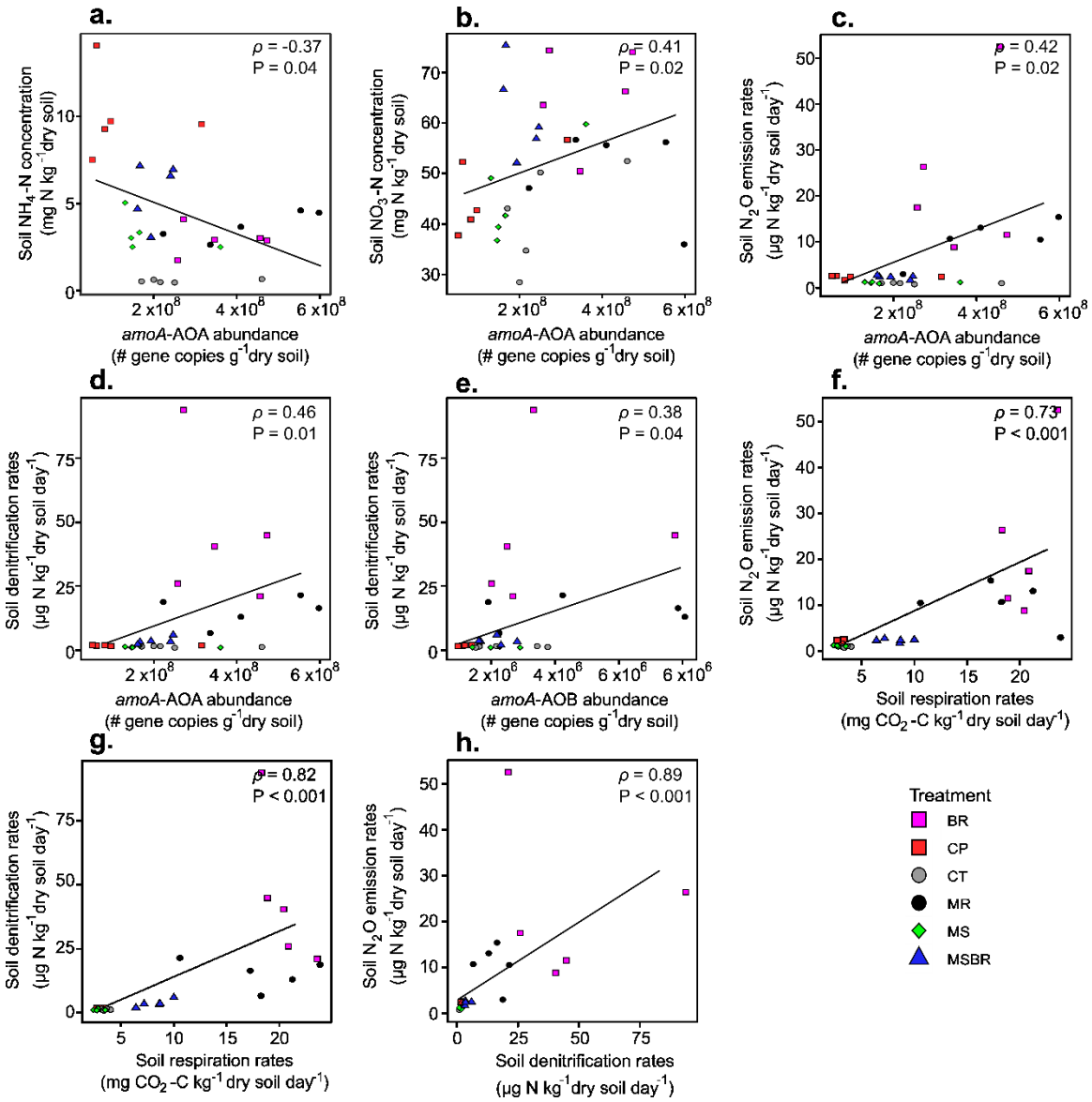

Figure 2. 5 Significant Spearman correlations (rho; ρ) between AOA-*amoA* abundance and (a) NH_4^+-N concentrations, (b) NO_3^--N concentrations, (c) N_2O emission rates, and (d) denitrification rates, (e) between AOB-*amoA* abundance and denitrification rates, between soil respiration rates and (f) N_2O emission rates and (g) soil denitrification rates, and (h) between soil denitrification rates and N_2O emission rates. Only significant correlations are presented. Treatments included: chemical fumigation with

chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Values represent each treatment averaged over time in each block ($n = 5$).

2.5 Discussion

2.5.1 Effect of chemical fumigation and biofumigation on soil net N mineralization

Low soil net N mineralization was observed in the CT and chemically fumigated (MS and CP) soil, likely reflecting the limited availability of labile C and N in these treatments. Chemical fumigation could have increased N mineralization due to the mineralization of cell lysates released from killed microbial biomass by the surviving microflora (De Neve et al., 2004; Yan et al., 2013a); however, there was not a significant effect of chemical fumigation with CP or MS on net N mineralization.

MSBR and BR increased net N mineralization compared to the CT-soil, which likely reflects labile N release from the decomposition of low C/N ratio barley plant residues. In contrast, there was no significant effect of the mustard residues on net N mineralization compared to the CT-soil. Under controlled conditions, Marchetti et al. (2015) also reported low N mineralization levels in soils amended with high GSL-*B. juncea* plant residues compared to low-GSL *Brassica carinata* defatted seed meal. In this study, the barley and mustard residues had similar C/N ratios and total tissue N contents; however, there are other soil organic matter chemical properties that are known to play a role in decomposition and N mineralization rates, including the form of tissue N (organic N vs. inorganic N), lignin/N ratio and lignin, cellulose, and polyphenol concentrations (Chen et al., 2014; Muhammad et al., 2011). Therefore, the

insignificant effect of MR compared to the significant effect of BR on soil net N mineralization may be due to differences in residue composition.

2.5.2 Effect of chemical fumigation and biofumigation on soil nitrification

Soil NH_4^+-N did not accumulate in the CT-soil, suggesting that nitrification occurred at a similar or greater rate than net N mineralization (Robertson & Groffman, 2007). Based on the accumulation of soil NH_4^+-N, CP fumigation inhibited nitrification for 64 days, and MS fumigation inhibited nitrification for 16 days. CP and MS-treated soil also had numerically lower NO_3^--N concentrations after 64 and 16 days of incubation, respectively, compared to CT-soil, further supporting that these treatments inhibited the conversion of NH_4^+-N to NO_3^--N. These findings align with Hypothesis 2 that chemical fumigation would inhibit nitrification. However, in contrast to Hypothesis 4, the addition of low C/N ratio barley plant residues to MS fumigated soil did not result in the early recovery of heterotrophic nitrification compared to MS used alone. Instead, MSBR-treated soil inhibited nitrification for 16 days, as indicated by the accumulation of NH_4^+-N and the numerically lower NO_3^--N concentrations compared to CT-soil after 16 days of incubation. MSBR-treated soil had greater NH_4^+-N concentrations than MS used alone, but this was likely related to labile N released from the barley residues and the significantly greater amount of net N mineralization observed in MSBR-treated soil. These findings are similar to previous incubation studies under controlled conditions that reported CP inhibited nitrification for 8 and 12 weeks after fumigation (Yan et al., 2013a; Yan et al., 2015) and MS inhibited nitrification for 7 and 13 days after fumigation (Li et al., 2017a; Yan et al., 2013b).

The addition of barley residues and biofumigation with mustard residues did not inhibit nitrification, as the rapid increase and decrease in NH_4^+-N concentrations between 3 and 32 days likely reflected the rapid mineralization of labile N released from plant residues followed by the biological oxidation of NH_4^+-N to NO_3^--N. However, BR-treated soil had numerically greater NH_4^+-N and NO_3^--N concentrations than MR-treated soil, likely relating to the numerically greater net N mineralization observed in BR-treated soil compared to MR-treated soil. In contrast to this study, Brown & Morra (2009) reported that biofumigation with mustard tissues inhibited nitrification for 24 days under controlled conditions, which was attributed to a high concentration of ITC-liberating GSLs. Biofumigant amendment rates may explain these inconsistencies: Wang et al. (2012) and Brown & Morra (2009) observed nitrification inhibition when mustard residues were applied at a rate of 2.5 and 1% w/w, respectively. Thus, the much lower application rate (~0.25% w/w) used in the current study may not have been sufficient to inhibit nitrification.

2.5.3 Effect of chemical fumigation and biofumigation on soil respiration, N_2O, and denitrification emissions

Chemical fumigation with CP and MS did not significantly affect denitrification compared to the CT-soil, as indicated by similar denitrification rates and cumulative denitrification emissions in CP and MS-treated soil compared to CT-soil. However, soil conditions in CP and MS-treated soil were likely not conducive to increasing denitrification, such as the low levels of net N mineralization and the fact that these treatments did not receive plant residues, which may increase denitrification through an increase in organic C and NO_3^--N availability. The significant nitrification inhibition and subsequently numerically lower soil NO_3^--N concentrations observed throughout

the duration of nitrification inhibition in CP and MS-treated soil may have also decreased denitrification by reducing available electron acceptors; however, denitrification in the CT-soil was low, and therefore, a decrease in denitrification would be difficult to detect. A previous study reported a significant increase in denitrification in CP fumigated soil; however, this study measured potential denitrification using additional inorganic N inputs (equivalent to 100 mg N kg^{-1} dry soil), thereby likely promoting denitrification through increased soil N availability (Yan et al., 2015).

Similarly, there were no significant differences in N_2O emissions between the control and chemical fumigation treatments, with the exception of CP-treated soil, which transiently increased N_2O emission rates after 3 days (~6-fold increase) and had significantly greater cumulative N_2O emissions after 160 days of incubation (~0.7-fold increase) compared to the CT-soil. Given CP fumigation significantly inhibited nitrification and did not have conditions conducive to denitrification, it is likely that neither of these processes contributed to the increased N_2O emissions. Previous studies also observed an increase in N_2O emissions following CP fumigation (Spokas & Wang, 2003; Spokas et al., 2005; Spokas et al., 2006; Yan et al., 2015). However, these studies showed that nitrification (Yan et al., 2015) and denitrification (Spokas et al., 2006) pathways did not significantly contribute to N_2O production but instead, N_2O was one of the breakdown products of the nitrogenous compound CP (Dungan & Yates, 2003; Spokas et al., 2006; Yan et al., 2015). Nonetheless, this increase was not a considerable source of N_2O compared to soils amended with labile organic amendments.

In the 2-day incubation, BR-treated soil had significantly greater denitrification and N_2O emission rates between 1.5 and 2.0 days than MR-treated soil. Similarly, in the 160-day incubation, BR-treated soil had numerically greater denitrification and N_2O emission rates, as well as significantly greater cumulative denitrification and N_2O emissions compared to MR-treated soil. Previous studies have reported similar findings, where the addition of GSL-hydrolysis products (ITCs) and *Brassica* tissues reduced urea-derived N_2O emissions compared to non-ITC-liberating plant tissues under controlled conditions (Balvert et al., 2017; Balvert et al., 2018). In the 160-day incubation, the lower N_2O emissions in MR-treated soil may be related to the numerically lower levels of net N mineralization and NO_3^--N concentrations compared to BR-treated soil, consequently reducing the pool of available inorganic N to feed N_2O production pathways. Nonetheless, these differences were not statistically significant. Therefore, based on this study, the mustard and barley plant residues had similar organic C availability, as indicated by soil respiration rates and cumulative respiration emissions, and similar NO_3^--N concentrations within the 2-day and 160-day incubations. Subsequently, these limiting factors cannot explain the lower denitrification and N_2O emissions in the MR-treated soil compared to BR, suggesting that biofumigation may transiently reduce N_2O production pathways.

The significant positive correlations between denitrification rates and N_2O emission rates suggests that denitrification was a significant source of N_2O production in this study. This is further supported by the significant positive correlations between soil respiration rates and N_2O emission and denitrification rates, indicating that an increase in available C was related to an increase in N_2O production pathways either

directly by increasing available electron donors or indirectly by reducing O_2 availability. Other processes such as nitrifier denitrification (ND) and dissimilatory nitrate reduction to ammonium (DNRA) may have contributed to N_2O emissions; however, these processes were not measured in this study.

2.5.4 Effect of chemical fumigation and biofumigation on soil nitrifier and denitrifier abundance

The CP-treated soil significantly reduced nitrifier abundance compared to all other treatments, likely contributing to the significant inhibition of nitrification sustained over a long duration of time in this treatment. The strong effect of CP fumigation on the nitrifying community likely reflects its high chemical volatility, toxicity, and, subsequently, ability to move and target microorganisms throughout the soil (Dungan & Yates, 2003; Gan et al., 2000). Previous studies also observed a significant effect of CP on soil nitrifiers, where AOA were the most sensitive to CP fumigation (Fang et al., 2018ab; Li et al., 2017b).

Although MS fumigation and biofumigation with MR release ITCs as their active component, MS and MSBR-treated soil had significantly lower AOA and AOB abundance than MR-treated soil, likely contributing to the significant inhibition of nitrification observed in MS and MSBR-treated soil compared to MR-treated soil. The primary ITC product released from *B. juncea* is 2-propenyl-ITC (Brown & Morra, 2009), whereas MS releases methyl-ITC. These ITCs have a common structural core entity, but their side-chains differ in structure (Matthiessen & Kirkegaard, 2006), resulting in differences in their volatility, toxicity, and movement throughout the soil profile (Gimsing & Kirkegaard, 2009; Sarwar et al., 1998). Moreover, the relative quantity of ITCs produced in the MS, MSBR, and MR treatments is unknown. In

theory, *Brassica* crops can contain equivalent amounts of GSLs to match levels of synthetic methyl-ITCs; however, this is assuming that all GSL material can be hydrolyzed during the incorporation process (Kirkegaard & Sarwar, 1998), which is dependant on the GSL tissue profile, level of tissue maceration, tissue myrosinase concentrations, and soil moisture (Gimsing & Kirkegaard, 2009). Therefore, the observed differences between MS, MSBR, and MR on the nitrifying community and nitrification may be related to different chemical properties and biological interactions of the ITC products themselves, as well as variations in ITC concentrations.

The CP-treated soil was the only treatment that decreased denitrifier abundance, once again likely reflecting its high chemical toxicity. Unlike soil nitrifiers, denitrifiers are represented by a diverse group of species (Wang et al., 2021); thus, these findings indicate that CP is toxic to a broad range of soil microorganisms. This aligns with a previous study that reported CP reduced the abundance of several denitrifying genes (Fang et al., 2018b). In contrast, MS did not affect denitrifier abundance, similar to previous findings (Li et al., 2017a). Treatments that increased denitrification rates and emissions (MR, BR, and MSBR) did not significantly increase denitrifier abundance, and there were no significant correlations between denitrifier abundance and denitrification, N_2O emission, or respiration rates. However, a correlation between gene abundance and the rate of the corresponding soil process is rarely observed (Rocca et al., 2015). Denitrification and N_2O emission rates, in particular, are controlled by environmental conditions that induce denitrifier activity rather than gene abundance (Attard et al., 2011; Liu et al., 2013; Salles et al., 2009). The results in our study suggest that the increase in denitrification and N_2O emission rates observed in MR, BR,

and MSBR-treated soils may be due to an increase in denitrifier gene expression and or enzyme activity rather than their abundance, likely resulting from the additional C source associated with residue addition.

2.5.5 Effect of chemical fumigation on plant residue decomposition

After 160 days, there was a significantly greater percentage (~2-fold increase) of plant residue dry matter that remained in the MSBR-treated soil compared to BR-treated soils, suggesting that MS, to some extent, inhibits the ability of the indigenous soil microorganisms to decompose plant residues. This is further supported by the delay in maximum soil respiration rates in MSBR-treated soil, which was ~0.7-fold smaller than the respiration rates observed in the BR-treated soil. This is the first study that has added plant residues in combination with chemical fumigants to soil microcosms, which has revealed that chemical fumigation may also be impacting the soil C cycle. A reduction in the rate of plant residue decomposition, if it persisted, would represent a significant impact on soil health and function.

2.6 Conclusions

Chemical fumigation with CP and MS influenced the soil N cycle by inhibiting nitrification. CP fumigation decreased the abundance of nitrifier and denitrifier abundance compared to CT-soil and slightly increased N_2O emissions due to the nitrogenous terminal breakdown products of CP. Biofumigation, unlike chemical fumigation, did not affect soil nitrification or the abundance of soil nitrifiers and denitrifiers. Biofumigation significantly increased soil respiration, N_2O, and denitrification cumulative emissions and rates compared to CT-soil, but decreased N_2O and denitrification emissions compared to BR-treated soil. Unexpectedly, MSBR-

treated soil had significantly lower soil respiration rates and a greater percentage of plant residue dry matter remaining in the soil microcosms after 160 days compared to BR-treated soil, indicating that MS limited the release of available C from barley plant residues and that chemical fumigation may also be affecting the soil C cycle. This phenomenon deserves further investigation.

The study of soil N cycling and N cycling microorganisms has intensified over the years, as these biochemical indicators are often used to assess the ecological, environmental, and soil health risks associated with agricultural management practices (Groenigen et al., 2015; Hills et al., 2020). This is the first study that has investigated the effects of chemical fumigation and biofumigation using high-GSL mustard plant residues on the soil N cycle and the abundance of N cycling microorganisms. Overall, these findings demonstrate that under controlled conditions, chemical fumigation, particularly with CP, had a greater impact on soil N cycling and N cycling microorganisms compared to biofumigation. Therefore, the minimal effect of biofumigation on the soil N cycle and abundance of N cycling microorganisms, along with its expectation to increase soil organic C, indicates that biofumigation may be a more sustainable option for disease management than chemical fumigation.

CHAPTER 3: EFFECT OF CHEMICAL FUMIGATION AND BIOFUMIGATION ON SOIL BACTERIAL COMMUNITY DIVERSITY

3.1 Introduction

Soil microorganisms play a central role in biogeochemical soil cycles and ecosystem maintenance (Fierer, 2017; van der Heijden et al., 2008). An increase in microbial diversity has been found to drive soil multi-functionality (Delgado-Baquerizo et al., 2016; Wagg et al., 2014), increase functional redundancy (Allison & Martiny, 2008; Philippot et al., 2013), and confer protection against soil-borne diseases (Mendes et al., 2011; van Elsas et al., 2012). Given the importance of microbial diversity in ecological functioning, there is a need to understand further how agricultural practices, such as soil chemical fumigation and biofumigation, influence the diversity of soil microbial communities.

Chemical fumigation is an agronomic practice that has been used globally to control soil pathogenic fungi, bacteria, nematodes, weeds, and insects in high-value crop systems (Huang et al., 2018; Huang et al., 2020; Zhang et al., 2019a). The two most commonly used chemical fumigants in Canadian potato (*Solanum tuberosum* L.) crop production systems are chloropicrin and metam sodium. Although chemical fumigation effectively reduces soil-borne diseases (Collins et al., 2006; De Cal et al., 2005), studies have shown that chemical fumigants are non-specific and impact non-target microorganisms, consequently altering the soil bacterial community diversity and decreasing bacterial species richness (Fang et al., 2018b; Li et al., 2017ab).

Biofumigation has been proposed as a plant-based alternative to chemical fumigation for disease management in potato production systems (Larkin & Honeycutt,

2006; Larkin et al., 2010). Biofumigation refers to the incorporation of glucosinolate (GSL)-containing fresh biomass from *Brassica* plant tissues into the soil, in which the GSLs are hydrolyzed to release natural fumigants known as isothiocyanates (ITCs) (Gimsing & Kirkegaard, 2009; Sarwar et al., 1998). Although biofumigation has been proposed as an alternative to chemical fumigation, there are several reported inconsistencies regarding the effects of biofumigation on the soil microbial community. For example, under controlled conditions, biofumigation has been found to alter bacterial and fungal β-diversity while decreasing bacterial and fungal species richness (Hollister et al., 2013). Alternatively, biofumigation has also been found to increase soil bacterial α-diversity in a field study (Shannon Index) (Wang et al., 2014a). Given these conflicting findings, further investigation is warranted regarding the effects of biofumigation on soil microbial community diversity.

Although several studies have examined the effects of chemical fumigation on bacterial communities, only three studies have directly compared the effects of chemical fumigation and biofumigation on soil bacterial communities (Omirou et al., 2011; Wang et al., 2014b; Wei et al. 2016). However, these studies either used microbial assessment tools that are not as reliable or powerful as high throughput amplicon-based next generating sequencing (NGS) (Omirou et al., 2011; Wang et al., 2014b) or used biofumigant-derived products, such as liquid *Brassica* seed meal pellets (Wei et al., 2016), which may not aptly reflect the current practice of biofumigation in agricultural production systems. Therefore, the objective of this study was to compare the effects of chemical fumigation with chloropicrin or metam sodium, used alone or combined with an organic amendment, and biofumigation with high GSL-containing

mustard plant residues on soil bacterial diversity and community composition using amplicon-based NGS in eastern Canadian agricultural soil under controlled conditions. In addition, relationships between bacterial phyla relative abundance or bacterial diversity indices and soil properties were also explored.

It was hypothesized that: 1) chemical fumigation with chloropicrin or metam sodium would change the soil bacterial community diversity and decrease species richness and evenness immediately after fumigation due to the fumigants killing a significant proportion of the soil bacterial community compared to the control; 2) biofumigation would rapidly change bacterial community diversity and decrease species richness and evenness compared to the addition of barley residues due to the release of volatile ITCs from mustard residues; 3) in soil fumigated with metam sodium in combination with low C/N barley plant residues, there would be an early recovery in bacterial community richness and evenness, as well as a dissimilar bacterial community diversity compared to metam sodium used alone due the rapid growth of surviving soil bacteria benefitting from labile C from barley residues; and 4) biofumigation would have less of an effect on bacterial community diversity and bacterial species richness and evenness compared to chemical fumigation used alone due to the addition of labile C from mustard residues providing substrates for rapid growth and recovery of the surviving bacteria.

3.2 Material and Methods

3.2.1 Experimental design

This study used samples collected from a subset of sampling time points in Study 1 previously reported in Chapter 2. The experiment was a laboratory incubation

study that consisted of six treatments in a randomized complete block design with five replicates that were blocked in time to manage a large number of soil microcosms. The six treatments included chemical fumigation with 1) chloropicrin (CP) and 2) metam sodium either used alone (MS) or 3) combined with barley plant residues (MSBR), 4) biofumigation with high GSL mustard plant residues (MR) (*Brassica juncea* cultivar Caliente 199), 5) the addition of barley plant residues (BR) (*Hordeum vulgare* L.), and 6) an untreated control (CT). Soil samples were collected on five of the nine destructive sampling time points reported in Chapter 2 on 3, 8, 32, 96, and 160 days of incubation.

Treatments CP and MS were selected because they are the most commonly used soil fumigants in Canadian potato crop production systems. The active component of MS is methyl-ITC, similar to the active components released from mustard residues; therefore, MSBR was selected to determine if any observed changes to soil bacterial diversity or bacterial community composition were due to the addition of organic C or the fumigant. Mustard cultivar Caliente 199 produces high concentrations of ITC-liberating GSLs (Rudolph et al., 2015; Waisen et al., 2020), and therefore was selected for the biofumigation treatment. BR was selected as a non-biofumigant organic amendment control.

3.2.2 Microcosm preparation

Microcosm establishment, including soil sampling, soil processing, greenhouse growth of plant tissues, biofumigation, and chemical fumigation were performed as previously described in detail in Chapter 2. Plant residue properties are listed in Table 2.1 of Chapter 2.

3.2.3 Soil properties

Soil respiration rate, an indicator of available organic C, and soil inorganic N concentrations (NH_4^+-N and NO_3^--N) were collected and measured as previously described in detail in Chapter 2.

3.2.4 DNA extraction and 16S rRNA gene PCR amplification, library preparation, and sequencing

At each sampling time point, soil sub-samples (~10 g) were collected and were stored at -80°C until the time of DNA extraction. Genomic DNA was extracted from 0.25 g of freeze-dried soil using the MagAttract PowerSoil DNA isolation kit (Qiagen, Germantown, MD) and Thermo Scientific KingFisher Flex magnetic particle processor according to manufacturer protocols. The extracted DNA was quantified with the PicoGreen Reagent using Invitrogen Quant-iT PicoGreen dsDNA assay kit according to manufacturer protocol.

The V4 region of the bacterial 16S rRNA gene was amplified using the forward (515F: GTGCCAGCMGCCGCGGTAA) and reverse (806R: GGACTACHVGGGTWTC) dual-indexed primers that were fused to Illumina sequence adapters (Caporaso et al., 2011). Library preparation and sequencing were performed following the Schloss MiSeq Wet Lab Standard Operating Procedure (SOP) (Kozich et al., 2013). Briefly, the PCR reaction was performed using 17 µL of Accuprime Pfx Supermix (Thermo Fisher Scientific, Waltham, USA), 1 µL (1:10 diluted) template DNA of each sample, and 2 µL of the paired set of dual-indexed primers for a reaction volume of 20 µL. DNA amplification was performed on a Mastercycler Nexus Thermal Cycler using the following conditions: 2 min at 95°C, 30 cycles of 95°C for 20 s, 55°C for 15 s, and 72° for 5 min; and 72°C for 10 min. PCR

products were cleaned and normalized using the Invitrogen SequalPrep Plate Normalization Kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol. Pooled libraries were quantified using quantitative PCR (qPCR) with an Applied Biosystems (Streetsville, Canada) ABI StepOnePlus thermal cycler and the NEBNext Library Quant Kit for Illumina (New England Biolabs Ltd., Ipswich, MA, USA) according to manufacturer protocol.

The library was sequenced using the MiSeq v2 Reagent kit (500 cycles) (Illumina, San Diego, CA, U.S.A.) and the Illumina MiSeq platform. Paired-end reads of 250 bp were generated and demultiplexing was done automatically on the MiSeq to generate read 1 and read 2 for all indexed sample. Inherent sequencing errors during DNA sequencing and data processing were evaluated using a bacterial mock community consisting of 20 bacterial species (catalog# HM783D, BEI Resources, Manassas, VA, USA).

3.2.5 Illumina MiSeq sequence data processing

The demultiplexed paired-end fastq sequence data were analyzed using mothur (v.1.44.3) and the Schloss MiSeq SOP (Kozich et al., 2013; Schloss, 2009). Briefly, paired-end sequences were merged into contigs, and contigs with ambiguous bases, erroneous length (> 275 bp), or >8 homopolymers were removed. Duplicate sequence reads were merged and then aligned to a reference alignment database (SILVA v.132) (Quast et al., 2013). Sequences that did not align to the correct region were removed, and sequence ends were trimmed (Schloss, 2013). The aligned sequences were used for amplicon sequence variant (ASV) identification using the pre.cluster command in mothur. The resulting sequences were screened for chimeras using UCHIME, which

were then removed. The Bayesian classifier was used to classify each sequence against the Ribosomal Database Project 16S rRNA gene training set (v.9) using an 80% confidence threshold (Wang et al., 2007). Sequences that were assigned to Eukaryota, Archaea, mitochondria, and chloroplasts were removed, and a consensus taxonomy table for each ASV was generated. In total, 7,177,577 high-quality sequences were obtained from 155 soil samples. The rarefaction curves were close to reaching a plateau, indicating that most of the community diversity was sequences (data not shown). The number of sequences was normalized across all samples to 7,787 reads using a random subsample method. There were 149,429 distinct ASVs among all soil samples.

Soil bacterial diversity was assessed using the Shannon index (H'), Pielou's evenness (J), and Chao1 species richness indices. These three diversity indices were calculated using the vegan package in R Studio software (v.4.0.5) (Oksanen et al., 2020). The β-diversity of the bacterial community was also evaluated using the vegan package in R Studio. Data were square-root transformed, and Bray-Curtis dissimilarities were generated. Differences in bacterial composition among treatments between sampling dates were ordinated using nonmetric multidimensional scaling (nMDS) generated from the ggplot2 package in R Studio (Wickham, 2016).

The relative abundances of the 11 most abundant phyla across all sampling dates were determined using MicrobiomeAnalyst (Chong et al., 2020; Dhariwal et al., 2017). The 10 bacterial species that exhibited the greatest increase and decrease in relative abundance between a given treatment and the control after 3 and 160 days of incubation were determined by calculating the $\log_2$ fold-change in relative abundance

for the corresponding ASV. Relative abundance was calculated as the number of counts for the corresponding ASV divided by the sum of counts for all ASVs and then averaged over the five replicates in each treatment. To better illustrate differences in bacterial species abundance, the ASVs that were evaluated had the following criteria: 1) an average relative abundance equal to or greater than 0.05%; and 2) a taxonomic affiliation identified at minimum at the phylum-level (i.e., ASVs that were identified as unclassified bacteria were not considered). The taxonomic affiliation of each ASV was assigned to the genus level or the closest known taxonomic level.

3.2.6 Statistical analyses

R Studio software was used to conduct statistical analyses. Analysis of variance (ANOVA) was performed to analyze soil inorganic N concentrations, soil respiration rates, the diversity indices, and the relative abundance of the 11 most abundant phyla with treatment, sampling time points, and blocks as fixed factors. Non-normal data were transformed using Box-Cox transformations and optimal lambda (λ) values. Treatment, time, and treatment x time interaction means were compared by performing post hoc Tukey Honest Significant Difference (HSD) tests. The treatment means presented in the figures and tables were calculated from untransformed data. Significant changes in the 10 greatest-fold increased and decreased ASVs in each treatment relative to the untreated control were determined using Kruskal-Wallis tests. The β-diversity of the bacterial community was analyzed using a permutational multivariate analysis of variance (PERMANOVA, permutations = 9999) with treatment, sampling time, and block using the Adonis function from the vegan package. Multilevel pairwise comparisons between treatment, sampling time, and treatment x

time interaction were performed using the pairwise Adonis wrapper function from vegan (Martinez Arbizu, 2020). Spearman's correlation test (rank coefficient ρ) was used to determine possible relationships between soil chemical properties and the relative abundance of phyla or diversity indices using the Hmisc package in RStudio (Harrell, 2021). Significance for all tests was accepted at $P \leq 0.05$.

3.3 Results

3.3.1 Soil bacterial diversity indices and bacterial β-diversity

There was a significant treatment x time interaction on the Shannon diversity (H'), Pielou's evenness (J), and Chao1 richness (all $P \leq 0.05$) (Figure 3.1; Table A.1 for ANOVA results). Throughout the incubation, MS- and BR-treated soil did not significantly affect Shannon diversity or Pielou's evenness compared to each other and the CT-treated soil (Figure 3.1a, b). After 3 days of incubation, there were no significant differences in Shannon diversity or Pielou's evenness among treatments except for MR-treated soil, which had significantly lower Shannon diversity and Pielou's evenness compared to all other treatments (~0.12-fold decrease compared to CT-soil) (Figure 3.1a, b). After 8 days of incubation and for the remainder of the incubation, both diversity indices in MR-treated soil did not differ from the CT-, MS-, MSBR-, and BR-treated soil (Figure 3.1a, b). After 8 days of incubation, MSBR-treated soil significantly decreased Shannon diversity and Pielou's evenness (average of 7.05 and 0.88, respectively) compared to the CT-treated soil (average of 7.44 and 0.92, respectively) and BR-treated soil (average of 7.40 and 0.92, respectively) (all $P \leq 0.05$) (Figure 3.1a, b). MSBR-treated soil also significantly decreased Pielou's evenness after 8 days of incubation compared to MS-treated soil (average of 0.91)

(Figure 3.1b). After 32 days of incubation and for the remainder of the incubation, both diversity indices in MSBR-treated soil did not differ from CT-, MS-, MR-, and BR-treated soil (Figure 3.1a, b). After 8 days of incubation and for the remainder of the incubation, CP-treated soil had significantly lower Shannon diversity and Pielou's evenness compared to all other treatments (Figure 3.1a, b). There were no significant differences in Chao1 richness among treatments throughout the incubation except for after 96 days of incubation, when CP-treated soil had significantly lower Chao1 richness compared to all other treatments (Figure 3.1c) (all $P \leq 0.001$). There were significant negative correlations between soil NH_4^+ concentrations and the Shannon diversity index ($\rho = -0.76$, $P < 0.001$), Chao1 richness ($\rho = -0.43$, $P = 0.017$), and Pielou's evenness ($\rho = -0.79$, $P < 0.001$) (Figure 3.5).

There was a significant treatment x time interaction on the β-diversity of the bacterial community (PERMANOVA $P \leq 0.001$; Table A.6). After 3 days of incubation, the β-diversity of the bacterial community in CP- and MR-treated soil were significantly different from each other and all other treatments (Figure 3.2a).

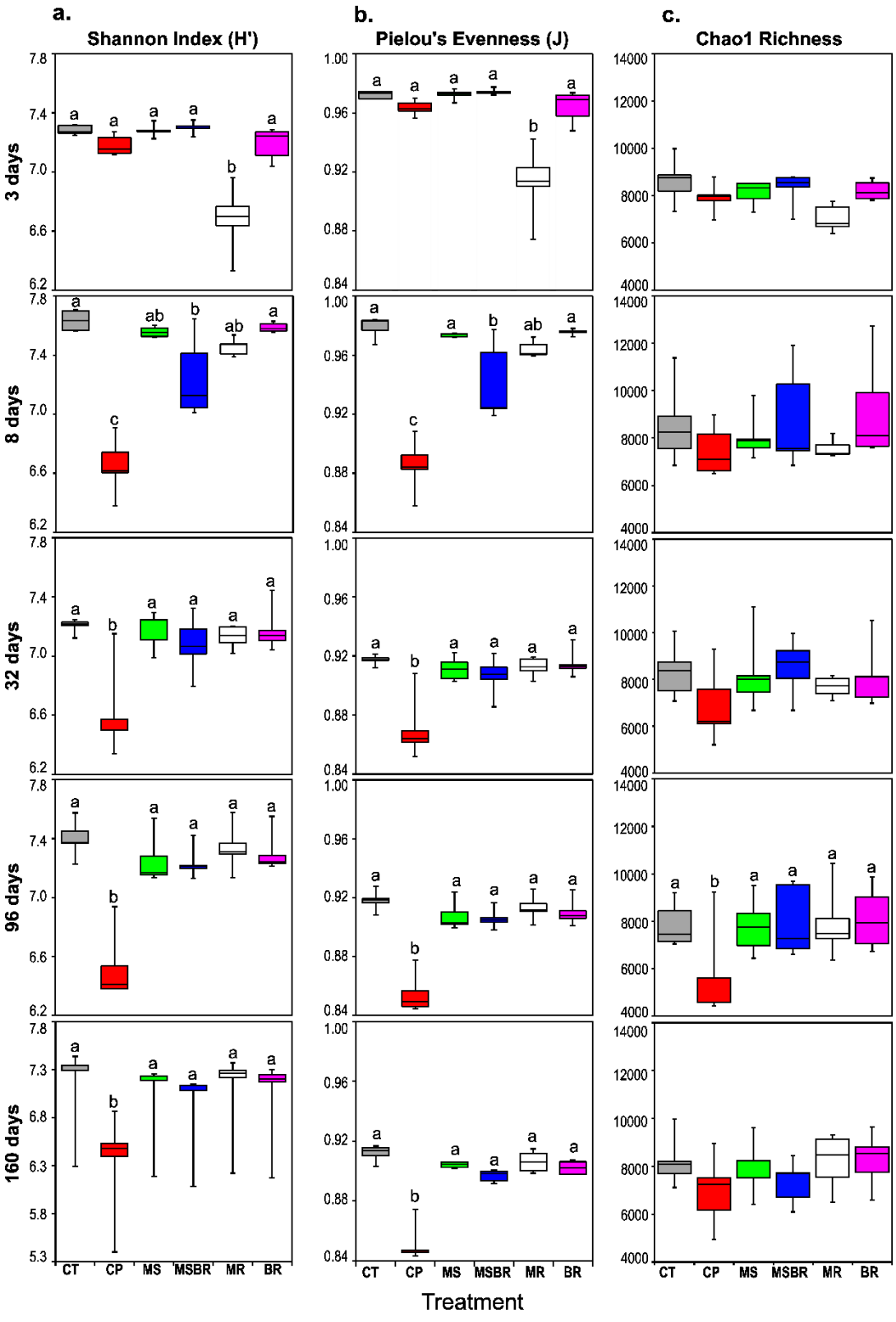

Figure 3. 1 Box and whisker plots showing the (a) Shannon diversity (H'), (b) Pielou's evenness (J), and (c) Chao1 richness in each treatment at each sampling time point.

Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Significant differences (HSD, $P \leq 0.05$) among treatment means ($n = 5$) for individual sampling points are represented by letters above the boxes. No letter indicates no significant difference between treatment means.

In contrast, the β-diversity of the bacterial community in MS-, MSBR-, and BR-treated soil was not significantly different from the CT-treated soil after 3 days of incubation. From 8 to 160 days of incubation, the β-diversity of the bacterial community of all treatments was significantly different from CT-treated soil and each other with some exceptions: the β-diversity of the bacterial community was similar between MS- and MSBR-treated soil after 8 days of incubation (Figure 3.2b), between MS- and MSBR-treated soil and MR- and BR-treated soil after 32 days of incubation (Figure 3.2c), and between MR- and BR-treated soil after 96 days of incubation (Figure 3.2d). The β-diversity of the bacterial community of MR-treated soil was the most dissimilar compared to all other treatments after 3 days of incubation, while the β-diversity of the bacterial community of CP-treated soil was the most dissimilar compared to all other treatments between 8 and 160 days of incubation (Figure 3.2b-e).

3.3.2 Relative abundance of the most abundant phyla

There was a significant treatment effect on the 11 most abundant phyla (Table 3.1). For CP-treated soil the relative abundance of all phyla were decreased compared to the CT-treated soil except for Proteobacteria, Planctomycetes, Armatimonadetes, and Candidate division WPS-1, where an increase in relative abundance was observed. The relative abundances of phyla in MS- and MSBR-treated soils were similar to the CT-treated soil except for greater relative abundance of Chloroflexi in the MS-treated soil

and Bacteroidetes in the MSBR-treated soil and lower relative abundance of Gemmatimonadetes in MSBR-treated soil compared to the CT-treated soil.

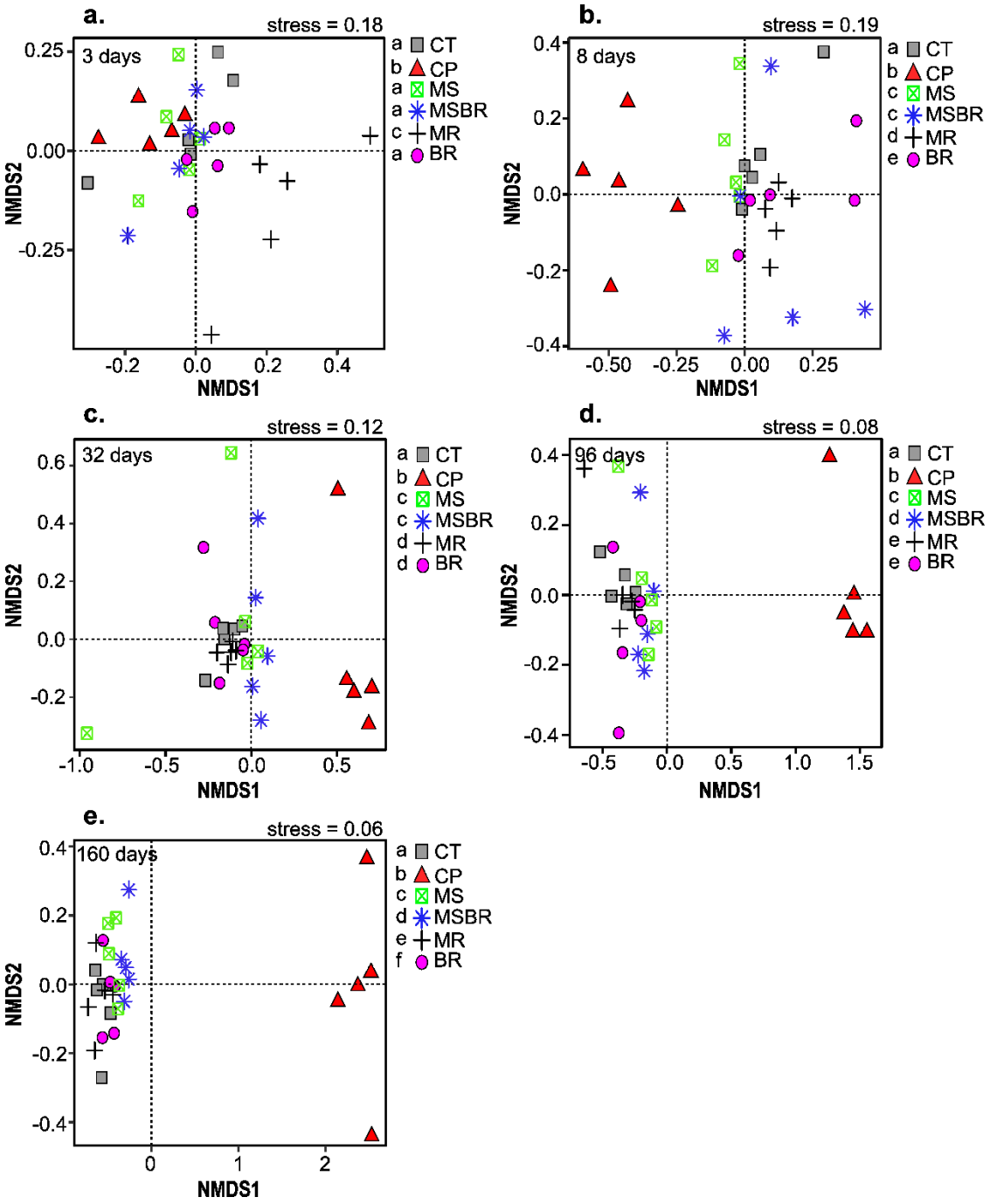

Figure 3. 2 Nonmetric multidimensional scaling ordination based on Bray-Curtis distances comparing the composition of bacterial communities among treatments at each sampling time point. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR),

biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Significant differences (PERMANOVA, permutations = 9999, $P \leq 0.05$) in β-diversity of the bacterial community among treatments at each sampling time point are represented by letters adjacent to treatments in legend.

MS- and MSBR-treated soil had similar relative abundances of phyla except for Actinobacteria and Bacteroidetes in MSBR-treated soil, which significantly decreased and increased, respectively, compared to MS-treated soil. The relative abundances of phyla in MR-treated soil were similar to the CT-treated soil except for Actinobacteria, Chloroflexi, and Verrucomicrobia, which were significantly decreased compared to the CT-treated soil, and Bacteroidetes and Planctomycetes, which were significantly increased compared to CT-treated soil. The relative abundances of all phyla in BR-treated soil were similar to the CT-treated soil. In BR-treated soil the relative abundance of Actinobacteria and Verrucomicrobia were significantly increased and the relative abundance of Bacteroidetes and Planctomycetes were significantly decreased compared to MR-treated soil.

There were significant negative correlations between soil NH_4^+ concentration and the relative abundance of Acidobacteria ($\rho = -0.66$, $P < 0.001$), Chloroflexi ($\rho = -0.58$, $P < 0.001$), Firmicutes ($\rho = -0.57$, $P = 0.001$), Gemmatimonadetes ($\rho = -0.61$, $P < 0.001$), and Verrucomicrobia ($\rho = -0.56$, $P = 0.01$), and positive correlations between soil NH_4^+ concentration and the relative abundance of Planctomycetes ($\rho = 0.47$, $P = 0.01$), Proteobacteria ($\rho = 0.40$, $P = 0.03$), and Candidate division WPS-1 ($\rho = 0.62$, $P = 0.002$). In addition, soil respiration rate was negatively correlated with the relative abundance of Armatimonadetes ($\rho = -0.58$, $P = 0.001$) and positively correlated with the abundance of Bacteroidetes ($\rho = 0.54$, $P = 0.002$) (Figure 3.5).

Table 3. 1 Relative abundances of the 11 most abundant phyla averaged across all sampling time points. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Values are means (n = 25) and standard errors (± SE). Significant differences in relative abundance (HSD, $P \leq 0.05$) among treatment means are represented by letters adjacent to mean values.

Phyla	CT	CP	MS	MSBR	MR	BR
Acidobacteria	22.5 ± 0.48**a**	19.6 ± 0.50**b**	22.0 ± 0.52**a**	21.8 ± 0.56**a**	21.9 ± 0.67**a**	22.2 ± 0.86**a**
Actinobacteria	15.9 ± 0.38**ab**	14.4 ± 0.44**cd**	17.2 ± 0.59**a**	15.8 ± 0.81**b**	13.5 ± 0.98**d**	15.7 ± 1.12**bc**
Armatimonadetes	2.80 ± 0.39**b**	3.70 ± 0.36**a**	3.20 ± 0.37**ab**	2.80 ± 0.37**b**	2.70 ± 0.38**b**	2.70 ± 0.40**b**
Bacteroidetes	7.30 ± 0.34**b**	6.40 ± 0.35**a**	7.40 ± 0.50**b**	9.30 ± 0.64**c**	9.10 ± 0.82**c**	7.30 ± 0.95**b**
Candidate div. WPS-1	1.90 ± 0.10**b**	3.30 ± 0.11**a**	2.00 ± 0.11**b**	2.00 ± 0.12**b**	1.90 ± 0.13**b**	1.90 ± 0.12**b**
Chloroflexi	2.10 ± 0.21**a**	1.40 ± 0.23**c**	1.60 ± 0.24**bc**	1.80 ± 0.26**ab**	1.60 ± 0.28**bc**	1.80 ± 0.29**ab**
Firmicutes	4.90 ± 0.29**a**	4.00 ± 0.30**b**	4.70 ± 0.31**a**	4.40 ± 0.30**ab**	4.50 ± 0.29**ab**	4.60 ± 0.32**ab**
Gemmatimonadetes	1.90 ± 0.16**a**	1.60 ± 0.15**b**	1.60 ± 0.15**ab**	1.50 ± 0.15**b**	1.70 ± 0.15**ab**	1.70 ± 0.16**ab**
Planctomycetes	9.20 ± 0.40**a**	12.3 ± 0.49**b**	8.80 ± 0.56**a**	9.40 ± 0.59**a**	11.6 ± 0.88**b**	9.70 ± 1.98**a**
Proteobacteria	24.7 ± 0.55**a**	27.3 ± 0.48**b**	24.5 ± 0.50**a**	24.8 ± 0.68**a**	25.2 ± 0.68**a**	25.0 ± 0.87**a**
Verrucomicrobia	6.90 ± 0.19**ab**	6.20 ± 0.23**c**	6.90 ± 0.25**ab**	6.40 ± 0.31**bc**	6.30 ± 0.35**c**	7.10 ± 0.42**a**

3.3.3 The 10 ASVs with the greatest-fold increase and decrease in relative abundance

Log_2 fold differences in the relative abundance of bacterial species between a given treatment and CT-treated soil after 3 and 160 days of incubation are presented in Figure 3.3 and Figure 3.4, respectively. After 3 days of incubation, CP-treated soil had six ASVs belonging to the order Bacillales that exhibited a change in relative abundance compared to CT-treated soil: two ASVs that increased (ASV031579 and ASV426342) and four ASVs that decreased (ASV167501, ASV198289, ASV56013, and ASV454275) (Figure 3.3). In MS-treated soil, five ASVs belonging to the phylum Proteobacteria (ASV318929, ASV296561, ASV134511, ASV361044, and ASV436145) increased in relative abundance compared to CT-treated soil. MSBR-treated soil had nine ASVs belonging to the phylum Proteobacteria that exhibited a shift in relative abundance compared to CT-treated soil: four ASVs that increased (ASV260308, ASV439362, ASV163702, and ASV517940) and five ASVs that

decreased (ASV322422, ASV469725, ASV190769, ASV014650, and ASV136008). MSBR-treated soil also had three ASVs belonging to the family Gemmataceae (ASV221587, ASV301603, and ASV106917) that increased in relative abundance compared to CT-treated soil. MR-treated soil had five ASVs belonging to the phylum Acidobacteria that changed in relative abundance compared to CT-treated soil: two ASVs that increased (ASV080708 and ASV279320) and three ASVs that decreased (ASV106727, ASV113033, and ASV092828). BR-treated soil had seven ASVs belonging to the phylum Proteobacteria that exhibited a change in relative abundance compared to CT-treated soil, including three ASVs that increased (ASV036859, ASV267556, and ASV095622) and four ASVs that decreased (ASV248828, ASV028050, ASV249036, and ASV136008).

Several treatments exhibited similar shifts in the relative abundance of particular ASVs after 3 days of incubation (Figure 3.3). For example, the ASV belonging to the family Bacillaceae (ASV031579) increased in CP and MS-treated soils (2.6 and 3.0 log_2 fold-change, respectively), the ASV identified as the genus *Paenibacillus spp.* (ASV198289) decreased in CP-, MS-, and MSBR-treated soil (-1.4, -0.7, and -1.1 log_2 fold-change, respectively), the ASV belonging to the order Solirubrobacterales (ASV296561) increased in MS and MSBR-treated soil (2.4 and 2.7 log_2 fold-change, respectively), the ASV belonging to the order Planctomycetales (ASV117360) and the family Isosphaeraceae (ASV021343) increased in MR-treated (6.1 and 5.6 log_2 fold-change, respectively) and BR-treated soil (4.0 and 3.3 log2 fold-change, respectively), and the ASV belonging to the order Rhizobiales (ASV014650) decreased in MS, MSBR, and MR-treated soil (-0.8, -1.3, and -1.8 log_2 fold-change, respectively).

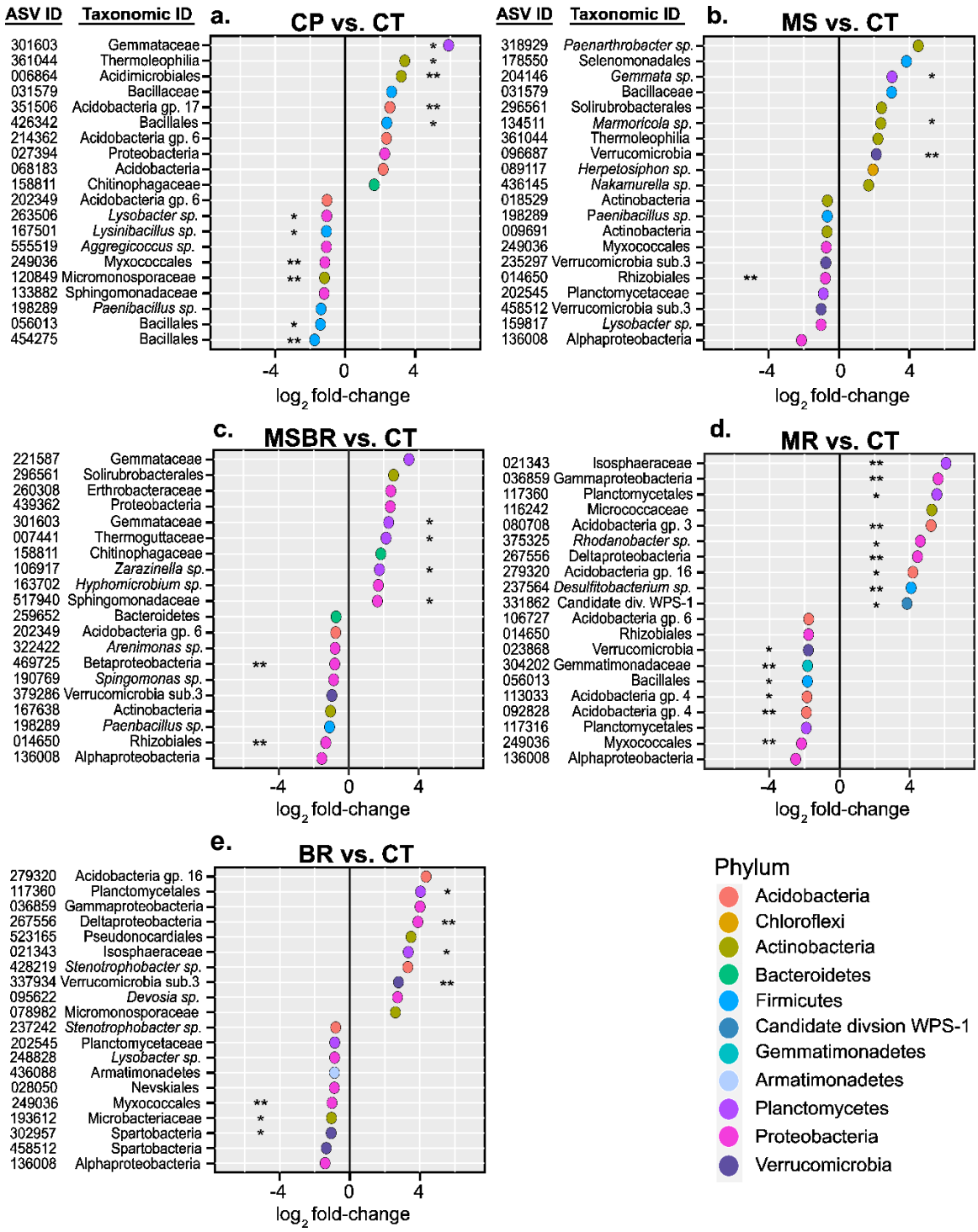

Figure 3. 3 The 10 greatest increased and decreased bacterial species (amplicon sequence variants, ASVs) after 3 days of incubation as determined by calculating the $\log_2$ fold-change in average ASV relative abundance between each treatment compared to the untreated control ($n = 5$). Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), barley residues (BR), and an

untreated control (CT). Significant changes in ASV relative abundance compared to the untreated control were determined using non-parametric Kruskal-Wallis tests adjusted for ties. Significant changes (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$) are indicated adjacent to the plotted ASV.

After 160 days of incubation, in the CP-treated soil, four ASVs belonging to the order Planctomycetales (ASV182445, ASV301603, ASV067528, and ASV029511) increased and four ASVs belonging to the phylum Actinobacteria (ASV321411, ASV009691, ASV571358, and ASV031617) decreased in relative abundance compared to CT-treated soil (Figure 3.4). In MS-treated soil, four ASVs belonging to the phylum Actinobacteria (ASV143916, ASV134511, ASV099754, and ASV112534) increased in relative abundance compared to CT-treated soil, as well as two ASVs that exhibited an increase after both 3 and 160 days of incubation: the ASV belonging to the order Selenomondales (ASV178550) and the ASV identified as the genus *Marmoricola spp.* (ASV134511) (Figure 3.3 and 3.4). In MSBR-treated soil, four ASVs belonging to the phylum Actinobacteria (ASV102641, ASV112534, ASV099754, and ASV143916) increased and two ASVs belonging to the family Chitinophagaceae (ASV363056 and ASV194228) decreased in relative abundance compared to CT-treated soil (Figure 3.3). In MR-treated soil, four ASVs belonging to the phylum Proteobacteria (ASV267556, ASV576390, ASV342116, and ASV442304) increased, whereas three ASVs belonging to the Phylum Actinobacteria (ASV491770, ASV018529, and ASV167638) and Acidobacteria (ASV443368, ASV528202, and ASV216673) decreased. MR-treated soil also had two ASVs exhibit an increase in relative abundance after both 3 and 160 days of incubation: the ASV belonging to the family Isosphaeraceae (ASV021343) and to the class Deltaproteobacteria (ASV267556) (Figure 3.3 and 3.4). In BR-treated soil,

three ASVs belonging to the phylum Actinobacteria increased (ASV099754, ASV112534, and ASV067598) and four ASVS belonging to the phylum Acidobacteria decreased (ASV528202, ASV046384, ASV132245, and ASV216673) compared to the CT-treated soil.

Several treatments exhibited similar shifts in the relative abundance of particular ASVs after 160 days of incubation (Figure 3.4). For example, the ASV belonging to the family Isosphaeraceae (021343) increased in BR- and MR-treated soils (2.4 and 4.5 $\log_2$ fold-change, respectively), the ASV belonging to the class Thermoleophilia (143916) increased in MS- and MSBR-treated soils (3.4 and 2.8 $\log_2$ fold-change, respectively), and the ASV identified as the genus *Phenlyobacterium spp.* (559274) decreased in CP-, MS-, and MSBR-treated soils (-2.8, -1.1, and -1.5 $\log_2$ fold-change, respectively).

3.4 Discussion

3.4.1 Effect of chemical fumigation and biofumigation on bacterial community diversity

This study examined the effect of a series of chemical and biofumigation treatments on the soil bacterial community diversity, as indicated by changes to the Shannon diversity index, Chao1 species richness, Pielou's species evenness, and the β-diversity of the bacterial community. For all of the treatments examined, at least one measure of bacterial diversity differed from the untreated control soil.

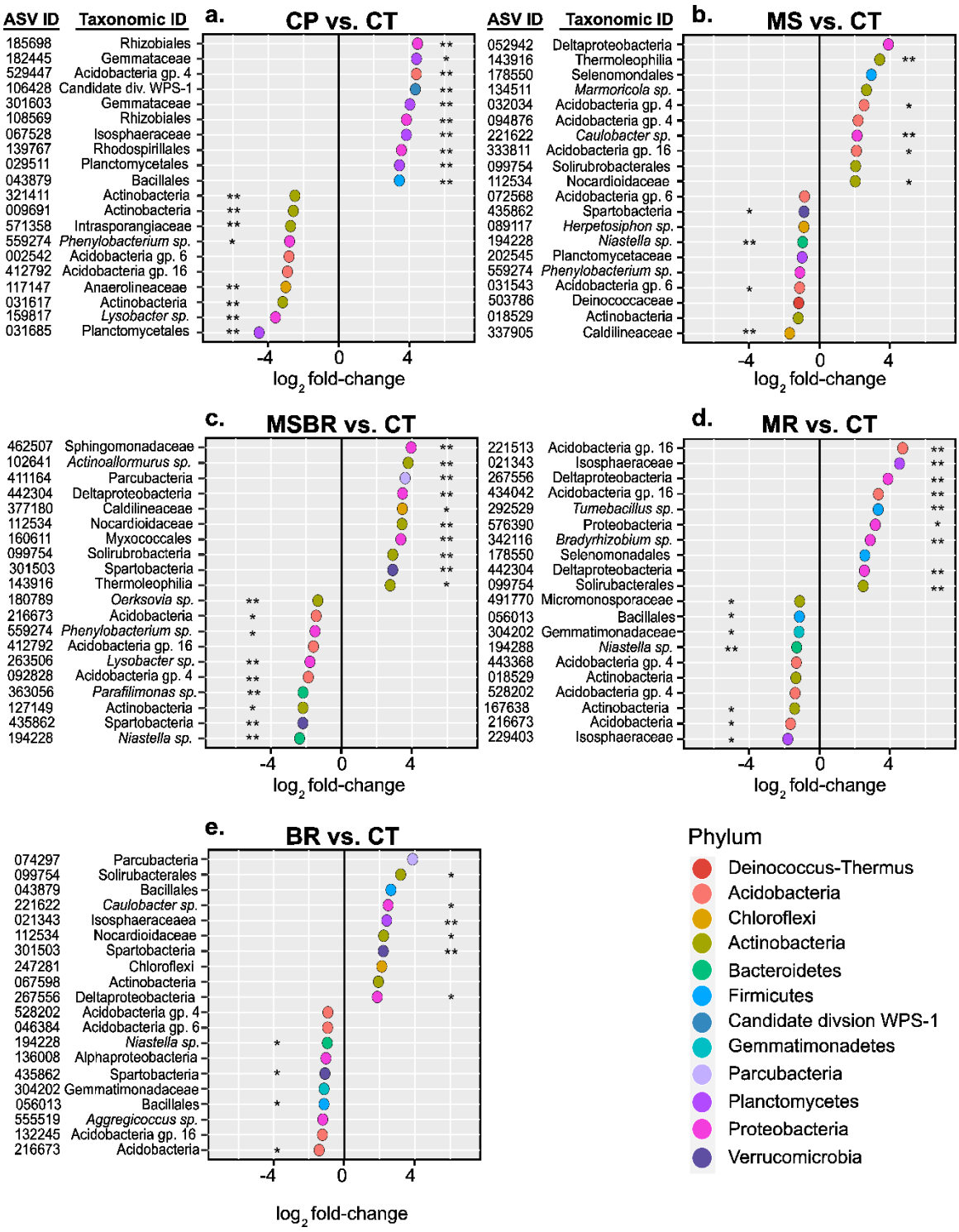

Figure 3. 4 The 10 greatest increased and decreased bacterial species (amplicon sequence variants, ASVs) after 160 days of incubation as determined by calculating the $\log_2$ fold-change in average ASV relative abundance between each treatment and the untreated control ($n = 5$). Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR),

biofumigation with mustard residues (MR), barley residues (BR), and an untreated control (CT). Significant changes in changes in ASV relative abundance compared to the untreated control were determined using non-parametric Kruskal-Wallis tests adjusted for ties. Significant changes (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$) are indicated adjacent to the plotted ASV.

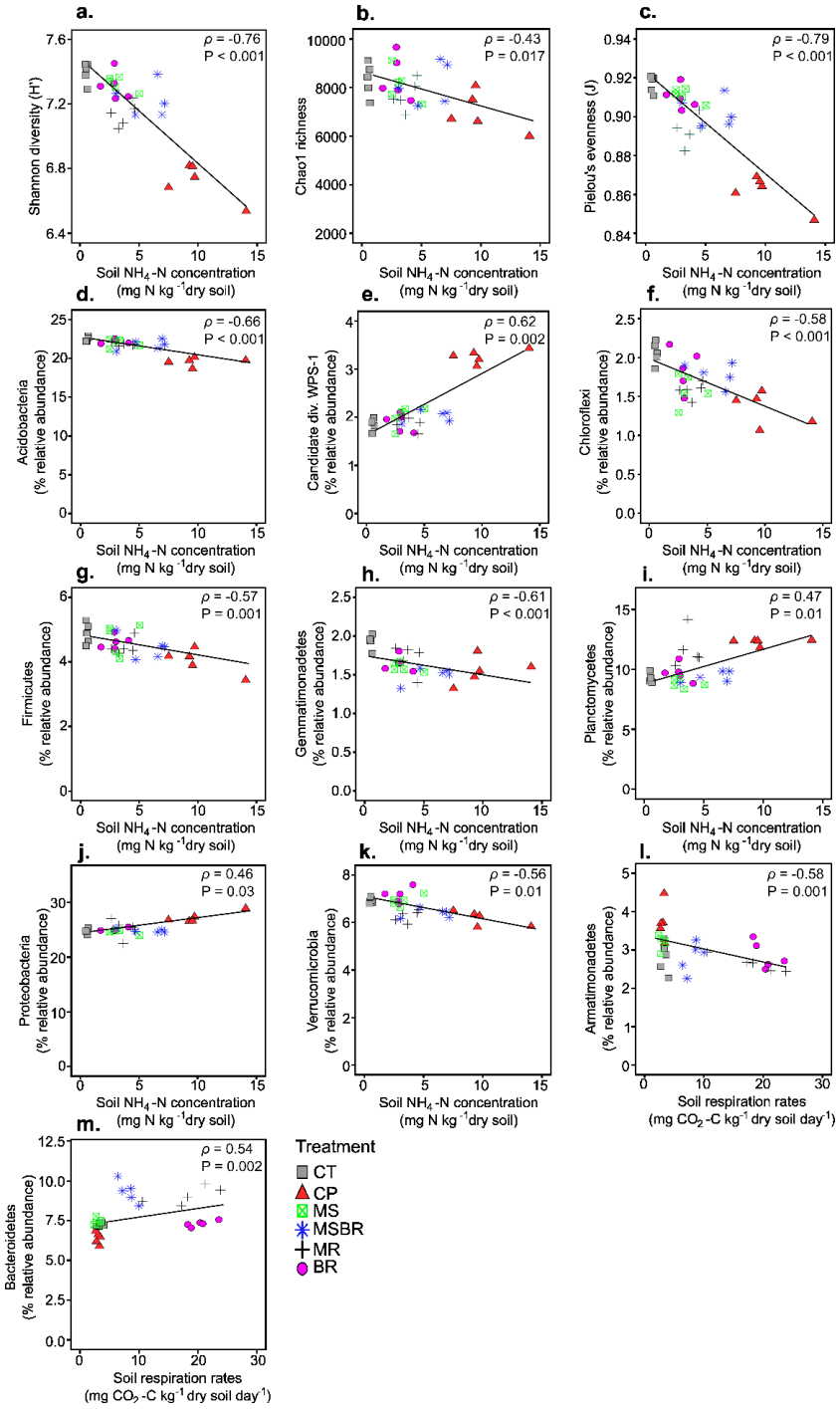

Figure 3. 5 Significant Spearman correlations (rho; ρ) between soil NH_4^+-N concentrations (mg N kg^{-1} dry soil) and (a) Shannon diversity index, (b) Chao1 richness, (c) Pielou's evenness, the relative abundance of (d) Actinobacteria, (e) Candidate division WPS-1, (f) Chloroflexi, (g) Firmicutes, (h) Gemmatimonadetes, (i) Planctomycetes, (j) Proteobacteria, and (k) Verrucomicrobia, and between soil respiration rates (mg CO_2-C kg^{-1} dry soil day^{-1}) and (l) Armatimonadetes and (m) Bacteroidetes. Treatments included: chemical fumigation with chloropicrin (CP), metam sodium used alone (MS) or combined with barley residues (MSBR), biofumigation with mustard residues (MR), the addition of non-biofumigant barley residues (BR), and an untreated control (CT). Values represent each treatment averaged over time in each block (n = 5).

The addition of young barley plant residues to soil did not affect the bacterial diversity indices used in this study, as indicated by the similar Shannon diversity, Pielou's evenness, and Chao1 richness between the BR- and CT-treated soils throughout the incubation. However, the significant effect of BR-treated soil on the β-diversity of the bacterial community over time indicates that the members that shaped the bacterial community were distinct from those in the CT-treated soil. Previous studies also reported that plant residue additions changed soil microbial community composition (Ceja-Navarro et al., 2010; Navarro-Noya et al., 2013; Nelson & Mele, 2006; Pascault et al., 2013), which was attributed to the predominance of bacteria that are capable of exploiting easily accessible C sources for energy and cell synthesis (Pascault et al., 2013).

Biofumigation exhibited a greater impact on bacterial diversity at early time points compared to chemical fumigation, as indicated by the significant, yet transient, decrease of Shannon diversity and species evenness in the MR-treated soil compared to all other treatments after 3 days of incubation. These findings were also consistent with the significantly dissimilar bacterial β-diversity in MR-treated soil compared to all

other treatments after 3 days, indicating that biofumigation yielded the most dissimilar community composition at early time points.

Given that the mustard and barley plant residues had similar C availability, as indicated by their similar C/N ratio and the similar respiration rate between MR and BR-treated soils (Chapter 2), the significantly dissimilar community composition between MR- and BR-treated soils after 160 days indicates that factors other than C availability impacted the bacterial community. These differences may be due to variation in organic C composition between the mustard and barley plant tissues, which has been shown to influence microbial diversity and composition due to varying substrate utilization and affinity among soil microorganisms (Kennedy, 1999; Larkin, 2003). Alternatively, the significant dissimilarities in community composition between MR- and BR-treated soils may also be associated with the toxic effect of ITCs released from the mustard plant residues. Under controlled conditions, the application of purified ITCs on soil has been found to target a diverse range of organisms, including bacteria (Friberg et al., 2009; Hu et al., 2015; Zhu et al., 2020). Our findings are similar to previous studies that reported the addition of GSL-containing oilseed meal significantly altered the bacterial community composition within three days of application (Hollister et al., 2013), and that the bacterial community was modified up to eight months after mustard residue incorporation (Friberg et al., 2009), compared to untreated soils or soils treated with non-biofumigant organic amendments.

Chemical fumigation with CP had the most marked effect on bacterial diversity, as evidenced by the significant decrease of Shannon diversity and species evenness in the CP-treated soil compared to all other treatments from 8 to 160 days of incubation.

This finding indicates that the bacterial diversity in the CP-treated soil could not recover to the level of diversity in the untreated soil, similar to previous studies under controlled conditions (Fang et al., 2018b; Li et al., 2017b). These findings were also consistent with the significant impact of CP fumigation on the β-diversity of the bacterial community throughout the incubation, indicating that CP fumigation yielded the most dissimilar bacterial community compared to all other treatments.

In contrast, MS fumigation used alone did not affect the diversity indices used in this study, as the Shannon diversity, species evenness, and species richness were not significantly different between the MS- and CT-treated soils throughout the incubation. Nonetheless, the significantly dissimilar β-diversity between the MS-treated soil and all other treatments at the end of the incubation indicates that MS fumigation altered bacterial community composition, similar to previous studies under controlled conditions (Li et al., 2017a; Sederholm et al., 2018).

The distinct differences between biofumigation and chemical fumigation with either MS or CP on the bacterial community may reflect the specific chemical properties and concentrations of their active components. For example, MR and MS release ITCs as their active components; however, the primary ITC product released from *B. juncea* is 2-propenyl-ITC (Brown & Morra, 2009), whereas MS releases methyl-ITC. These ITCs have a common structural core entity, but their side-chains differ in structure (Matthiessen & Kirkegaard, 2006), resulting in differences in their volatility, toxicity, and movement throughout the soil profile (Gimsing & Kirkegaard, 2009; Sarwar et al., 1998). Regardless, ITCs are not as volatile as CP (Dungan & Yates, 2003; Gan et al., 2000), indicating that CP may more readily diffuse and target

microorganisms throughout the soil environment. In addition, although the mode of action of ITC-based fumigants and CP has yet to be elucidated, the different impacts on the bacterial community between MR, MS, and CP fumigation may also reflect their specific cellular targets. Finally, the amount of ITCs released from biofumigation depends on the GSL tissue profile, level of tissue maceration, tissue myrosinase concentration, and soil moisture (Gimsing & Kirkegaard, 2009). Similarly, unlike CP, MS must undergo a hydrolysis reaction to release its active component methyl-ITC (Di Primo et al., 2003; Fang et al., 2016). Therefore, the observed differences between MR-, MS-, and CP-treated soil on the bacterial community composition may also reflect variations in concentrations of their active components.

Given that MS fumigation and mustard plant residues release ITCs as their active components, MSBR was used to mimic biofumigation and differentiate between organic soil enrichments (i.e., the addition of barley residues), ITC-based fumigation, and the combined effect of organic amendments and ITC-fumigation. Similar to biofumigation, the transient decrease of Shannon diversity and species evenness in MSBR-treated soil, but not in MS- or BR-treated soils, may reflect the combined effect of methyl-ITC on the bacterial community and the proliferation of C-benefitting bacteria that survived or were resistant to MS fumigation. Previous studies have demonstrated that methyl-ITC degradation is a biological process (Warton et al., 2001; Zhang et al., 2005) and that the addition of organic amendments to soil treated with purified methyl-ITC promoted the growth and activity of methyl-ITC degrading microorganisms (Di Primo et al., 2003; Dungan et al., 2002; Dungan & Yates, 2003),

supporting the theory that certain C-benefitting bacteria are indeed resistant to MS fumigation.

The combined effect of methyl-ITC and organic C amendments may also further explain the observed differences in bacterial community composition between MSBR-, MS-, and BR-treated soil. For example, the similarities between MSBR- and MS-treated soil but not the BR-treated soil after 8 and 32 days suggest that at early time points, the bacterial community in MSBR-treated soil was predominately shaped by methyl-ITC rather than barley residues. However, the effect of methyl-ITC likely weakened over time, while the release of labile C from the barley residues likely promoted the proliferation of species that survived MS fumigation, thereby, yielding a community in MSBR-treated soil that was dissimilar to MS- and BR-treated soil at the end of the incubation.

3.4.2 Effect of chemical fumigation and biofumigation on the relative abundance of bacterial phyla and species

The addition of young barley residues only affected the bacterial community at the species level, as there were no significant differences in the relative abundance of the 11 most abundant phyla between the BR- and CT-treated soils. In contrast, biofumigation greatly affected the bacterial community structure at the phyla level, which was evident based on the significant increase of Bacteroidetes and Planctomycetes and decrease of Actinobacteria and Verrucomicrobia in MR-treated soil compared to CT- and BR-treated soils. The distinct impact of MR-treated soil compared to BR-treated soil on bacterial phyla relative abundance further supports a biofumigation effect on the bacterial community, likely reflecting the dominance of

bacteria responding to the specific organic C source of the mustard plant residues, along with the toxic impact of ITCs on the bacterial community.

Chemical fumigation with CP fumigation had the greatest effect on the bacterial community at the phyla level, as the relative abundance of all phyla except for Armatimonadetes, Candidate division WPS-1, Planctomycetes, and Proteobacteria significantly decreased in the CP-treated soil compared to the CT-treated soil. Changes at the phyla level in CP-treated soil were also well-represented at the species level, including the increase of bacteria belonging to Planctomycetes after 160 days of incubation. Based on this study, CP fumigation appears to target a broad range of bacteria, which likely contributed to the significant compositional dissimilarities between the CP-treated soil and all other treatments. Previous studies also reported CP fumigation significantly decreased the relative abundance of Chloroflexi, Acidobacteria, Verrucomicrobia, and Firmicutes and increased the abundance of Proteobacteria (Li et al., 2017b; Wang et al., 2014b). However, in contrast to our study, it was also reported that Firmicutes, Actinobacteria, Bacteroidetes, and Gemmatimonadetes increased in CP-fumigated soil (Fang et al., 2018b; Li et al., 2017b), but this likely reflects differing soil physicochemical properties, which are known to influence the bacterial abundance and community structure (Mhete et al., 2020; Wei et al., 2018).

MS fumigation did not greatly affect the relative abundance of bacterial phyla, as it only significantly decreased the relative abundance of Chloroflexi compared to CT-treated soil. In contrast to MS- and BR-treated soil, MSBR-treated soil greatly affected bacterial phyla relative abundance compared to the CT-soil, significantly increasing

and decreasing the relative abundance of Bacteroidetes and Verrucomicrobia, respectively. MSBR also had a significantly greater relative abundance of Bacteroidetes compared to both the MS- and BR-treated soils, likely contributing to the compositional dissimilarities between the MSBR-, MS-, and BR-treated soils at the end of the incubation and reflecting the proliferation of C-benefitting bacteria that survived MS fumigation. For example, Bacteroidetes are considered specialists for the degradation of high molecular weight organic matter (Lapébie et al., 2019). Therefore, their proliferation in MSBR-treated soil and not MS or BR-treated soil may suggest their resistance to MS fumigation and their ability to readily utilize C substrates released from the barley plant residues.

3.4.3 Correlations between bacterial diversity indices or phyla relative abundance and soil properties

Unexpectedly, there were several negative correlations between soil NH_4^+-N concentration and the bacterial diversity indices and phyla relative abundance. Soil NH_4^+-N concentration increased in soils either due to the mineralization of labile N following plant residue addition (Chapter 2) or due to chemical fumigation, most notably CP fumigation, due to the release of cell lysates following fumigation (Yan et al., 2013a) or the inhibition of nitrification (Chapter 2). Therefore, it is unlikely that soil NH_4^+-N concentration caused these changes in diversity or phyla relative abundance. Instead, bacterial diversity, the relative abundance of bacterial phyla, and soil NH_4^+-N concentration were either driven by the addition of plant residues and or chemical fumigation; thus, a change in diversity or bacterial phyla relative abundance was indirectly associated with soil NH_4^+-N concentration.

3.5 Conclusions

This is the first study that has investigated both the effects of chemical fumigation and biofumigation with high-GSL mustard plant residues on the bacterial diversity using amplicon-based NGS. This study showed that biofumigation exhibited a greater impact on bacterial diversity indices at early time points, whereas chemical fumigation with CP had a profound and enduring impact on the bacterial diversity indices throughout the incubation. Interestingly, the addition of barley plant residues or chemical fumigation with MS used alone did not affect the diversity indices compared to untreated soil throughout the incubation, yet MS fumigation in combination with barley plant residues transiently decreased Shannon diversity and species evenness compared to the untreated soil after 8 days of incubation. These findings indicate that there was a significant combined effect between ITC-based fumigation and soil organic amendments on bacterial diversity. Although all treatments in this study progressed towards an alternative community assemblage compared to the untreated control over time, CP fumigation yielded the most dissimilar community composition at the end of the incubation.

Chapter 2 demonstrated that chemical fumigation (i.e., CP, MS, and MSBR treatments) significantly inhibited nitrification, and that the MSBR treatment also significantly decreased soil respiration and plant residue decomposition compared to BR-treated soil. These findings collectively provide evidence that although biofumigation, the addition of non-biofumigant barley residues, and chemical fumigation all altered bacterial community composition, only chemical fumigation significantly affect soil function whether used alone or alone combined with barley

plant residues. Overall, this study demonstrates that under controlled conditions, chemical fumigation with CP had the greatest and most persistent impact on bacterial diversity and community composition, whereas MS fumigation and biofumigant led to transient decreases in bacterial diversity.

CHAPTER 4: EFFECT OF CHEMICAL FUMIGATION AND SUBSTRATE CARBON AVAILIBILITY ON SOIL RESPIRATION, SOIL NITROUS OXIDE EMISSIONS, AND SOIL INORGANIC NITROGEN CONCENTRATIONS

4.1 Introduction

Chemical fumigation is an agronomic practice used to mitigate soil-borne diseases and pathogens in high-value crop systems, including potato (*Solanum tuberosum* L.) production systems. Currently, the two most commonly used chemical fumigants in Canadian potato crop production systems are chloropicrin and metam sodium. Chemical fumigation has been found to effectively reduce soil-borne diseases (Collins et al., 2006; De Cal et al., 2005); however, due to its non-specificity and broad-spectrum properties, chemical fumigation may impact non-target microorganisms and other essential soil processes mediated by soil microorganisms.

In Chapter 2, there was evidence that chemical fumigation may impact soil carbon (C) dynamics. For example, soil fumigated with metam sodium combined with the addition of fresh labile barley (*Hordeum vulgare* L.) plant residues significantly reduced soil respiration rates and had a significantly greater percentage of added plant residue dry matter remaining at the end of the 160-day incubation compared to non-fumigated soil amended with labile barley plant residues. Increased rates of soil respiration (i.e., emissions of carbon dioxide, CO_2) often indicate increased soil organic C (SOC) decomposition, C availability, and microbial activity (Liu et al., 2006; Xu & Shang, 2016; Zhang et al., 2019b). Thus reduced respiration rates following chemical fumigation may indicate an inhibitory effect on the indigenous soil microbial community that readily decomposes plant residues. However, soil respiration is also the

primary pathway of C moving from soil to the atmosphere (Li et al., 2019; Xu & Shang, 2016), and therefore, reduced respiration rates following chemical fumigation, if persistent, may present a significant impact on SOC storage, soil productivity, and function.

In addition to soil C dynamics, several studies have reported that chemical fumigation with metam sodium or chloropicrin used alone also affects the soil nitrogen (N) cycle by significantly inhibiting nitrification (Yan et al., 2013ab; Yan et al. 2015). Similarly, there was evidence that fumigation with metam sodium combined with the addition of fresh labile barley residues significantly inhibited nitrification and reduced soil nitrous oxide (N_2O) emissions compared to non-fumigated soil amended with barley residues (Chapter 2). N_2O is a potent greenhouse gas with a significantly greater global warming potential than CO_2 (Tian et al., 2020; Wang et al., 2021). Therefore, reduced N_2O emissions following plant residue incorporation in chemically fumigated soil may increase soil inorganic N pools and thereby present benefits for both soil health and agricultural production.

Further investigation is warranted regarding the effect of chemical fumigation on the soil C and N dynamics. The objective of this study was to investigate the effects of chemical fumigation with either chloropicrin or metam sodium compared to non-fumigated soil amended with substrates of contrasting C availability (young vs. mature barley plant residues) on soil respiration rates, N_2O emission rates, ammonium (NH_4^+-N) concentrations, and nitrate (NO_3^--N) concentrations.

It was hypothesized that: 1) for soils receiving chemical fumigation, amendment with young barley plant residues would have greater respiration rates, N_2O emission

rates, and inorganic N concentrations due to more readily available C and N from the plant residues when compared with mature barley residues; and 2) across residue amendment treatments, chemical fumigation would result in lower respiration rates and N_2O emission rates due to a reduction in microbial metabolism resulting from microbial death following fumigation and greater NH_4^+-N concentrations and lower NO_3-N concentrations due to significant nitrification inhibition by the chemical fumigation, when compared with non-fumigated soil.

4.2 Material and Methods

4.2.1 Experimental design

This microcosm experiment consisted of a 3 x 3 factorial arrangement of treatments in a randomized complete block design (RCBD) with five replicates blocked in time to manage a larger number of microcosms. Factors included three fumigation treatments [chloropicrin (CP), metam sodium (MS), or an untreated control (CT)] and three soil amendment treatments [young barley tissue (Y), mature barley tissue (M), or unamended soil (U)]. In total, there were nine treatment combinations: CP-U, CP-Y, CP-M, MS-U, MS-Y, MS-M, CT-U, CT-Y, and CT-M.

Fumigants CP and MS were selected because they are the most commonly used soil fumigants in Canadian potato crop production systems. In New Brunswick, a two-year crop rotation consisting of potato-barley crops is frequently used, and chemical fumigation is performed in early fall following the barley crop. Therefore, barley plant residues were selected as the substrates of contrasting C availability, where the young and mature barley plant residues served as high and low available C substrates, respectively.

4.2.2 Soil sampling

Soil (0-20 cm) was collected from a field under continual potato crop rotations at the Fredericton Research and Development Centre of Agriculture and Agri-Food Canada located in Fredericton, New Brunswick, Canada (45°55'15"N, 66°36'30"W). The field had no previous history of chemical fumigation. The soil texture was sandy loam (590 g sand kg^{-1}, 310 g silt kg^{-1}, 100 g clay kg^{-1}) as measured using the pipette method with organic matter removal (Kroetsch & Wang, 2007). Soil organic C and total N concentrations were 23.6 g C kg^{-1} and 2.20 g N kg^{-1}, respectively, as determined by dry combustion (Elementar varioMACRO, Skjemstad & Baldock, 2007). Soil pH (1:1 water dilution) was 5.8.

The soil was collected on fives dates between mid-September and early October 2019 after potato harvest. Soil collected at each sampling date was used for an experimental replicate for the preparation of soil microcosms. The field moist soil was passed through a 4.75 mm sieve at each sampling date and stored at room temperature (~20°C) for five days before implementing the experimental treatments to avoid a potential flush of microbial activity following a change in soil temperature or the sieving of soil. The gravimetric water content at the time of soil collection for each experimental replicate ranged between 0.23-0.26 g g^{-1} dry soil.

4.2.3 Plant residues

The young barley plants were grown in a greenhouse with a daytime temperature of 20-24°C, a nighttime temperature of 18-22°C, and a 16 hr photoperiod. The mature barley plants were collected from a field under potato-barley crop rotation at the Fredericton Research Development Centre. The young and mature barley plants were

harvested 5 cm above the soil once they reached the Zadoks growth stage of 37 and 91, respectively. For the young barley plants, all of the above-ground plant tissue was used, whereas for the mature barley plants, only the straw was used.

Following harvest, the plant tissues were dried for 72 h at 55°C and ground to pass through a 2 mm screen using a Thomas Model 4 Wiley Mill. Plant residue sub-samples were sent to PEI Analytical Laboratories (PEIAL, Charlottetown, PE, Canada) for the analysis of total tissue C, N, acid detergent fibre (ADF), and neutral detergent fibre (NDF) content. The tissue C/N ratio is not always reliable in inferring tissue C structural complexity or availability (Chen et al., 2014; Muhammad et al., 2011); therefore, to ensure that the young and mature barley plant residues were of contrasting C availability, ADF and NDF analyses were used to quantify the least digestible plant components, including cellulose and lignin content (ADF) and hemicellulose content (NDF). The young barley residues had a C/N ratio of 10.3, ADF content of 276 g kg^{-1}, and NDF content of 422 g kg^{-1}, whereas the mature barley residues had a C/N ratio of 70.1, ADF content of 502 g kg^{-1}, and NDF content of 763 g kg^{-1} (Table 4.1).

4.2.5 Microcosm preparation

A 500 mL glass canning jar served as an individual soil microcosm. Soil (275 g equivalent of oven-dry soil) was loosely added to the microcosms, and the water content was adjusted such that when the soil was packed to the target bulk density, a water-filled pore space (WFPS) of 55% would be achieved. The soil remained loosely packed for the first 48 hours of the incubation following treatment implementation to ensure that: 1) a fumigation effect was effectively achieved by promoting the diffusion of gaseous fumigant products throughout the soil during a 24-hour sealed period; and

2) any remaining toxic fumigant products were able to escape the soil during a 24-hour ventilated period to proceed safely with the experiment.

Table 4. 1 Characteristics of young and mature barley (*Hordeum vulgare* L.) plant residues.

Plant Residue	Zadoks growth scale	Dry matter added per microcosm (g)	C (g kg^{-1})	N (g kg^{-1})	C/N	Tissue C added per microcosm (mg)	Tissue N added per microcosm (mg)	ADF[+] (g kg^{-1})	NDF[++] (g kg^{-1})
Young barley	37 (28 days of growth)	0.70	400	39.0	10.3	275	26.9	276	422
Mature barley	91 (80 days of growth)	0.60	470	6.70	70.1	275	3.95	502	763

[+] Acid detergent fibre

[++] Neutral detergent fibre

Prior to fumigation, young barley plant residues (0.70 g dry matter per microcosm) and mature barley plant residues (0.60 g dry matter per microcosm) were added to the microcosms to achieve an application rate of 1000 mg C kg^{-1} dry soil, which falls within the range of expected organic C additions in agricultural soils after the plow-down of plant residues (Bolinder et al., 2002; Miller et al., 2012). The barley plant residues were gently mixed into the soil. Chloropicrin (19.5 µL of PIC100 per microcosm, Trinity Manufacturing Inc.) and metam sodium (87.5 µL of VAPAM per microcosm, AMVAC Chemical Corporation) were added to the microcosms based on field recommended application rates specific to eastern Canadian potato production (235 L ha^{-1} and 700 L ha^{-1}, respectively). The chemical fumigants were applied to the top of the soil using a micropipette and sealed immediately to retain the fumigant within the soil. This approach was used to simulate common commercial practices,

where chloropicrin is commonly injected into the soil followed by soil compaction, and metam sodium is commonly banded into the soil and the soil wetted by irrigation to retain the fumigant within the root zone.

The sealed microcosms were incubated in a pesticide fume hood at room temperature (~20°C) for a 24-hour period. After 24 hours, the lids were removed, and the microcosms were ventilated for a 24-hour period. The soil in the microcosms was then packed to a pre-drawn line on the microcosm to establish a bulk density of 1.1 Mg m^{-3}. Next, the microcosms were covered with Parafilm pierced with holes to reduce water evaporation and allow for gas exchange and then placed in incubators (model MIR-553, Sanyo Scientific, Japan) at 20°C for the remainder of the experiment. The microcosms were weighed weekly, and deionized distilled water was added to maintain the desired water content.

Two sets of microcosms were used to quantify all desired parameters: the first set was continuously sampled to quantify soil respiration and N_2O emission rates, while the second set was destructively sampled to quantify soil inorganic N concentrations, where an individual treatment, time, and replicate combination served as an individual destructive sample that was discarded after sampling. The first set of microcosms were sampled after 3, 4, 8, 16, 30, 44, 58, 72, 86, 100, 114, and 128 days of incubation, whereas the second set of microcosms were destructively sampled after 3, 8, 16, 30, 44, 72, 100, and 128 days of incubation. To ensure that the entire duration of nitrification inhibition was captured in this study, the incubation length of 128 days was selected based on previous studies that investigated the effects of chemical fumigation on soil N

dynamics (i.e., the duration of nitrification inhibition based on soil NH_4^+-N concentrations) (De Neve et al., 2004; Yan et al., 2013a; Yan et al., 2015).

4.2.6 Soil respiration and N_2O emissions

At each sampling time point, the first set of microcosms were sealed with a screw-top lid fitted with a rubber septum, and 10% of headspace volume was replaced with compressed air to provide sufficient volume of gas for sampling. To quantify soil respiration (i.e., emissions of CO_2) and N_2O emission rates, headspace gas (20 mL) was collected at 15, 30, 45, and 60 min and transferred to a pre-evacuated 12 mL glass vial (Exetainers; Labco, High Wycombe, U.K.). Headspace gas samples were analyzed for CO_2 and N_2O concentrations using a Varian Star 3800 gas chromatograph (Varian, Mississauga, ON) described by Burton et al. (2008). Rates of soil respiration and N_2O emissions were calculated as the change in mass of CO_2-C or N_2O-N in the microcosm headspace over 60 min, as described by Zebarth et al. (2012). Cumulative gas emissions were calculated over two different time periods: from 0-16 days and 0-128 days of incubation. This was done because day 16 was the last date in which emission rates differed significantly among treatments. Cumulative emissions were calculated using the linear trapezoidal integration method, as described by Burton et al. (2008). After gas sampling, the microcosms were re-covered with Parafilm pierced with holes and placed back into incubators set at 20°C.

The apparent decomposition of the barley residues added to the fumigant treatments examined for the time periods 0 to 16 days (i.e., the time period over which soil respiration differed significantly among treatments) and 0 to 128 days (i.e., the entire incubation). The apparent decomposition (%) was calculated as the cumulative

respiration in amended soil averaged across young and mature residues (i.e., CT-Y/CT-M, MS-Y/MS-M, or CP-Y/CP-M), minus the cumulative respiration in unamended soil (i.e., CT-U, MS-U, or CP-U), divided by the total quantity of C added from the barley plant residues (i.e., 1000 mg C kg^{-1}), and expressed as a percentage.

4.2.7 Soil inorganic N concentrations

At each sampling time point, sub-samples of 20 g moist soil were collected from the second set of microcosms, and concentrations of NH_4^+-N and NO_3^--N were determined by shaking 20 g of moist soil for 30 min with 100 mL 2 M KCl. Extracts were filtered and stored at -20°C pending analysis. Extracts were analyzed for NH_4^+-N and NO_3^--N concentrations on a QuikChem 8500 Series 2 Flow Injection Auto-Analyzer (Lachate Instruments) following QuikChem Method 90-107-06-3-A and Method 90-107-04-2-A, respectively.

The potential rate at which NH_4^+-N can be depleted in soil by nitrification is commonly greater than the potential for soil to produce NH_4^+-N through net N mineralization (Roberston & Groffman, 2007); subsequently, soil NH_4^+-N concentrations do not commonly accumulate in agricultural soils. In this study, the incubation conditions (i.e., WFPS adjusted to 55% and temperature of 20°C) were favourable for nitrification (Norton & Ouyang, 2019), and therefore, nitrification inhibition was inferred by an accumulation of soil NH_4^+-N over time, which has been used as evidence in previous studies (Chaves et al., 2006; Chen et al., 2010; Yamamoto et al., 2008; Yan et al., 2013a).

4.2.8 Statistical analyses

The RStudio base statistic software was used to conduct statistical analyses (v.4.0.5) (RStudio Team, 2020). Data were assessed for normality (i.e., normal and independent distribution) using homoscedasticity diagnostic plots and Shapiro-Wilks tests. Non-normal data were transformed using Box-Cox transformations with the optimal lambda (λ) value using the MASS package in RStudio (Venables, 2002). For the gaseous emission rates, a general linear model analysis of variance (ANOVA) was performed based on a factorial arrangement of 3 fumigant treatments, 3 residue amendments, and 12 incubation lengths in an RCBD with all fixed factors. An ANOVA was performed for the soil inorganic N concentrations based on a factorial arrangement of 3 fumigant treatments, 3 residue amendments, and 8 incubation lengths in an RCBD with all fixed factors. Treatment, time, and interaction means were tested using post-hoc Tukey Honest Significant Difference (HSD) tests. Spearman correlations (rho [ρ] rank coefficient) were used to determine possible relationships between gas emission rates and soil inorganic N concentrations and between cumulative gas emissions and ammonium exposure (cumulative NH_4^+-N concentration) or nitrate exposure (cumulative NO_3^--N concentration) using the Hmisc package in RStudio (Harrell, 2021). Only scatter plots representing significant correlations are presented. The treatment means and standard errors presented in the figures were calculated from untransformed data. Significance was accepted at $P \leq 0.05$.

4.3 Results

4.3.1 Soil respiration and N_2O emission rates

There was a significant fumigant x amendment x time interaction on soil respiration rates ($P < 0.001$) (Figure 4.1a). The MS-U and CP-U-treated soils had low soil respiration rates throughout the incubation and were not significantly different from each other or from the CT-U-treated soil (average over time of 5.28 mg CO_2-C kg^{-1} dry soil day^{-1}). The CT-Y-treated soil had significantly greater respiration rates after 3 and 4 days of incubation compared to all other treatments (all $P < 0.05$) and had the greatest rate after 3 days of incubation (40.4 mg CO_2-C kg^{-1} dry soil day^{-1}) compared to all other time points. After 8 days of incubation, soil respiration rates in the CT-Y treated soil returned to rates similar to those in the CT-U treated soil. In contrast to CT-Y treated soil, the MS-Y soil had the greatest respiration rate after 8 days of incubation compared to all other time points (24.1 mg CO_2-C kg^{-1} dry soil day^{-1}). The respiration rate in MS-Y soil after 8 days was significantly greater than MS-U, CP-U, MS-M, and CT-U treated soils (all $P < 0.05$). Similar to MS-Y treated soil, the CP-Y soil also had the greatest respiration rate after 8 days of incubation compared to all other time points (13.6 mg CO_2-C kg^{-1} dry soil day^{-1}). The respiration rate in CP-Y-treated soil after 8 days was significantly greater than MS-U, CP-U, and CT-U-treated soil (all $P < 0.05$). The MS-Y and CP-Y-treated soils had similar rates to those in the CT-U-treated soil after 16 days of incubation. Throughout the incubation, CT-M, MS-M, and CP-M treated soils had respiration rates that were relatively stable over time and were not significantly different from each other (average over time of 7.34 mg CO_2-C kg^{-1} dry soil day^{-1}) or significantly different from MS-U, CP-U, and CT-U-

treated soils, except for after 16 days of incubation, when CT-M-treated soil had a significantly greater respiration rate compared to CP-U-treated soil (~5-fold increase). After 30 days of incubation, all treatments had respiration rates that were not significantly different from each other for the remainder of the incubation.

There was a significant fumigant x amendment x time interaction on N_2O emission rates ($P < 0.001$) (Figure 4.1b). The MS-U and CP-U treated soils had low N_2O emission rates throughout the incubation and were not significantly different from each other or from the CT-U treated soil (average over time of 3.10 μg N_2O-N kg^{-1} dry soil day^{-1}) (Figure 4.1b). The CT-Y soil had significantly greater N_2O emission rates after 3 and 4 days of incubation compared to the CT-U-treated soil, where the greatest emission rate (average of 36.2 μg N_2O-N kg^{-1} dry soil day^{-1}) was achieved after 3 days of incubation and was significantly greater than all other treatments (Figure 4.1b). The MS-Y soil had the greatest N_2O emission rate after 8 days of incubation (average of 41.5 μg N_2O-N kg^{-1} dry soil day^{-1}) compared to all other time points, which was significantly greater than N_2O emission rates in all other treatments except for CP-Y-treated soil. The CP-Y soil also had the greatest N_2O emission rate after 8 days of incubation (average of 25.1 μg N_2O-N kg^{-1} dry soil day^{-1}) compared to all other time points, which was significantly greater than N_2O emission rates in CT-U, CT-U, CT-M, CP-U, CP-M, and MS-M treated soils (Figure 4.1b). After 16 days of incubation, MS-Y and CP-Y-treated soils had N_2O emission rates that were similar to the CT-U-treated soil. CT-M, MS-M, and CP-M-treated soils had N_2O emission rates that were not significantly different from each other (average over time of 3.30 μg N_2O-N kg^{-1} dry soil day^{-1}) or significantly different from MS-U, CP-U, and CT-U- treated soil

throughout the incubation. After 16 days of incubation, all treatments had N_2O emission rates that were not significantly different from each other for the remainder of the incubation (Figure 4.1b).

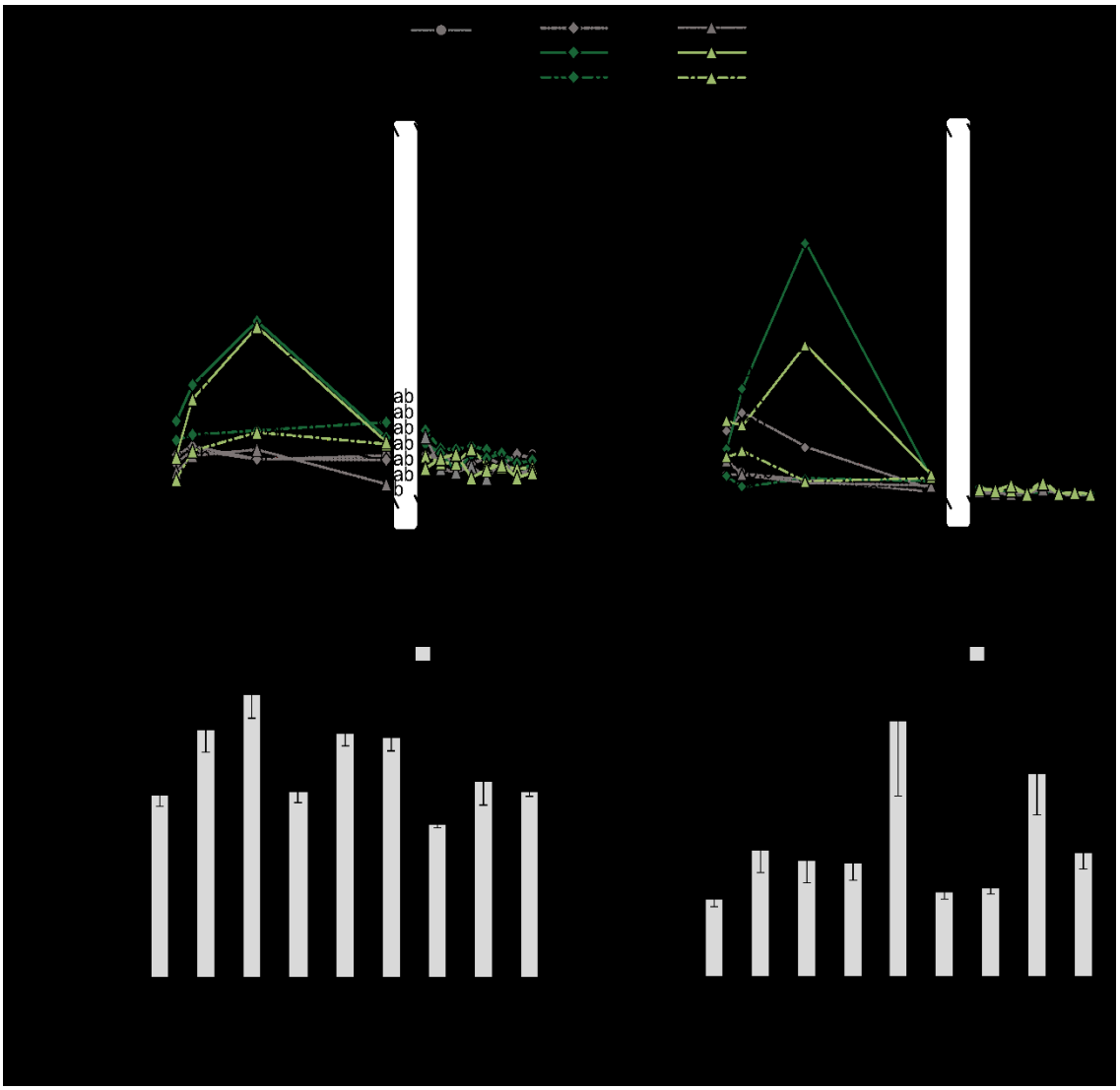

Figure 4. 1 Soil (a) respiration rate (mg CO_2-C kg^{-1} dry soil day^{-1}) and (b) nitrous oxide emission rate (µg N_2O-N kg^{-1} dry soil day^{-1}) throughout the 128-day incubation. Cumulative (c) respiration (mg CO_2-C kg^{-1} dry soil) and (d) N_2O emissions (µg N_2O-N kg^{-1} dry soil) from 0-16 and 0-128 days of incubation. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U). Values are

means (n = 5) and error bars are the standard error (± SE). For the gas emission rates, significant differences among mean values for individual time points are represented by a letter adjacent to the time points. For the cumulative gas emissions, significant differences among mean values from 0-16 days of incubation are represented by a lower case letter above the mean value, whereas significant differences among values from 0-128 days of incubation are presented by an upper case letter above the mean value.

4.3.2 Cumulative soil respiration and N_2O emissions

From 0 to 16 days of incubation, the CT-U, MS-U, MS-M, CP-U, and CP-M treated soils did not have significantly different cumulative respiration (average of 78, 95, 149, 78, and 112 mg CO_2-C kg^{-1} dry soil, respectively) (Figure 4.1c; Table A.2 for ANOVA results). Similarly, the CT-M, MS-Y, and CP-Y treated soils did not have significantly different cumulative respiration (average of 221, 255, and 225 mg CO_2-C kg^{-1} dry soil, respectively); however, they all had significantly greater cumulative respiration than the CT-U, MS-U, MS-M, CP-U, and CP-M treated soils (Figure 4.1c). In contrast, the CT-Y soil had significantly greater cumulative respiration (313 mg CO_2-C kg^{-1} dry soil) compared to all other treatments except for the MS-Y soil (Figure 4.1c).

From 0 to 128 days of incubation, the CT-U, MS-U, CP-Y, and CP-M treated soils did not have significantly different cumulative respiration (average of 689, 706, 741, and 704 mg CO_2-C kg^{-1} dry soil, respectively) (Table A.3 for ANOVA results). Similarly, the CT-Y, CT-M, MS-Y, and MS-M treated soils also did not have significantly different cumulative respiration (939, 1071, 922, and 908 mg CO_2-C kg^{-1} dry soil, respectively); however, they all had significantly greater cumulative respiration than all other treatments. In contrast, the CP-U soil had significantly lower

cumulative respiration compared to all other treatments (average of 585 mg CO_2-C kg^{-1} dry soil).

From 0 to 16 days, the CT-U, CT-Y, CT-M, MS-U, MS-M, CP-U, and CP-M treated soils did not have significantly different cumulative N_2O emissions (average of 87μg N_2O-N kg^{-1} dry soil, respectively) (Figure 4.1d; Table A.2 for ANOVA results). The MS-Y soil had significantly greater cumulative N_2O emissions (average of 557 μg N_2O-N kg^{-1} dry soil) compared to all other treatments except for the CP-Y soil, whereas the CP-Y soil had significantly greater cumulative N_2O emissions (average of 329 μg N_2O-N kg^{-1} dry soil, respectively) compared to all other treatments except for the CT-Y and MS-U treated soils (Figure 4.1d). From 0 to 128 days, the CT-U, CT-M, MS-U, MS-M, and CP-U treated soils did not have significantly different cumulative N_2O emissions (average of 315 μg N_2O-N kg^{-1} dry soil, respectively) (Figure 4.1d; Table A.3 for ANOVA results). The CT-Y and CP-M treated soils did not have significantly different cumulative N_2O emissions (average of 413 and 406 μg N_2O-N kg^{-1} dry soil, respectively), but both treatments had significantly greater cumulative emissions than the CT-U soil. The MS-Y and CP-Y treated soils did not have significantly different cumulative emissions (average of 833 and 661 μg N_2O-N kg^{-1} dry soil, respectively), but both treatments had significantly greater cumulative N_2O emissions than all other treatments except for CT-Y and CP-M treated soils, which did not significantly differ from the CP-Y soil (Figure 4.1d).

From 0 to 16 days and 0 to 128 days of incubation, there were significant differences in apparent decomposition of barley plant residues among the amended CT-, MS-, and CP-treated soils (both $P < 0.05$) (Figure 4.2; Table A.4 and A.5 for ANOVA

results). From 0 to 16 days of incubation, both the MS- and CP-treated soils had a significantly lower percentage of apparent decomposition (11% and 9%, respectively, of C added in the barley residues) compared to the CT-treated soils (18%). From 0 to 128 days of incubation, the MS- and CT-treated soils had similar percentages of apparent decomposition compared to each other (24% and 32%, respectively), whereas the CP-treated soils had a significantly lower percentage of apparent decomposition (13%) compared to the MS- and CT-treated soils (Figure 4.2).

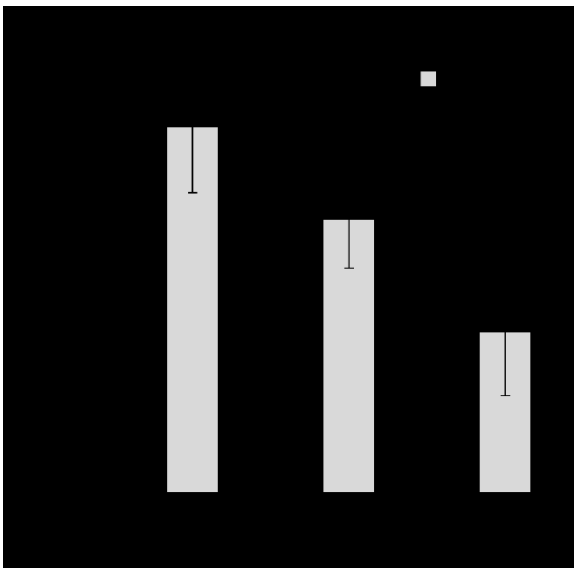

Figure 4. 2 The apparent decomposition of the barley residues added to the fumigant treatments examined for the time periods 0 to 16 days (i.e., the time period over which soil respiration differed significantly among treatments) and 0 to 128 days (i.e., the entire incubation). The apparent decomposition (%) was calculated as the cumulative respiration in amended soil averaged across young and mature residues (i.e., CT-Y/CT-M, MS-Y/MS-M, or CP-Y/CP-M), minus the cumulative respiration in unamended soil (i.e., CT-U, MS-U, or CP-U), divided by the total quantity of C added from the barley plant residues (i.e., 1000 mg C kg^{-1}) and expressed as a percentage. Significant differences among mean percentages (HSD, $P \leq 0.05$) are represented by a letter above mean values (± standard error, SE) ($n = 5$).

4.3.3 Soil inorganic N concentrations

There was a significant fumigant x amendment x time interaction on soil NH_4^+-N concentrations ($P < 0.001$) (Figure 4.3a). The CT-U-treated soil had low NH_4^+-N concentrations throughout the incubation (average over time of 0.63 mg N kg^{-1} dry soil). The MS-U soil had a maximum NH_4^+-N concentration of 8 mg N kg^{-1} dry soil after 8 days of incubation, whereas the CP-U soil had a maximum NH_4^+-N concentration of 16 mg N kg^{-1} dry soil after 16 days of incubation, both of which were significantly greater than the CT-U soil. The CT-Y soil had a maximum NH_4^+-N concentration of 15 mg N kg^{-1} dry soil after 3 days of incubation, which was significantly greater than all other treatment except for the MS-Y soil. The MS-Y soil had a maximum NH_4^+-N concentration of 29 mg N kg^{-1} dry soil after 8 days of incubation, whereas the CP-Y soil had a maximum NH_4^+-N concentration of 57 mg N kg^{-1} dry soil after 30 days of incubation, both of which were significantly greater than the CT-U and CT-Y treated soils. The CT-M soil had low NH_4^+-N concentrations throughout the incubation that did not significantly differ from the CT-U soil (average over time of 0.74 mg N kg^{-1} dry soil). The MS-M soil had a maximum NH_4^+-N concentration of 8 mg N kg^{-1} dry soil after 8 days of incubation, whereas the CP-M soil had a maximum NH_4^+-N concentration of 6 mg N kg^{-1} dry soil after 30 days of incubation, both of which were significantly greater than the CT-U and CT-M treated soils. After 72 days of incubation, soil NH_4^+-N concentrations were not significantly different among all treatments for the remainder of the incubation.

There was no significant fumigant x amendment x time interaction on NO_3^--N concentrations ($P = 0.136$); however, there was a significant fumigant x amendment

interaction (P = 0.002). The CT-M, MS-M, and CP-M-treated soils had similar NO_3^--N concentrations compared to each other (average over time of 48, 46, and 48 mg N kg^{-1} dry soil, respectively) that were significantly lower than all other treatments (all P < 0.05) (Figure 4.3b). In contrast, the CT-Y, MS-Y, and CP-Y-treated soils had similar NO_3^--N concentrations compared to each other (average over time of 105, 99, and 80 mg N kg^{-1} dry soil, respectively) that were significantly greater than all other treatments except for CP-Y treated soil, which had similar NO_3^--N concentrations to MS-U treated soil (Figure 5.2b). The CT-U, MS-U, and CP-U treated soils had similar NO_3^--N concentrations compared to each other (average over time of 60, 62, and 80 mg N kg^{-1} dry soil, respectively) (Figure 5.2b). There was also a significant time effect on soil NO_3^--N concentrations (P < 0.001), where NO_3^--N concentrations significantly increased over time from an average of 43 mg N kg^{-1} dry soil after 3 days of incubation to an average of 118 mg N kg^{-1} after 128 days of incubation (data not shown).

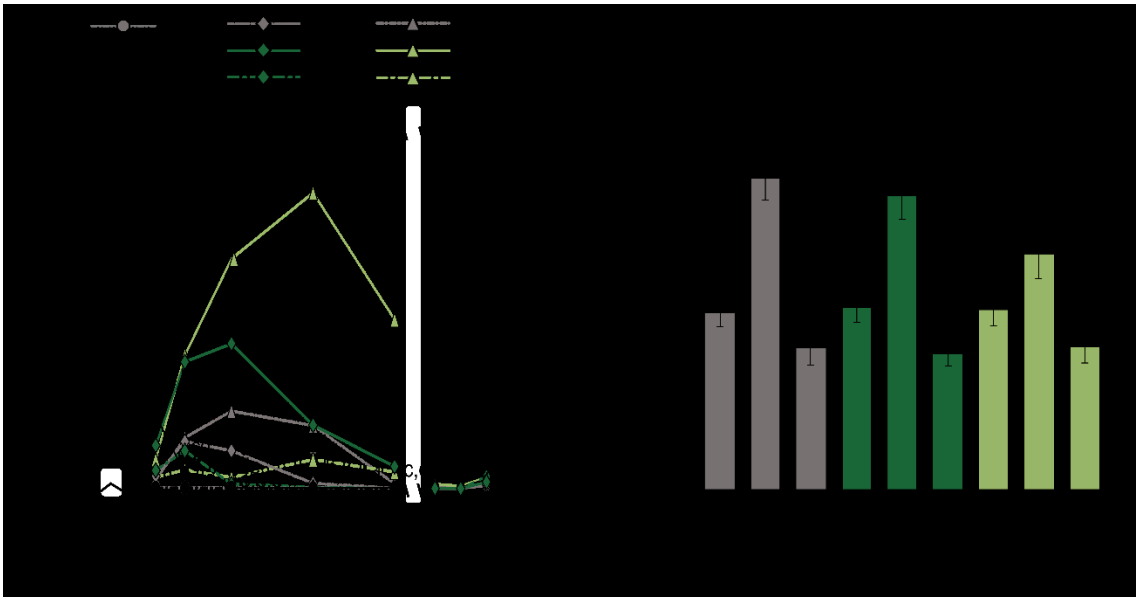

Figure 4. 3 Soil (a) ammonium (mg NH_4^+-N kg^{-1} dry soil) throughout the 128-day incubation and (b) nitrate (mg NO_3^--N kg^{-1} dry soil) concentrations averaged over time. Treatments included: chemical fumigation with metam sodium (MS-U) and

chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U). Values are means ([NH_4^+-N, $n = 5$], [NO_3^--N, $n = 40$]) and error bars are the standard error (± SE). Significant differences among mean values are presented in two ways (HSD, $P \leq 0.05$): (i) differences among treatment means for individual time points are represented by letter adjacent to the time points, and (ii) differences among treatment means averaged over time are represented by letters above mean values.

There were significant positive correlations between soil respiration rate and N_2O emission rate ($\rho = 0.44$, $P = 0.002$) and between soil NO_3^--N concentration and N_2O emission rate ($\rho = 0.51$, $P < 0.001$) (Figure 4.4). There were no other significant correlations between gas emission rates and inorganic N concentrations or between cumulative gas emissions and NH_4^+-N or NO_3^--N exposure.

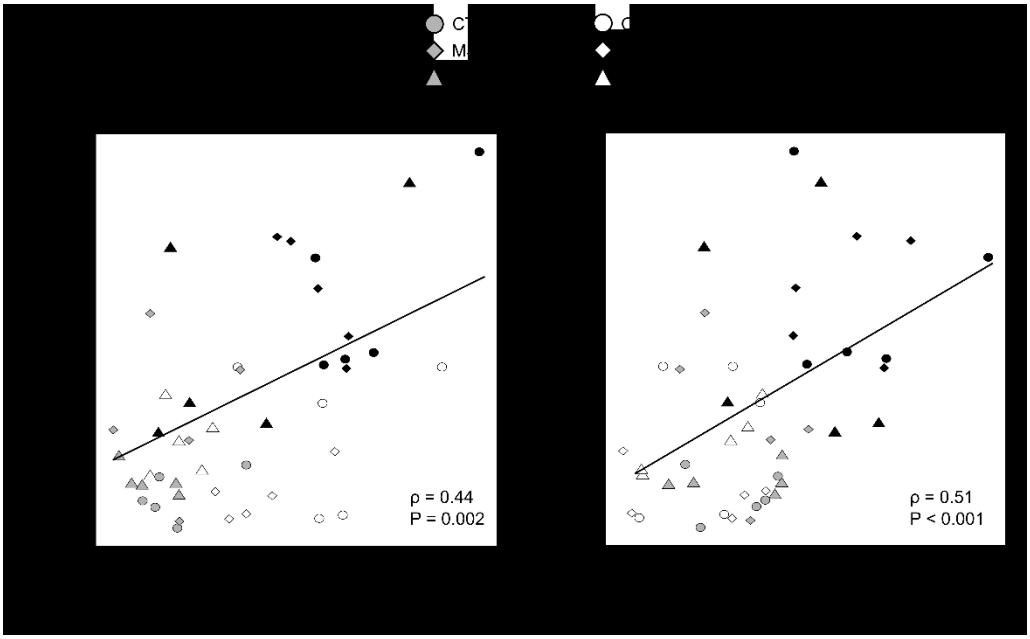

Figure 4. 4 Significant Spearman correlations (rho; ρ) (a) between soil respiration rate and nitrous oxide (N_2O) emission rate and (b) between soil nitrate (NO_3^--N) concentration and N_2O emission rate. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley

plant residues (CT-M), and an untreated control (CT-U). Values represent each treatment averaged over time in each block (n = 5).

4.4 Discussion

4.4.1 Effect of chemical fumigation and substrate C availability on soil respiration

This study investigated the effects of chemical fumigation in combination with plant residues of contrasting substrate C availability on soil respiration. In non-fumigated soil, the addition of young barley residues led to greater soil respiration than the addition of mature barley residues with the same quantity of added C, which was supported by the significantly greater maximum respiration rate achieved at earlier time points and the significantly greater cumulative respiration from 0 to 16 days of incubation in the CT-Y soil compared to the CT-M soil. However, since respiration could not be measured during the first two days of incubation and the maximum measured respiration rate for the CT-Y soil was achieved after 3 days of incubation, the amount of respiration in this treatment may be significantly underestimated. Nonetheless, these results were consistent with the lower C/N ratio, ADF content, and NDF content of the young barley residues and thereby likely reflect contrasting tissue C availability between the young and mature barley plant residues. Previous studies have reported that soil organic amendments with a lower C/N ratio and a greater proportion of soluble organic C led to greater respiration rates than amendments with a higher C/N ratio and a greater proportion of insoluble C due to the fast metabolic turnover of labile SOC fractions (Bond-Lamberty & Thomson, 2010; Wang et al., 2003; Wei et al., 2015). Therefore, the greater soil respiration in the CT-Y soil than the CT-M soil likely reflects the greater proportion of labile organic C rapidly promoting microbial-mediated decomposition.

Chemical fumigation reduced the decomposition of the added barley residues, regardless of substrate C availability, compared to non-fumigated soil amended with young and mature barley residues. This finding is supported by the lower maximum respiration rates achieved 5-days later in the MS-Y and CP-Y treated soils compared to the CT-Y soil and the lower cumulative emissions from 0-16 days of incubation in the MS-Y and CP-Y treated soils compared to the CT-Y soil and in the MS-M and CP-M treated soils compared to the CT-M soil. Reduced plant residue decomposition in fumigated soils, regardless of C availability, is further supported by the lower percentages of apparent decomposition in MS and CP-treated soils compared to CT-treated soils from 0-16 and 0-128 days of incubation. These findings are similar to those observed in Chapter 2, where MS fumigation in combination with fresh young barley residue amendments exhibited a 13-day delay in maximum soil respiration rates and had a significantly greater percentage of added plant residue dry matter remaining at the end of the 160-day incubation compared to non-fumigated soil amended with labile barley plant residues.

This is the first study that has compared the impact of chemical fumigation with CP or MS in combination with soil organic C amendments on soil C dynamics. The reduced soil respiration in chemically fumigated soil indicates that chemical fumigation influences the decomposition and release of available C from labile and mature plant residue amendments. In addition to plant residue chemical composition, the decomposition rate and release of nutrients from organic soil amendments are also influenced by the nature and abundance of microbial decomposers (Cleveland et al., 2007; de Graff et al., 2010; Marschner et al., 2015). Therefore, the delayed

decomposition of labile and mature barley residues in chemically fumigated soil compared to non-fumigated soil may reflect a decrease of microbial biomass and the subsequent reduction in the metabolic turnover of SOC fractions.

Chemical fumigation with CP had a greater effect on plant residue decomposition than MS fumigation. This was evident based on the significantly lower cumulative respiration from 0-128 days of incubation in the CP-Y and CP-M treated soils compared to MS-Y, MS-M, CT-Y, and CT-M treated soils, and the lower percentage of apparent decomposition in the CP-treated soils compared to both the MS- and CT-treated soils. Interestingly, CP fumigation in unamended soil also yielded significantly lower cumulative respiration compared to all other treatments. Collectively, these findings indicate that reduced respiration in CP-treated soils amended with barley plant residues was likely a direct toxic effect of CP fumigation on the soil microbial community, which has previously been shown to decrease soil bacterial diversity, as observed in Chapter 3 and previous studies (Fang et al., 2018b; Li et al., 2017b), and microbial biomass (Fang et al., 2018b; Yan et al., 2015); thereby, likely reducing the metabolic capacity of the soil microbial community.

4.4.2 Effect of chemical fumigation and substrate C availability on soil inorganic N concentrations

Throughout the incubation, the low NH_4^+-N and NO_3^--N concentrations in the CT-U soil likely represent low net N mineralization and nitrification due to the absence of barley plant residue amendments. Conversely, the transient increase and decrease of NH_4^+-N concentration in the CT-Y soil likely reflect the rapid mineralization of labile N released from the young barley plant residues followed by the consumption of NH_4^+-

N through the process of nitrification, which is further supported by the significantly greater NO_3^--N concentration in the CT-Y soil compared to the CT-U-treated soil.

In contrast, the accumulation of NH_4^+-N concentrations over time in the MS-U, MS-Y, CP-U, and CP-Y treated soils indicates that chemical fumigation used alone or combined with young barley residue amendments inhibited nitrification until 8, 16, 16, and 30 days of incubation, respectively. However, the eventual consumption of NH_4^+-N in MS-U, MS-Y, CP-U, and CP-Y treated soils indicates that nitrification inhibition was temporary, and thereby, led to similar NO_3^--N concentrations when averaged over time between fumigated and non-fumigated soils except for the CP-Y soil, which had a significantly lower NO_3^--N concentrations than the CT-Y and MS-Y treated soils, likely reflecting the inhibition of nitrification sustained over a long duration of time in this treatment. These findings are similar to those observed in previous studies (Li et al., 2017a; Yan et al., 2013a, b; Yan et al., 2015) and in Chapter 2, where MS and CP fumigation inhibited nitrification for 16 and 64 days, respectively.

Conversely, the significantly lower NH_4^+-N and NO_3^--N concentrations in the CT-M, MS-M, and CP-M treated soils compared to all other treatments suggests that the addition of mature barley plant residues promoted net N immobilization, regardless of chemical fumigation. These findings likely reflect the low amount of available N in the mature barley plant residues, which is supported by the higher tissue C/N ratio of the mature barley plants compared to the young barley plants. Soil net N immobilization is characteristic of soils when an organic substrate undergoes decomposition, and the quantity of N within the substrate is not sufficient to sustain microbial growth (Jackson et al., 2009; Roberston & Groffman, 2007). Therefore, the

reduced inorganic N concentrations in soils amended with mature barley plant residues in the current study were most likely due to low levels of microbial-available N followed by the assimilation of alternative mineral N sources, rather than an effect of chemical fumigation.

4.4.3 Effect of chemical fumigation and substrate C availability on soil N_2O emissions

The significant positive correlations between soil N_2O emission rate and respiration rate and NO_3^--N concentration suggest that heterotrophic denitrification was the dominant N_2O production pathway in this study. These findings are consistent with labile C additions that increased N_2O emissions either directly by providing available electron donors or indirectly by reducing available oxygen (O_2) concentrations, and that labile N additions that increased N_2O emissions directly by providing available electron acceptors (Miller et al., 2008).

Given the soil system used in the study was under good soil aeration (based on the maintained WFPS of 55%), an increase in denitrification would likely be expected to occur as a result of increased C availability, which would increase microbial activity, and thereby, decrease O_2 availability and create anoxic soil microsites (Chen et al., 2013; Miller et al., 2008; Scherbak et al., 2014). Therefore, the similar N_2O emission rates between CT-U, MS-U, CP-U, CT-M, MS-M, and CP-M treated soils were consistent with the absence of readily available organic C. The significant nitrification inhibition in MS-U and CP-U-treated soils may have also decreased denitrification by reducing the pool of available NO_3^--N; however, N_2O emissions in CT-U-treated soil were low; therefore, a decrease in denitrification would be difficult to detect.

Conversely, the increased N_2O emissions in the CT-Y, MS-Y, and CP-Y treated soils were consistent with labile C additions, where the addition of young barley plant residues likely increased N_2O production via denitrification through increased available electron (C) donors and O_2 consumption (Chen et al., 2013; Miller et al., 2008; Scherbak et al., 2014). This is further supported by the increased respiration in the CT-Y, MS-Y, and CP-Y treated soils compared to the CT-U, MS-U, and CP-U treated soils.

Unlike the findings observed in Chapter 2, where MS-fumigated soils amended with fresh young barley residues decreased N_2O emissions compared to non-fumigated soil amended with fresh barley residues, both the MS-Y and CP-Y treated soils had greater N_2O emissions than the CT-Y soil. This was unanticipated, as the MS-Y and CP-Y-treated soils significantly decreased soil respiration rates and inhibited nitrification compared to CT-Y-treated soil; consequently, it would be expected that MS-Y and CP-Y-treated soils would also exhibit lower N_2O emissions.

Nonetheless, the MS-Y and CP-Y treated soils greatly affected the flux of N_2O compared to the CT-Y soil, which was evident based on the 5-day delay in maximum N_2O emission rates achieved in MS-Y and CP-Y treated soils compared to the CT-Y soil. These findings are consistent with the reduced decomposition of the added young barley plant residues in the MS and CP-fumigated soils, consequently resulting in low levels of readily available C to serve as electron donors in the denitrification pathway. This is supported by the lower maximum respiration rates achieved 5-days later in the MS-Y and CP-Y treated soils compared to the CT-Y soil, the significantly lower cumulative respiration between 0-16 days in the MS-Y and CP-Y treated soils

compared to the CT-Y soil, and the lower percentages of apparent decomposition in MS and CP-treated soils compared to CT-treated soils. Therefore, this study provides evidence that chemical fumigation delayed the flux of N_2O due to a reduction in plant residue decomposition.

4.5 Conclusions

This study demonstrates that chemical fumigation significantly affects soil C and N dynamics in soils amended with labile and recalcitrant C substrates. In particular, soil C dynamics were greatly influenced by chemical fumigation, predominantly CP fumigation, which significantly reduced respiration compared to non-fumigated soil amended with barley plant resides, regardless of substrate C availability. This study confirms the findings observed in Chapter 2 and shows that chemical fumigation reduced the decomposition of the added barley plant residues. Reduced decomposition in chemically fumigated soil during the fall season may have implications for soil C and N cycling in agricultural production systems, possibly reducing CO_2 emissions and increasing SOC storage and N concentrations. However, since most soil microorganisms have the metabolic capacity to utilize C substrates, reduced plant residue decomposition may indicate reduced soil microbial activity and significant changes to the functional capacity and diversity of the soil bacterial and fungal community.

CHAPTER 5: EFFECT OF CHEMICAL FUMIGATION AND SUBSTRATE C AVAILIBILITY ON SOIL BACTERIAL AND FUNGAL DIVERSITY

5.1 Introduction

Chemical fumigation is an agronomic practice used to mitigate soil-borne diseases and pathogens in high-value crop systems, including potato (*Solanum tuberosum* L.) production systems. The two most commonly used chemical fumigants in Canadian potato crop production systems are chloropicrin and metam sodium. Although chemical fumigation effectively reduces soil-borne diseases (Collins et al., 2006; De Cal et al., 2005; Wang et al., 2014b), fumigants are non-specific and have been shown to impact non-targeted microorganisms, consequently altering the soil bacterial community diversity, decreasing bacterial species richness, and disturbing essential soil processes mediated by soil microorganisms (Fang et al., 2018ab; Li et al., 2017ab; Sederholm et al., 2018).

In Chapters 2 and 4, there was evidence chemical fumigation significantly affected soil N and C dynamics. For example, chemical fumigation with chloropicrin or metam sodium, used alone or combined with labile barley plant residues, significantly inhibited nitrification and significantly reduced soil respiration compared to non-fumigated soil. The soil N and C cycles are controlled by a diverse network of soil microorganisms (Spohn et al., 2016; Trivedi et al., 2016; Xiao et al., 2018). Therefore, these findings indicate that chemical fumigation may be altering both the bacterial and fungal community diversity and composition.

Previous studies have investigated the effects of chemical fumigation with chloropicrin or metam sodium on soil bacterial and fungal diversity (Fang et al., 2020;

Li et al., 2017ab; Sederholm et al., 2018; Zhu et al., 2021). In Chapter 3, there was evidence that chemical fumigation with metam sodium combined with labile barley residues had a greater effect on soil bacterial diversity than metam sodium used alone, transiently decreasing bacterial species evenness. This was the first study to investigate the effects of chemical fumigation combined with plant residue amendments on the soil bacterial diversity. However, both bacteria and fungi play an important role in the decomposition of labile and recalcitrant organic matter. Currently, there are no studies that have investigated the effects of chemical fumigation combined with soil amendments of contrasting C availability on the soil bacterial and fungal diversity. Further investigation is therefore needed regarding the effects of chemical fumigation on the soil microbial community.

The objective of this study was to investigate the effects of chemical fumigation with either chloropicrin or metam sodium compared to non-fumigated soil amended with substrates of contrasting C availability (young vs. mature barley plant residues) on the soil bacterial and fungal community diversity using amplicon-based next-generation sequencing.

It was hypothesized that: 1) across residue amendment treatments, chemical fumigation would change the soil bacterial and fungal community diversity and result in lower species richness and evenness immediately after fumigation due to microbial death when compared with non-fumigated soil; and 2) the addition of organic amendments to chemically fumigated soil would change the soil bacterial and fungal community diversity compared to chemical fumigation used alone due to the

proliferation of microorganisms that survived fumigation and are capable of utilizing labile or recalcitrant C substrates.

5.2 Material and Methods

5.2.1 Experimental design

This study used samples collected from a subset of sampling dates in Study 3 previously reported in Chapter 4. The microcosm experiment consisted of a 3 x 3 x 6 factorial arrangement in a randomized complete block design (RCBD) with five replicates blocked in time to manage a large number of microcosms. Factors included three fumigation treatments [chloropicrin (CP), metam sodium (MS), or an untreated control (CT)], three soil amendment treatments [young barley tissue (Y), mature barley tissue (M), or unamended soil (U)], and six destructive incubation lengths (3, 8, 16, 30, 72, and 128 days of incubation). In total, there were nine treatment combinations: CP-U, CP-Y, CP-M, MS-U, MS-Y, MS-M, CT-U, CT-Y, and CT-M.

5.2.2 Microcosm preparation

Microcosm establishment, including soil sampling, soil processing, plant material growth and processing, plant material amendment rates, and chemical fumigation were performed as previously described in detail in Chapter 4. Plant residue properties are listed in Table 4.1 of Chapter 4.

5.2.4 DNA extraction

At each sampling time point, soil sub-samples (~10 g) were collected and stored at -80°C until processed. Genomic DNA was extracted from 0.25 g of freeze-dried soil using the MagAttract PowerSoil DNA isolation kit (Qiagen, Germantown, MD) and Thermo Scientific KingFisher Flex magnetic particle processor according to

manufacturer protocols. According to manufacturer protocol, the extracted DNA was quantified with the PicoGreen Reagent using Invitrogen Quant-iT PicoGreen dsDNA assay kit.

5.2.5 Bacterial 16S rRNA gene PCR amplification, library preparation, and sequencing

The V4 region of the bacterial 16S rRNA gene was amplified using the forward (515F: GTGCCAGCMGCCGCGGTAA) and reverse (806R: GGACTACHVGGGTWTC) dual-indexed primers that were fused to Illumina sequence adapters (Caporaso et al., 2011; Kozich et al., 2013). Library preparation and sequencing were performed following the Schloss MiSeq Wet Lab Standard Operating Procedure (SOP) (Kozich et al., 2013), as described in detail in the Materials and Methods section of Chapter 3. The library was sequenced using the MiSeq v2 Reagent kit (500 cycles) (Illumina, San Diego, CA, U.S.A.) and the Illumina MiSeq platform. Paired-end reads of 250 bp were generated, and demultiplexing was done automatically on the MiSeq to generate read 1 and read 2 for all indexed samples. Inherent sequencing errors during DNA sequencing and data processing were evaluated using a bacterial mock community consisting of 20 bacterial species (catalog# HM783D, BEI Resources, Manassas, VA, USA).

5.2.6 Fungal ITS2 sequencing

Extracted DNA samples were submitted to Dalhousie University's Integrated Microbiome Resource (IMR) (Dalhousie University, Halifax, NS Canada) for sequencing of the fungal-specific internal transcribed spacer (ITS2) region using the forward (ITS86F: GTGAATCATCGAATCTTTGAA) and reverse (ITS4R: TCCTCCGCTTATTGATATGC) dual-indexed primers that were fused to Illumina

sequence adapters (Op De Beeck et al., 2014). PCR amplification, library preparation, and sequencing are detailed by Comeau et al. (2017) and the IMR wet-lab protocol. The library was sequenced using the MiSeq v3 Reagent kit (600 cycles) (Illumina, San Diego, CA, U.S.A.) and the Illumina MiSeq platform. Demultiplexing was done automatically on the MiSeq to generate 300 bp paired-end reads for all indexed samples. Inherent sequencing errors during DNA sequencing and data processing were evaluated using a fungal mock community consisting of 10 fungal species (catalog# MSA-1010, ATCC Microbiome Standards, Manassas, VA, USA).

5.2.7 Illumina MiSeq bacterial and fungal sequence data processing

The demultiplexed paired-end fastq bacterial sequence data were analyzed using mothur (v.1.44.3) and the Schloss MiSeq SOP (Schloss, 2009; Kozich et al., 2013), as described in detail in Chapter 3. In total, 9,685,266 high-quality sequences were obtained from 270 soil samples. The rarefaction curves were close to reaching a plateau, indicating that most of the community diversity was sequenced (data not shown). The number of sequences was normalized across all samples to 11,962 reads using a random subsample method. There were 176,093 distinct ASVs among all soil samples.

The demultiplexed paired-end fastq fungal sequence data were analyzed using the PIPITS automated pipeline (v.2.7) following the Gweon PIPITS protocol (Gweon et al., 2015) (https://github.com/hsgweon/pipits). Briefly, the ITS2 sub-region was extracted, and the extracted ITS2 sequences were re-inflated to reflect their original abundances. The sequences were de-replicated, and short (< 100 bp) and unique sequences were removed before clustering them into operational taxonomic units

(OTUs) at a defined 97% similarity threshold using VSEARCH (Rognes et al., 2016). The resulting representative sequences for each cluster were subjected to chimera detection and removal using the UNITE UCHIME reference data set (Kõljalg et al., 2013). The input ITS sequences were then mapped onto the chimera-free representative sequences at the defined threshold, and the representatives were taxonomically assigned with the RDP Classifier (Wang et al., 2007) against the UNITE fungal ITS reference data set. The resulting sequences were then translated into an OTU and phylotype abundance table. In total, 10,158,523 high-quality sequences were obtained from 270 soil samples. The rarefaction curves were close to reaching a plateau (data not shown), indicating that most of the community diversity was sequenced. The number of sequences was normalized across all samples to 4,953 reads using a random subsample method. There were 4,255 distinct OTUs among all soil samples.

Soil bacterial and fungal diversity was assessed using Chao1 species richness and Pielou's species evenness (J) diversity indices, which were calculated using the vegan package in R Studio software (v.4.0.5) (Oksanen et al., 2020). The β-diversity of the bacterial and fungal community was also evaluated using the vegan package in R Studio. Data were square-root transformed, and Bray-Curtis dissimilarities were generated. Differences in bacterial and fungal composition among treatments between sampling dates were ordinated using nonmetric multidimensional scaling (nMDS) generated from the ggplot2 package in R Studio (Wickham, 2016).

The relative abundances of the 11 most abundant bacterial phyla and the six most abundant fungal phyla across all sampling dates were determined using MicrobiomeAnalyst (Chong et al., 2020; Dhariwal et al., 2017). The five bacterial and

fungal species that exhibited the greatest increase and decrease in relative abundance between a given treatment and the control after 3 days (immediately after fumigation) and 128 days (at the end of the incubation period) were determined by calculating the $\log_2$ fold-change in relative abundance for the corresponding ASV (bacteria)/OTU (fungi). Relative abundance was calculated as the number of counts for the corresponding ASV/OTU divided by the sum of counts for all ASVs/OTUs and then averaged over the five replicates in each treatment. To exclude noise related to sampling sequence depth and better illustrate differences in bacterial and fungal species abundance, the ASVs/OTUs that were evaluated had the following criteria: 1) an average relative abundance equal to or greater than 0.05% (bacteria) or 0.01% (fungi); and 2) a taxonomic affiliation identified at minimum at the phylum-level (i.e., ASVs/OTUs that were identified as unclassified bacteria/fungi were not considered). The taxonomic affiliation of each ASV/OTU was assigned to the genus level or the closest known taxonomic level.

The abundance of commonly occurring fungal pathogens in potato production systems was investigated using the FunGuild database (Nguyen et al., 2016), which assigns fungal taxa to trophic guilds. Fungal OTUs corresponding to *Verticillium spp*., *Fusarium spp*., *Rhizoctonia spp*., *Spongospora spp*., and *Phytophthora spp*. were investigated; however, only OTUs belonging to *Fusarium* and *Verticillium* pathogenic species were detected. The change in the relative abundance of pathogenic fungal species between a given treatment and the untreated control was determined by calculating the $\log_2$ fold-change in relative abundance for the corresponding OTU. Relative abundance was calculated as the number of counts for the corresponding OTU

divided by the sum of counts for all OTUs and then averaged over all sampling time points and replicates ($n = 30$).

5.2.8 Statistical analyses

RStudio base statistics software was used to conduct statistical analyses (v.4.0.5) (RStudio Team, 2020). Analysis of variance (ANOVA) was performed to analyze the diversity indices and the relative abundance of the most abundant bacterial and fungal phyla. ANOVAs were performed based on a 3 x 3 x 6 factorial arrangement in an RCBD with all fixed factors. Data were assessed for normality (i.e., normal and independent distribution) using homoscedasticity diagnostic plots and Shapiro-Wilks tests. Non-normal data were transformed using Box-Cox transformations with the optimal lambda (λ) value using the MASS package in RStudio (Venables, 2002). Treatment, time, and interaction means were tested using post-hoc Tukey Honest Significant Difference (HSD) tests. The treatment means presented in the figures and tables were calculated from untransformed data. Significant changes in the five greatest-fold increased and decreased ASVs/OTUs, and significant fold-changes in the relative abundance of pathogenic fungal OTUs in each treatment relative to the untreated control were determined using non-parametric Kruskal-Wallis tests. The β-diversity of the bacterial and fungal community was analyzed using a permutational multivariate analysis of variance (PERMANOVA, permutations = 9999) using the Adonis function from the vegan package. Multilevel pairwise comparisons between treatment, time, and interaction factors were performed using the pairwise Adonis wrapper function from vegan (Martinez Arbizu, 2020). Significance for all tests was

accepted at $P \leq 0.05$ except for the PERMANOVAs, whereby significance was accepted at $P \leq 0.10$.

5.3 Results

5.3.1 Soil bacterial and fungal diversity indices and community β-diversity

5.3.1.1 Bacterial diversity

There were significant fumigant x amendment x time interactions on Pielou's species evenness (J) and Chao1 species richness in the bacterial community (both $P \leq 0.05$) (Figure 5.1a, b). Throughout the incubation, the CT-U, CT-M, MS-U, and MS-M treated soils had similar species evenness compared to each other except for after 30 days, when MS-M had significantly lower species evenness than the CT-U and CT-M-treated soils (Figure 5.1a). After 3 days of incubation, the CT-Y treated soil was the only treatment with significantly lower species evenness than all other treatments (~1.0-fold decrease compared to CT-U). After 16 days, the species evenness in the CT-Y soil did not differ from the CT-U and CT-M treated soils for the remainder of the incubation. After 8 and 16 days, the MS-Y soil had significantly lower species evenness compared to the CT-U, CT-Y, MS-U, and MS-M treated soils (~1.0-fold decrease compared to CT-U), and after 72 days, the MS-Y soil had significantly lower species evenness than the MS-U and MS-M treated soils. After 128 days, the species evenness in MS-Y treated soil did not differ from CT-U, CT-Y, CT-M, MS-U, and MS-M treated soils. Throughout the incubation, the CP-U, CP-Y, and CP-M treated soils had similar species evenness compared to each other except for after 16 days, in which the CP-Y and CP-M soils had significantly lower species evenness compared to the CP-U soil, and after 72 days, in which the CP-Y soil had significantly lower species

evenness compared to the CP-U soil. From 8 to 128 days of incubation, CP-U treated soil had significantly lower species evenness than the CT-U and MS-U treated soils. Similarly, from 8 to 128 days, CP-Y and CP-M treated soils had significantly lower species evenness compared to the CT-U, CT-Y, CT-M, and MS-M treated soils, except for after 8 days, where the CP-M soil had similar evenness to the CT-Y and MS-M treated soils. After 128 days of incubation, the CP-U, CP-Y, and CP-M treated soils had significantly lower species evenness than all other treatments.

All of the treatments had similar species richness compared to each other throughout the incubation with some exceptions: 1) after 8 days, CP-Y soil had significantly lower species richness compared to CT-U soil; 2) from 30 to 72 days, CP-Y and CP-M treated soils had significantly lower species richness compared to CT-U, CT-M, and MS-U treated soils; and 3) after 128 days the CP-U, CP-Y, and CP-M treated soils had significantly lower species richness than the CT-U, CT-Y, CT-M, MS-U, and MS-M treated soils (Figure 5.1b).

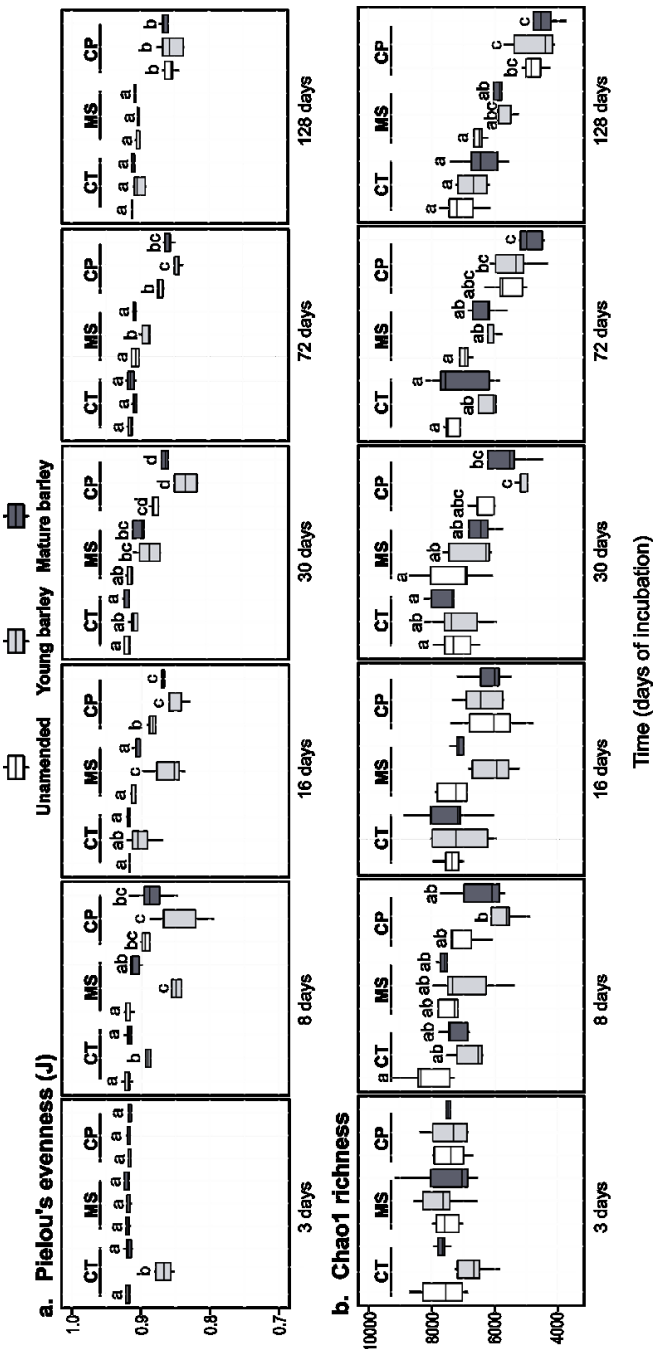

Figure 5. 1 Box and whisker plots showing the bacterial (a) Pielous evenness (J) and (b) Chao1 richness in each treatment and sampling time points. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U). Significant differences (HSD, $P \leq 0.05$) among treatments means ($n = 5$) are

represented by letters above the boxes. No letter indicates no significant difference between treatment means.

There was a significant fumigant x amendment x time interaction on the β-diversity of the bacterial community (PERMANOVA P = 0.09; Table A.7). After 3, 8, and 16 days, the bacterial β-diversity in the CT-Y treated soil was significantly dissimilar from all other treatments, except for the MS-U soil after 16 days (Figure 5.2a-c). Similarly, the bacterial β-diversity was similar between the CP-U, CP-Y, and CP-M treated soils but significantly dissimilar from all other treatments except for after 8 days, when the β-diversity was similar between CP-M and MS-M treated soils (Figure 5.2a-c). After 3, 8, and 16 days, the bacterial β-diversity was also similar between CT-U, CT-M, MS-U, MS-Y, and MS-M treated soils, except for after 8 days, in which the β-diversity in CT-M and MS-Y treated soils were significantly different from each other and all other treatments (Figure 5.2b), and after 16 days, in which the β-diversity in MS-Y and MS-M treated soils was significantly different from all other treatments except for MS-U soil (Figure 5.2c). After 30, 72, and 128 days of incubation, the bacterial β-diversity was significantly different among all treatments with a few exceptions, where the β-diversity was similar: 1) between CT-U and CT-M treated soils, CT-Y and CT-M treated soils, and MS-U and MS-M treated soils after 72 days incubation (Figure 5.2e); and 2) between CT-U and CT-M treated soils, MS-U and MS-Y treated soils, CP-Y and CP-M treated soils, and CP-Y and CP-M treated soils after 128 days of incubation (Figure 5.2f). Although most treatments established a significantly dissimilar bacterial community β-diversity compared to each other, the CP-U, CP-Y, and CP-M treated soils had the most dissimilar β-diversity compared to all other treatments between 16 and 128 days of incubation (Figure 5.2c-f).

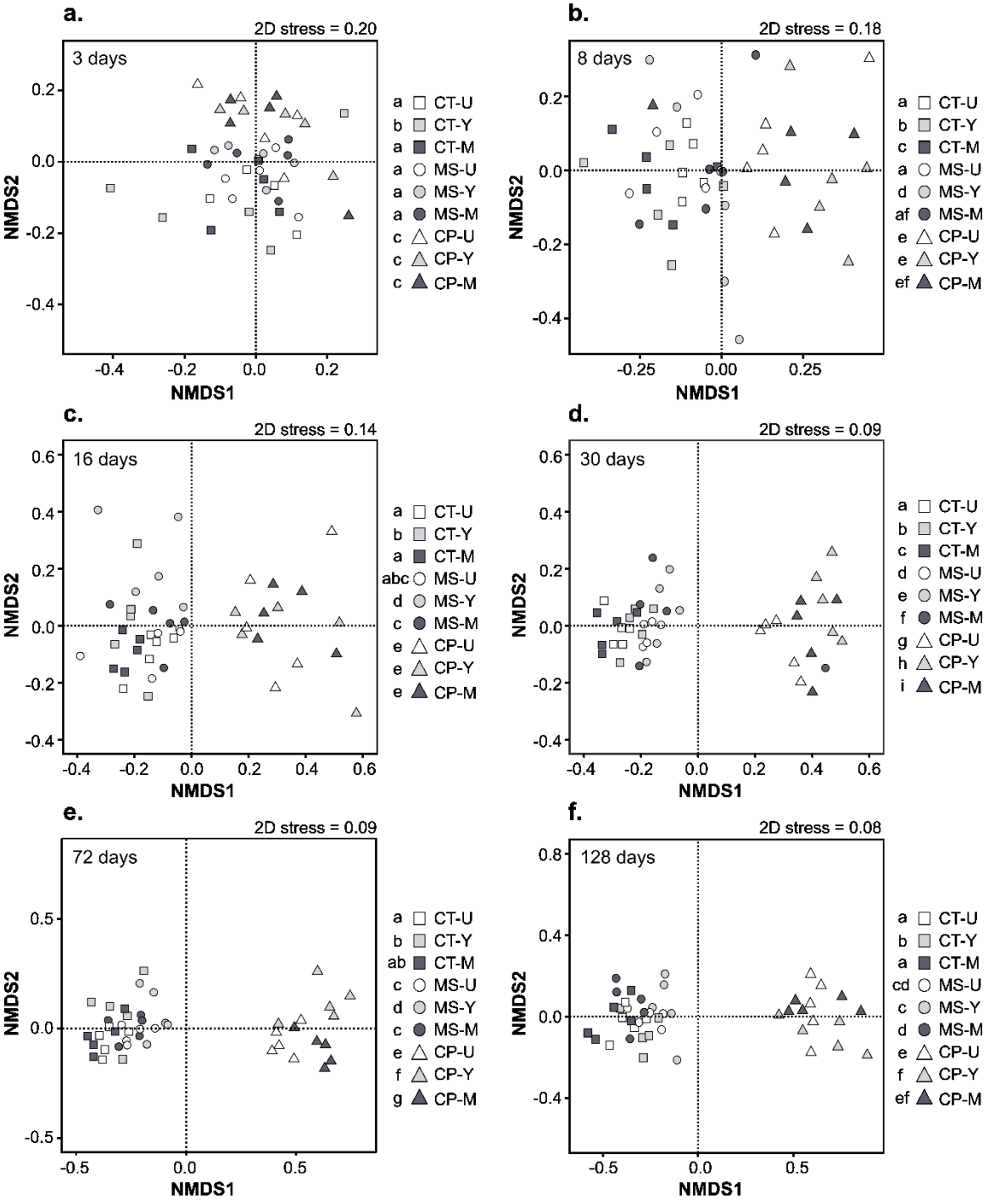

Figure 5. 2 Nonmetric multidimensional scaling ordination based on Bray-Curtis distances comparing the composition of the bacterial communities among treatments at each sampling time point. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-

M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U). Significant differences (PERMANOVA, permutations = 9999, $P \leq 0.10$) in β-diversity of the bacterial community among treatments at each sampling time point are represented by letters adjacent to treatments in legend.

5.3.1.2 Fungal diversity

There was a significant fumigant x amendment x time interaction on Pielou's species evenness (J) in the fungal community ($P < 0.05$) (Figure 5.3a). Throughout the entire incubation, the CT-U, CT-M, MS-U, MS-Y, MS-M, and CP-U treated soils had similar species evenness compared to each other. After 3 days, the CT-Y treated soil was the only treatment with significantly lower species evenness than all other treatments (0.5-fold decrease compared to CT-U soil). For the remainder of the incubation, CT-Y soil had significantly lower species evenness compared to the CT-U soil except for after 16 days. Throughout the entire incubation, the CP-M and CT-M treated soils and the CP-M and CP-Y treated soils had similar species evenness except for after 128 days of incubation, when the CP-M soil had significantly lower species evenness compared to the CT-M soil (~0.4-fold decrease). From 8 to 128 days of incubation, the CP-Y and CP-M treated soils had significantly lower species evenness compared to the CT-U, MS-U, MS-Y, MS-M, and CP-U treated soils with some exceptions: 1) after 16 days, CP-M, CP-Y, and MS-Y had similar species evenness; 2) after 30 days, CP-M, MS-M, and MS-Y had similar species evenness, and CP-Y and MS-M had similar species evenness; and 3) after 128 days, CP-U, CP-Y, and CP-M had similar species evenness.

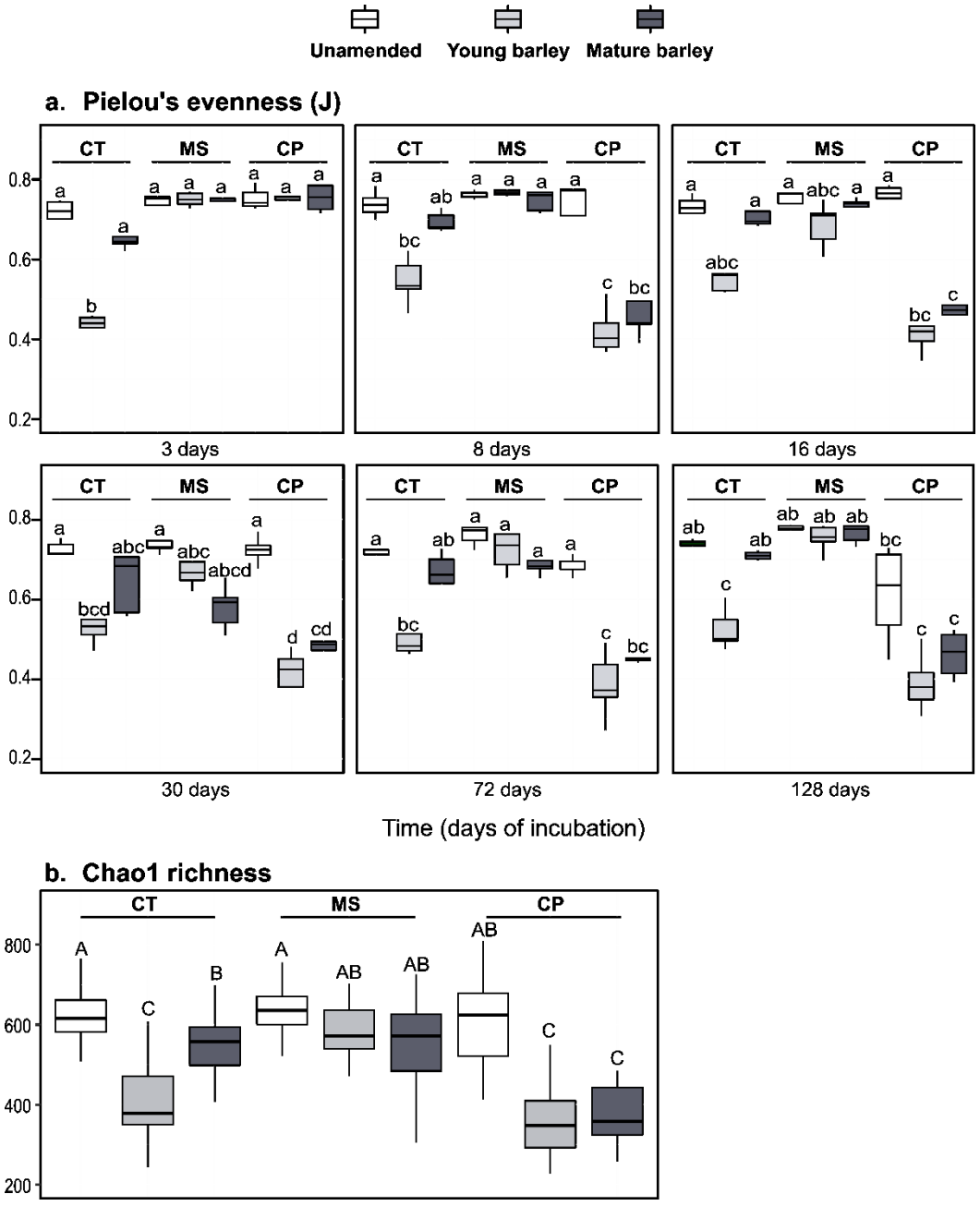

Figure 5. 3 Box and whisker plots showing the fungal (a) Pielou's evenness (J) and (b) Chao1 richness. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U).Significant differences (HSD, $P \leq 0.05$) among treatments means (evenness $n = 5$; richness $n = 30$) are represented by letters above the boxes. No letter indicates no significant difference between treatment means.

There was no significant fumigant x amendment x time interaction on Chao1 fungal species richness ($P = 0.24$); however, there was a significant fumigant x amendment interaction on fungal species richness ($P < 0.001$) (Figure 5.3b). The CT-U, MS-U, MS-Y, MS-M, and CP-U treated soils had similar species richness compared to each other. The CT-M soil had significantly lower species richness than the CT-U and MS-U treated soils (~0.2-fold decrease compared to CT-U). The CT-Y, CP-Y, and CP-M had similar species richness compared to each other, which was significantly lower than all other treatments.

There was a significant fumigant x amendment x time interaction in the β-diversity of the fungal community (PERMANOVA $P < 0.001$; Table A.8). After 3 days of incubation, the fungal β-diversity was similar between CT-U, MS-U, and MS-Y treated soils, between CP-U, CP-Y, and CP-M treated soils, between MS-Y, CP-U, and CP-M treated soils, and between MS-M and CP-M treated soils (Figure 5.4a). In contrast, the CT-Y and CT-M treated soils had a significantly different β-diversity compared to each other and all other treatments (Figure 5.4a). After 8 days of incubation, the fungal β-diversity was similar between MS-U, MS-Y, and MS-M treated soils, between CP-U, CP-Y, and CP-M treated soils, and between CP-U, MS-U, and MS-M treated soils (Figure 5.4b). Conversely, the CT-U, CT-Y, and CT-M treated soils had significantly different β-diversity compared to each other and all other treatments (Figure 5.4b). After 16 days of incubation, the β-diversity was similar between CT-U, CT-Y, and MS-U treated soils, between MS-Y and MS-M-treated soils, between CP-U and CP-Y treated soils, and between CP-Y and CP-M treated soils (Figure 5.4c). In contrast, the fungal β-diversity in CT-M treated soil differed

significantly from all other treatments (Figure 5.4c). From 30 to 128 days of incubation, all of the treatments had a significantly different fungal β-diversity compared to each other with a few exceptions, where the β-diversity was similar: 1) between CT-Y and CT-M treated soils after 72 days; and 2) between MS-Y and MS-M treated soils after 128 days (Fig. d-f). The fungal β-diversity of the CT-Y and CT-M treated soils was the most dissimilar compared to all other treatments after 3 days of incubation (Figure 5.4a), whereas the β-diversity of the CP-U, CP-Y, and CP-M treated soils was the most dissimilar compared to all other treatments at the end of the incubation (Figure 5.4f).

5.3.2 Relative abundance of the most abundant bacterial and fungal phyla

5.3.2.1 The 11 most abundant bacterial phyla

The relative abundances of the 11 most abundant bacterial phyla are listed in Table 5.1. The CT-U, CT-M, MS-U, and MS-M treated soils had similar relative abundances of all phyla, except for MS-M soil, which had a significantly lower relative abundance of Actinobacteria compared to CT-M treated soil. The relative abundance of Proteobacteria was increased, whereas the relative abundances of Bacteroidetes and Chloroflexi were decreased, in the CT-Y soil compared to the CT-U soil. Only the relative abundance of Acidobacteria decreased in the MS-Y soil compared to the CT-U soil. The relative abundances of Firmicutes, Proteobacteria, and Verrucomicrobia were significantly increased in the CP-U soil, whereas the relative abundances of Actinobacteria, Armatimonadetes, and Bacteroidetes were significantly decreased in the CP-U soil, compared to the CT-U soil.

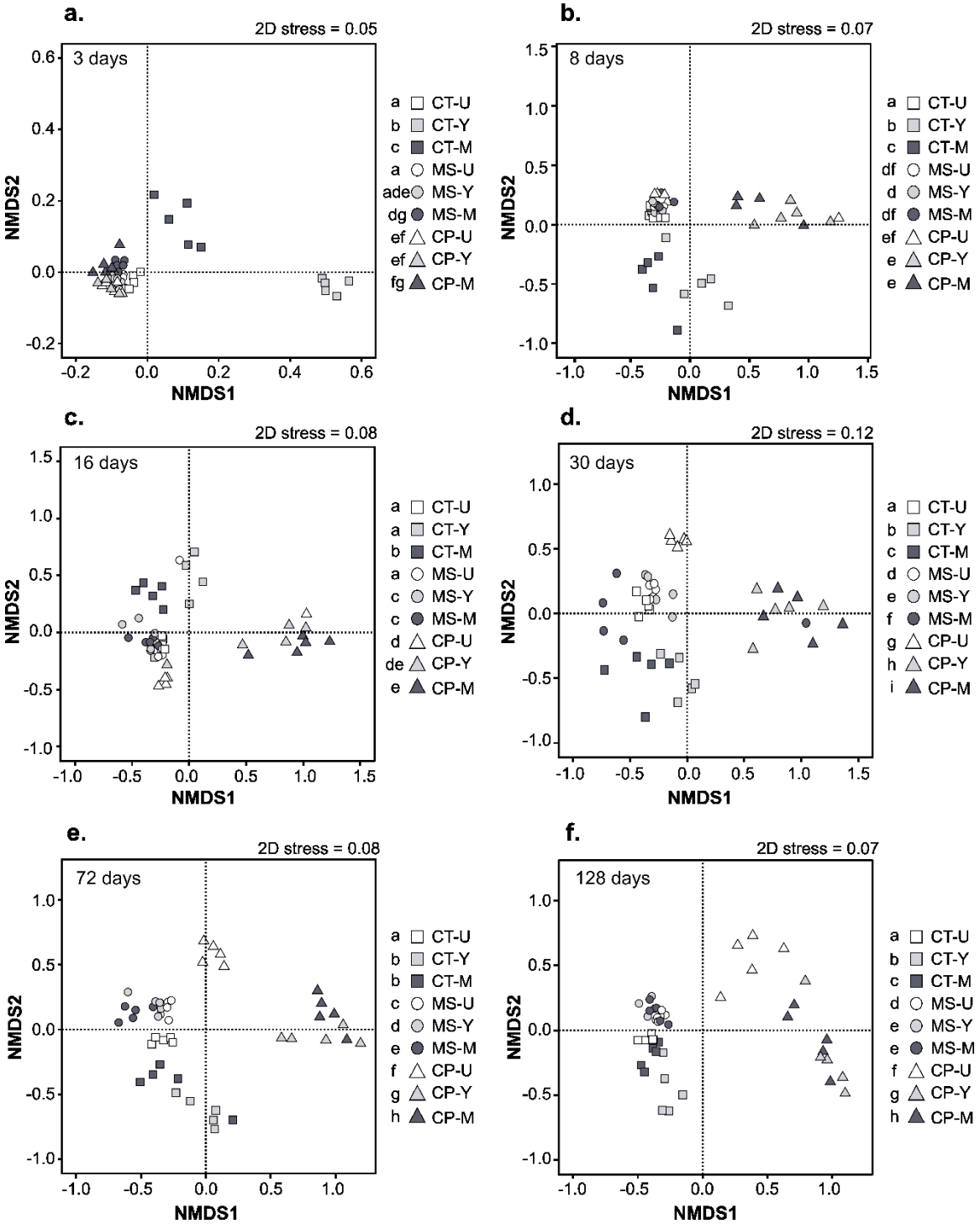

Figure 5. 4 Nonmetric multidimensional scaling ordination based on Bray-Curtis distances comparing the composition of the fungal communities among treatments at each sampling time point. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-

fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U).Significant differences (PERMANOVA, permutations = 9999, $P \leq 0.10$) in β-diversity of the bacterial community among treatments at each sampling time point are represented by letters adjacent to treatments in legend

The relative abundances of Firmicutes, Proteobacteria, and Verrucomicrobia significantly increased in the CP-Y soil, whereas the relative abundances of Actinobacteria and Bacteroidetes significantly decreased in the CP-Y soil compared to the CT-U soil. The relative abundances of Chloroflexi, Firmicutes, and Proteobacteria were significantly increased in the CP-M soil, whereas the relative abundances of Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, and Candidate division WPS-1 were significantly decreased in the CP-M soil compared to the CT-U soil.

5.3.2.2 The six most abundant fungal phyla

The relative abundances of the six most abundant fungal phyla are listed in Table 5.2. The relative abundance of Mortierellomycota significantly increased in CT-Y soil, whereas the relative abundance of all other phyla except for Mucoromycota significantly decreased, compared to the CT-U soil. Only the relative abundance of Ascomycota significantly increased in the CT-M soil compared to the CT-U soil. There were no other significant differences in fungal phyla relative abundance between the CT-M and CT-U soil. The relative abundances of Ascomycota and Mortierellomycota significantly increased and decreased, respectively, in the MS-U, MS-Y, and MS-M treated soils compared to the CT-U soil. Among the MS-treated soils, the relative abundance of Ascomycota and Mortierellomycota was significantly lower and greater, respectively, in the MS-U soil compared to the MS-Y and MS-M treated soils. The

relative abundances of Aphelidiomycota, Ascomycota, and Mucoromycota were significantly increased in the CP-U soil, whereas the relative abundances of Basidiomycota and Mortierellomycota were decreased in the CP-U soil compared to the CT-U soil. The relative abundance of Ascomycota significantly increased in the CP-Y and CP-M treated soils, whereas the relative abundances of Basidiomycota, Chytridiomycota, and Mortierellomycota were significantly decreased in the CP-Y and CP-M treated soils compared to the CT-U soil. In the CP-M soil, the relative abundance of Ascomycota and Mortierellomycota were significantly greater and lower, respectively, compared to the CP-Y soil.

5.3.3 The bacterial ASVs and fungal OTUs with the greatest-fold increase and decrease in relative abundance

Log_2 fold-changes in the relative abundance of bacterial and fungal species between a given treatment and the CT-U soil after 3 and 128 days of incubation are presented in Figure 5.5 and Figure 5.6, respectively. After 3 days of incubation, there were distinct fold-changes in relative abundances (Figure 5.5). For example, the relative abundance of four fungal OTUs belonging to the phylum Mortierellomycota (OTU0975, OTU0815, OTU1082, and OTU0915) increased in the CT-Y soil compared to the CT-U soil. In addition, the relative abundance of four bacterial ASVs belonging to the phylum Proteobacteria (ASV019962, ASV316310, ASV266373, and OTU158973) increased, while five fungal OTUs belonging to the phylum Ascomycota (OTU2927, OTU2088, OTU2746, OTU1996, and OTU3145) decreased, in the CP-Y soil compared to the CT-U soil (Figure 5.5).

Table 5. 1 The relative abundance of the 11 most abundant bacterial phyla averaged across all sampling time points. Values are means (n = 30) and standard errors (± SE). Significant differences in relative abundance (HSD, $P \leq 0.05$) among treatment means are represented by letters adjacent to mean values.

Phyla	CI-U	CI-Y	CI-M	MS-U	MS-Y	MS-M	CP-U	CP-Y	CP-M
Acidobacteria	24.3 ± 0.74 bc	24.2 ± 0.79 bc	24.1 ± 0.82 bc	24.4 ± 0.77 bc	26.0 ± 0.81 a	24.8 ± 0.76 ab	23.3 ± 0.67 cd	23.9 ± 0.79 bc	22.8 ± 0.79 d
Actinobacteria	13.0 ± 0.39 ab	13.3 ± 0.36 a	13.2 ± 0.34 a	12.8 ± 0.32 ab	12.4 ± 0.43 bc	12.4 ± 0.37 b	11.6 ± 0.40 cd	10.5 ± 0.47 e	11.3 ± 0.44 d
Armatimonadetes	1.13 ± 0.05 ab	1.13 ± 0.05 ab	1.18 ± 0.04 a	1.15 ± 0.05 ab	1.06 ± 0.06 ab	1.04 ± 0.04 ab	0.79 ± 0.05 d	1.01 ± 0.05 bc	0.86 ± 0.05 cd
Bacteroidetes	7.78 ± 0.22 a	6.50 ± 0.27 b	7.34 ± 0.24 a	7.57 ± 0.24 a	7.35 ± 0.30 a	7.61 ± 0.26 a	6.54 ± 0.28 b	5.75 ± 0.30 c	6.51 ± 0.27 b
Can. div. WPS-1	1.75 ± 0.05 abc	1.55 ± 0.06 cd	1.60 ± 0.05 abcd	1.83 ± 0.06 a	1.82 ± 0.10 ab	1.80 ± 0.06 a	1.61 ± 0.10 abcd	1.61 ± 0.06 bcd	1.47 ± 0.06 d
Chloroflexi	1.98 ± 0.07 b	1.67 ± 0.08 c	1.97 ± 0.08 b	1.83 ± 0.08 b	2.08 ± 0.10 b	2.04 ± 0.07 b	2.17 ± 0.07 ab	2.10 ± 0.07 b	2.45 ± 0.11 a
Firmicutes	11.6 ± 0.39 cd	11.0 ± 0.41 d	11.8 ± 0.35 c	11.8 ± 0.08 c	11.6 ± 0.10 cd	12.2 ± 0.07 c	13.6 ± 0.56 b	15.0 ± 0.64 a	14.8 ± 0.56 a
Gemmatimonadetes	3.36 ± 0.11 ab	3.60 ± 0.18 ab	3.62 ± 0.11 a	3.51 ± 0.14 ab	3.04 ± 0.15 b	3.70 ± 0.19 a	3.73 ± 0.17 a	3.53 ± 0.21 ab	3.55 ± 0.25 ab
Planctomycetes	7.00 ± 0.47 a	7.25 ± 0.55 a	6.80 ± 0.45 a	6.92 ± 0.48 a	5.94 ± 0.42 b	6.63 ± 0.44 a	7.31 ± 0.63 a	7.24 ± 0.58 a	7.05 ± 0.60 ab
Proteobacteria	28.0 ± 1.39 d	29.8 ± 1.41 a	28.3 ± 1.45 cd	28.0 ± 1.38 d	28.7 ± 1.53 bcd	27.6 ± 1.35 d	29.3 ± 1.25 abc	29.4 ± 1.29 ab	29.2 ± 1.38 abc
Verrucomicrobia	10.3 ± 2.71 cd	10.4 ± 2.80 cd	10.5 ± 2.79 bc	10.6 ± 2.89 cd	10.5 ± 2.99 d	10.4 ± 2.82 cd	11.7 ± 3.23 ab	12.3 ± 3.39 a	10.9 ± 2.99 bc

Table 5. 2 The relative abundance of the six most abundant fungal phyla averaged across all sampling time points. Values are means (n = 30) and standard errors (± SE). Significant differences in relative abundance (HSD, $P \leq 0.05$) among treatment means are represented by letters adjacent to mean values.

Phyla	CT-U	CT-Y	CT-M	MS-U	MS-Y	MS-M	CP-U	CP-Y	CP-M
Aphelidiomycota	0.10 ± 0.01 **bc**	0.02 ± 0.01 **d**	0.04 ± 0.01 **cd**	0.12 ± 0.01 **b**	0.13 ± 0.02 **b**	0.11 ± 0.02 **bc**	0.31 ± 0.06 **a**	0.04 ± 0.01 **cd**	0.06 ± 0.01 **bcd**
Ascomycota	54.6 ± 1.25 **f**	33.2 ± 1.87 **g**	64.6 ± 2.08 **e**	65.6 ± 1.95 **e**	78.0 ± 2.32 **bc**	74.3 ± 2.53 **cd**	70.9 ± 2.10 **de**	81.6 ± 2.70 **b**	88.8 ± 2.70 **a**
Basidiomycota	17.1 ± 1.13 **a**	4.85 ± 0.68 **c**	13.3 ± 1.62 **ab**	16.2 ± 1.16 **a**	12.8 ± 1.20 **ab**	13.5 ± 1.45 **ab**	10.0 ± 0.99 **b**	4.16 ± 1.41 **c**	4.34 ± 1.31 **c**
Chytridiomycota	1.89 ± 0.16 **ab**	0.29 ± 0.07 **d**	1.14 ± 0.36 **bc**	2.45 ± 0.20 **a**	1.50 ± 0.15 **b**	1.54 ± 0.23 **b**	1.77 ± 0.20 **ab**	0.66 ± 0.21 **cd**	0.66 ± 0.18 **cd**
Mortierellomycota	26.0 ± 1.34 **b**	61.6 ± 2.41 **a**	20.7 ± 2.17 **bc**	15.5 ± 2.19 **cd**	7.51 ± 1.40 **cf**	10.4 ± 1.52 **def**	16.3 ± 2.02 **cd**	13.3 ± 1.94 **de**	5.98 ± 1.45 **f**
Mucoromycota	0.04 ± 0.01 **b**	0.03 ± 0.01 **b**	0.02 ± 0.01 **b**	0.02 ± 0.01 **b**	0.02 ± 0.01 **b**	0.02 ± 0.01 **b**	0.49 ± 0.2 **a**	0.12 ± 0.02 **b**	0.18 ± 0.03 **b**

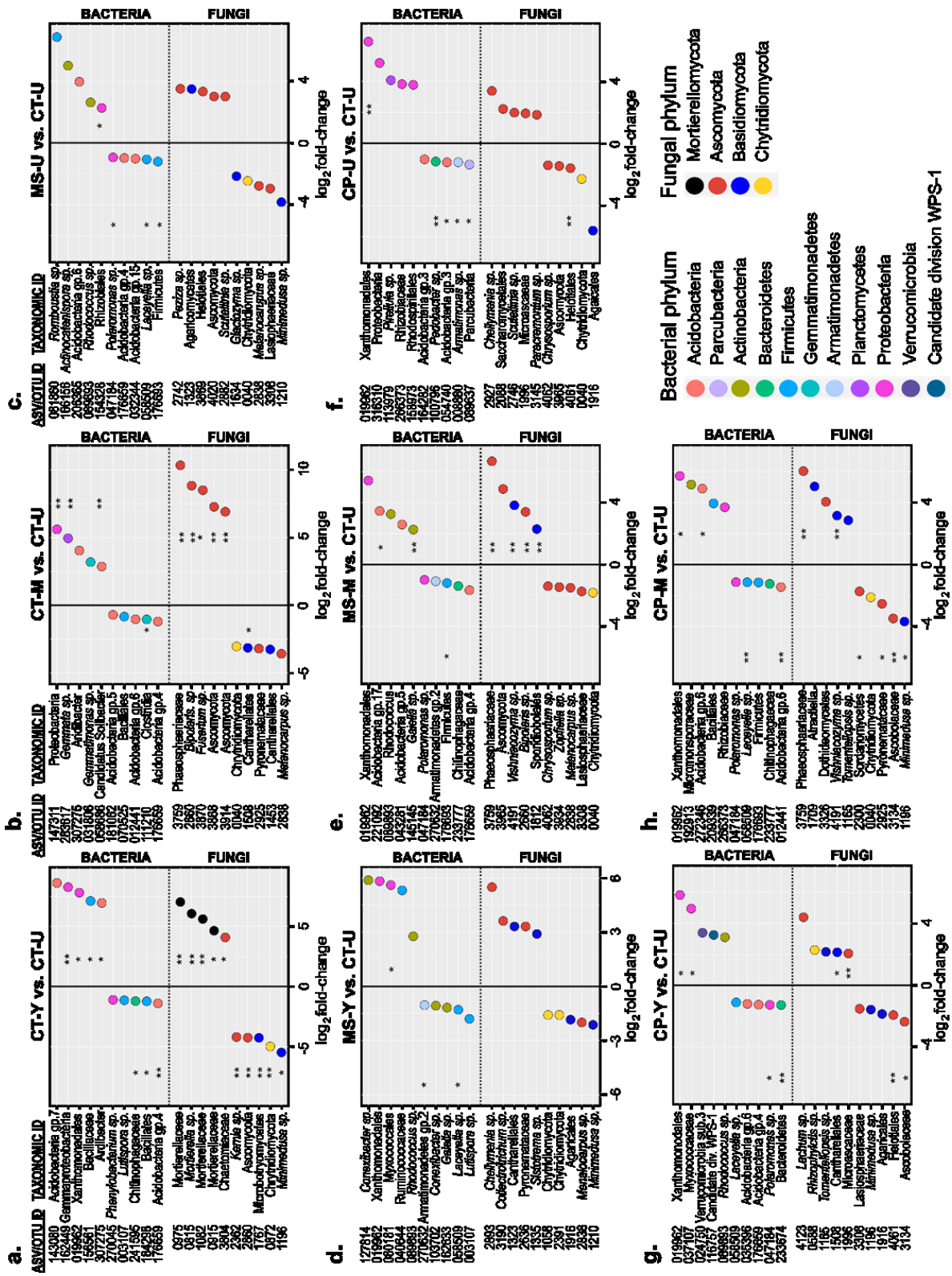

Figure 5. 5 The 5 greatest increased and decreased bacterial species (amplicon sequence variants, ASVs) and fungal species (operational taxonomic units, OTUs) after 3 days of incubation as determined by calculating the $\log_2$ fold-change in average

ASV/OTU relative abundance ($n = 5$) in each treatment relative to the untreated control. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U).

Several treatments exhibited similar shifts in the relative abundance of particular bacterial ASVs and fungal OTUs after 3 days of incubation (Figure 5.5). For example, the bacterial ASV belonging to the order Xanthomonadales (ASV019962) increased in all treatments compared to the CT-U soil except for the MS-U vs. CT-U soil, while two fungal OTUs identified as *Minimeduas spp*. (OTU1210 and OTU1196) decreased in all treatments compared to the CT-U soil except for CT-M, MS-M, and CP-U vs. CT-U. The bacterial ASV identified as *Polaramonas spp*. (ASV047184) decreased in MS-M and CP-M treated soils compared to the CT-U soil, whereas the fungal OTUs identified as *Vishniacozyma spp*. (OTU4191) and the family Phaeosphaeriaceae (OTU3759) increased in the MS-M and CP-M treated soils compared to the CT-U soil (Figure 5.5).

After 128 days of incubation, there were distinct fold-changes in relative abundances (Figure 5.6). For example, the relative abundance of four fungal OTUs belonging to the phylum Mortierellomycota (OTU0975, OTU0815, OTU1082, and OTU0737) and Ascomycota (OTU3323, OTU2707, OTU3114, and OTU2893) increased and decreased, respectively, in the CT-Y soil compared to the CT-U soil. Moreover, in the CT-Y soil, two bacterial ASVs belonging to the order Myxococcales (ASV127857 and ASV111352) exhibited the greatest increase in relative abundance compared to CT-U soil. The relative abundance of four fungal OTUs belonging to the

phylum Ascomycota (OTU2660, OTU3590, OTU3804, and OTU2925) increased in the CT-M soil compared to the CT-U soil. The relative abundance of four ASVs belonging to the phylum Acidobacteria (ASV025040, ASV058686, ASV162005, and ASV135514) increased in the MS-Y soil compared to the CT-U soil. Several fungal OTUs belonging to the phylum Ascomycota increased in the MS-U (OTU4122, OTU3981, OTU4062, OTU2479, and OTU3934), MS-Y (OTU3805, OTU2965, OTU4062, OTU3953, and OTU3804), and MS-M (OTU3346, OTU3953, OTU3308, and OTU3804) treated soils. Similarly, several fungal OTUs belonging to the phylum Ascomycota increased in CP-U (OTU2666 and OTU2692), CP-Y (OTU2666, OTU3805, OTU3665, and OTU3431), and CP-M (OTU2805, OTU3178, OTU2692, OTU2666, and OTU3665) treated soils compared to the CT-U soil. In contrast, several OTUs belonging to the phylum Mortierellomycota decreased in the MS-U (OTU1025, OTU0798, OTU0880, and OTU0845), MS-Y (OTU0880 and OTU1025), and MS-M (OTU0880) treated soils compared to the CT-U soil (Figure 5.6).

Several treatments exhibited similar shifts in the relative abundance of particular bacterial ASVs and fungal OTUs after 128 days (Figure 5.6). For example, the bacterial ASVs identified as *Hydrogenispora spp*. (ASV116295) and *Rombousita spp.* (ASV081860) decreased in the CT-Y and CT-M soils, whereas the fungal OTU identified as *Minimedusa spp.* (OTU1210) decreased in CT-Y and CT-M soils compared to the CT-U soil. The bacterial ASV belonging to the order Rhizobiales (ASV105407) and the fungal OTU identified as *Trichoderma spp*. (OTU2666) increased in the CP-U, CP-Y, and CP-M treated soils compared to the CT-U soil. The bacterial ASV belonging to the family Streptosporangiaceae (ASV131513) and fungal

OTUs identified as *Mrakia spp*. (OTU1191 or OTU1237) decreased in MS-U, MS-Y, and MS-M treated soils compared to the CT-U soil (Figure 5.6).

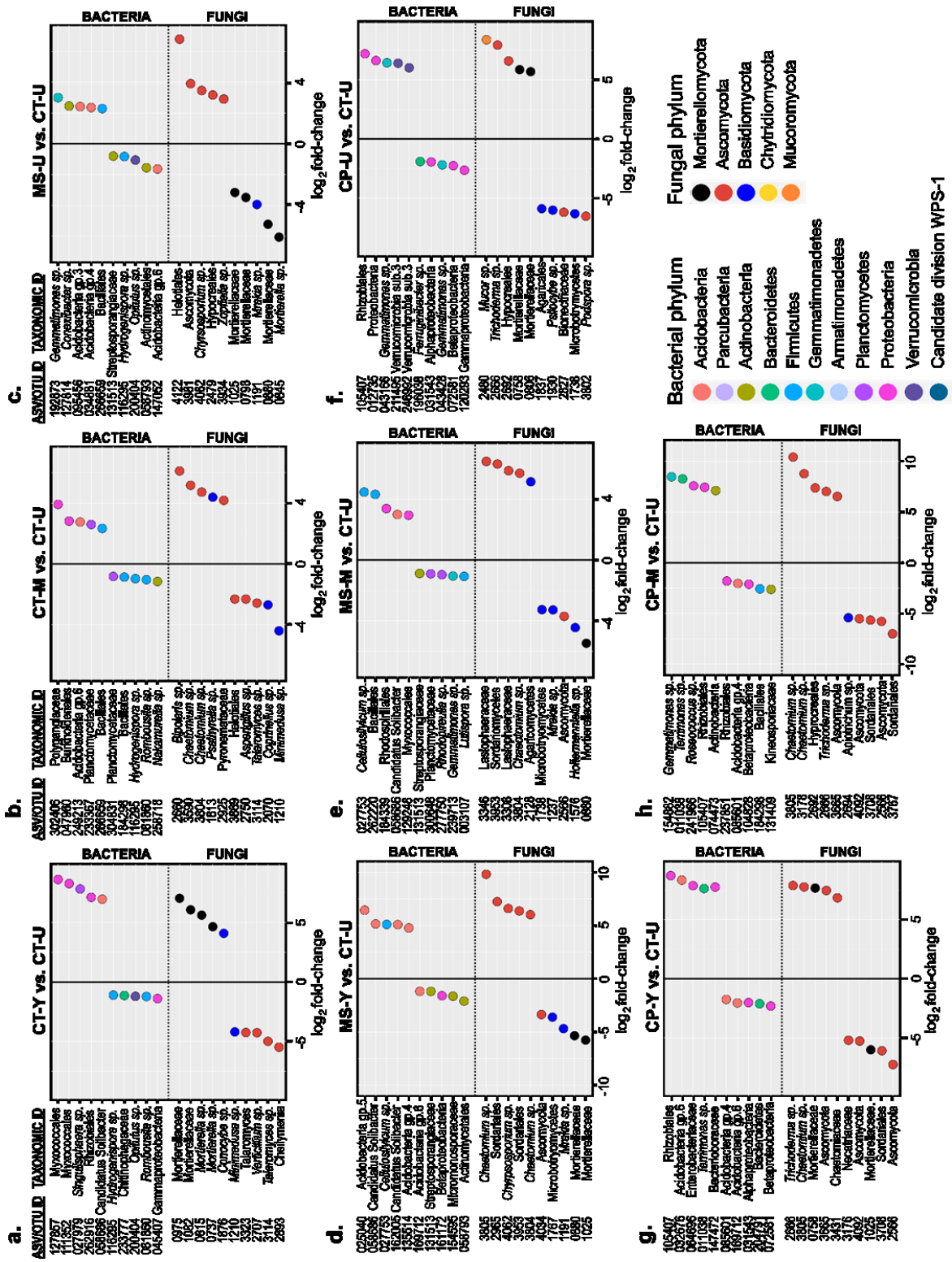

Figure 5. 6 The 5 greatest increased and decreased bacterial species (amplicon sequence variants, ASVs) and fungal species (operational taxonomic units, OTUs) after 128 days of incubation as determined by calculating the $\log_2$ fold-change in average ASV/OTU relative abundance ($n = 5$) in each treatment relative to the untreated control. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U).

5.3.4 The $\log_2$ fold-change in relative abundance of pathogenic *Verticillium spp.* and *Fusarium spp.*

There were three OTUs identified as pathogenic *Verticillium spp*. (OTU2707, OTU2886, and OTU2748) and four OTUs that were identified as pathogenic *Fusarium spp*. (OTU2627, OTU2609, OTU2631, and OTU3871) (Figure 5.7). The relative abundances of *Verticillium spp*. and *Fusarium spp*. decreased in all treatments relative to the CT-U soil except for certain *Fusarium spp*. that increased in the MS-U soil (OTU2609, OTU2631, and OTU2627), MS-Y soil (OTU2609), MS-M soil (OTU2627), and the CP-U soil (OTU2631). The CP-Y, CP-M, and CT-Y treated soils exhibited the greatest decreases in the relative abundance of both *Verticillium spp*. and *Fusarium spp*., where $\log_2$ fold-changes ranged between -1.7 to -4.8 in the CP-Y soil, between -1.6 to -2.7 in the CP-M soil, and between -0.7 to -2.5 in the CT-Y soil (Figure 5.7).

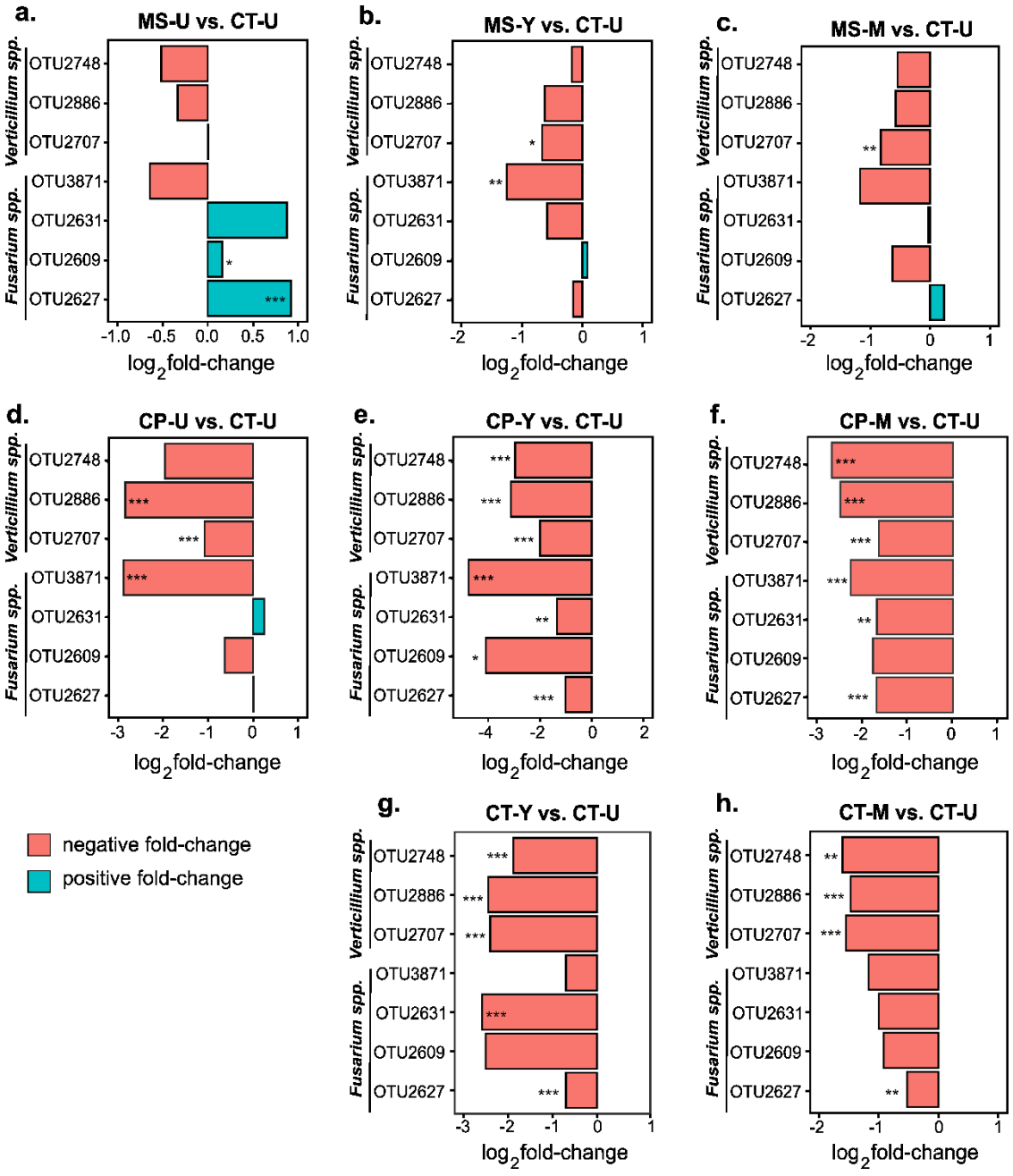

Figure 5. 7 The log_2 fold-change in relative abundance of three operational taxonomic units (OTUs) identified as pathogenic *Verticillium spp*. and four OTUs identified as pathogenic *Fusarium spp*. between a given treatment and the untreated control. Treatments included: chemical fumigation with metam sodium (MS-U) and chloropicrin (CP-U) used alone, metam sodium (MS-Y) and chloropicrin (CP-Y) combined with young barley plant residues, metam sodium (MS-M) and chloropicrin (CP-M) combined with mature barley plant residues, non-fumigated soil amended with young barley plant residues (CT-Y) and mature barley plant residues (CT-M), and an untreated control (CT-U). Significant changes in OTU abundance relative to the control were determined using non-parametric Kruskal-Wallis tests adjusted for ties.

Significant changes (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$) in OTU abundance are indicated adjacent to the plotted OTU.

5.4 Discussion

5.4.1 Effect of chemical fumigation and substrate C availability on bacterial diversity

This study examined the effect of chemical fumigants combined with amendments of contrasting C availability on the soil bacterial and fungal community diversity, as indicated by changes in Pielou's species evenness (J), Chao1 species richness, and the β–diversity of the bacterial and fungal community.

In non-fumigated soil, substrate C availability greatly affected the soil bacterial diversity at early time points. This was evident based on the significant yet transient decrease in bacterial species evenness in the CT-Y soil but not the CT-M soil compared to the CT-U soil. A reduction of bacterial evenness was unexpected, as labile C amendments have been shown to increase microbial richness and evenness (Bending et al., 2002; Lienhard et al., 2013; Wang et al., 2020); however, this effect is similar to what has been observed in the soil rhizosphere, where the microbial diversity decreases compared to bulk soil due to high-levels of microbial competition for available root exudates (Garcia-Salamanca et al., 2012; Marilley et al., 1998). The change in bacterial species evenness was consistent with the significantly dissimilar bacterial β-diversity between the CT-Y soil and the CT-M and CT-U soils and the similar β-diversity between the CT-M and CT-U treated soils throughout the incubation, indicating that only the addition of young barley residues to non-fumigated soil significantly changed the bacterial community composition. The significant effect of CT-Y but not CT-M on the soil bacterial diversity was likely related to differences in substrate quality. For

example, most heterotrophic bacteria can rapidly turnover labile C substrates; however, not all bacteria have the enzymatic capacity to biophysically degrade recalcitrant C compounds (DeAngelis et al., 2011; Nannipieri et al., 2012; Pold et al., 2013; Sinsabaugh, 2010). Therefore, the change of bacterial diversity in CT-Y soil likely reflects the proliferation of copiotrophic bacteria responding to labile C additions, similar to previous studies (Navarro-Noya et al., 2013; Pascault et al., 2013).

Similarly, substrate C availability greatly affected the bacterial diversity in MS-fumigated soils, as MS-treated soil only decreased species evenness compared to the CT-U soil when amended with young barley plant residues (after 8, 16, and 72 days). This was consistent with the similar β-diversity between the MS-Y, MS-U, and MS-M treated soils after 3 days, but the significantly dissimilar β-diversity between the MS-Y soil and the MS-U and MS-M treated soils after 8, 16, and 72 days of incubation. In addition, MS-fumigated soil amended with young barley residues had a greater impact on the bacterial diversity than non-fumigated soil amended with young barley residues, as the MS-Y soil had significantly lower species evenness compared to the CT-Y soil after 8, 16, and 72 days of incubation. The impact of MS-Y on soil bacterial diversity may reflect a combined effect of methyl-ITC (the active component of MS fumigation) that shaped the community and the proliferation of C benefitting bacteria that were either resistant to or survived MS fumigation. This aligns with previous studies that demonstrated that methyl-ITC degradation is a biological process (Warton et al., 2001; Zhang et al., 2005) and that the addition of organic amendments to soil treated with purified methyl-ITC promoted the growth and activity of methyl-ITC degrading microorganisms (Di Primo et al., 2003; Dungan et al., 2002; Dungan & Yates, 2003).

In contrast to MS-Y, the MS-M soil did not have a sufficient labile C source to promote rapid and extensive change in bacterial community diversity, as demonstrated by the absence of change in species evenness and richness and the slow establishment of a distinctive bacterial community composition in the MS-M soil compared to the MS-U soil. Nonetheless, at the end of the incubation, the MS-U, MS-Y, and MS-M treated soils had a significantly dissimilar β-diversity compared to the CT-U, CT-Y, and CT-M treated soils, indicating MS fumigation altered the bacterial community composition, similar to previous studies that applied MS to unamended soil (Li et al., 2017a; Sederholm et al., 2018).

Chemical fumigation with CP had the greatest effect on the soil bacterial diversity, regardless of soil amendments or substrate C availability. This was evident based on the significant decrease in species evenness and richness in the CP-U, CP-Y, and CP-M treated soils compared to the CT-U, CT-Y, and CT-M treated soils at the end of the incubation, indicating that the bacterial community in CP-treated soils could not recover to a similar diversity observed in the untreated soil. These findings are similar to the results observed in Chapter 3 and to previous studies that applied CP to unamended soil (Fang et al., 2018b; Li et al., 2017b).

There are currently no studies that have investigated the combined effect of CP fumigation and plant residue amendments on soil bacterial diversity. However, a previous study reported that the addition of biochar to CP-fumigated soil shortened the recovery time of bacterial diversity and resulted in communities that were more similar to untreated soils over time (Fang et al., 2020). However, this was not the case in the current study. Instead, CP fumigation had the greatest effect on the bacterial β-

diversity, regardless of soil amendments or substrate C availability, consequently resulting in a continual divergence of CP-treated soils from all other treatments, thereby yielding a bacterial community that was the most dissimilar from all other treatments throughout the incubation.

5.4.2 Effect of chemical fumigation and substrate C availability on fungal diversity

Similar to bacterial diversity, the addition of young barley residues to non-fumigated soil had the greatest effect on the fungal community diversity at early time points, as indicated by the significant decrease in fungal species evenness in the CT-Y treated soil compared to all other treatments after 3 days of incubation. This was also consistent with the significantly dissimilar β-diversity of the CT-Y treatment compared to all other treatments after 3 days, indicating that the fungal community composition was the most dissimilar in the CT-Y treated soil at early time points. However, unlike bacterial diversity, the CT-Y soil had an enduring impact on fungal diversity, as the fungal species richness was significantly lower in the CT-Y treated soil compared to the CT-U soil and the fungal species evenness in the CT-Y treated soil did not recover to the similar species evenness observed in the untreated soil. Although it is generally accepted that fungi primarily decompose recalcitrant organic matter (Andresen et al., 2014; Fabian et al., 2017), certain saprotrophic fungi have ruderal characteristics, including rapid growth and the ability to use relatively simple C compounds (Boer et al., 2005). In addition, ^{13}C-studies have demonstrated that fungal contribution to the decomposition of labile C substrates increases with high substrate loading rates (Griffiths et al., 1999; Lundberg et al., 2001); therefore, the significant effect of CT-Y

on the fungal diversity was likely related to the proliferation of saprotrophic fungi responding to high inputs of readily degradable C.

The addition of mature barley residues to non-fumigated soil had a greater effect on the fungal community diversity than bacterial community diversity. This was evident based on the significant decrease in fungal species richness in the CT-M soil compared to the CT-U soil and the significantly dissimilar fungal β-diversity between the CT-M and CT-U soils throughout the incubation. Unlike most bacteria, fungi have a greater ability to depolymerize complex C compounds through extracellular enzyme production (Banerjee et al., 2016; Boer et al., 2005; Shahbaz et al., 2020); therefore, changes to the fungal community composition in the CT-M soil likely reflects shifts in recalcitrant C degrading fungi.

Unlike bacterial diversity, chemical fumigation with MS did not affect the fungal community diversity, regardless of soil amendments or substrate C availability. These findings are similar to a previous study that reported MS fumigation used alone did not affect fungal species richness compared to non-fumigated soil (Montiel-Rozas et al., 2020). Given that the CT-Y and CT-M treated soils decreased fungal evenness and or richness, the insignificant effect of the MS-Y and MS-M treatments may suggest that MS fumigation reduced the community of fungi that could readily utilize C substrates. This is further supported by the significantly dissimilar β-diversity between the MS-U, MS-Y, and MS-M treated soils compared to the CT-U, CT-M, and CT-Y soils from 8 to 128 days incubation, indicating that MS fumigation significantly changed the composition of the fungal community.

The addition of barley plant residues to CP-fumigated soils, regardless of substrate C availability, had a greater effect on fungal diversity than CP used alone and all other treatments. This was evident based on the significantly lower fungal species evenness and richness in the CP-Y and CP-M soils compared to the CT-U soil and the similar fungal species evenness and richness between the CP-U and CT-U soils throughout the incubation. Conversely, previous studies observed that CP used alone significantly decreased fungal diversity (Li et al., 2021; Zhu et al., 2021). This is the first study that has investigated the effects of CP fumigation combined with plant residue amendments on soil fungal diversity. The findings of this study demonstrate that CP fumigation had a more significant impact on soil fungal species evenness and richness when combined with young or mature barley residues.

Since CP used alone did not affect the diversity indices used in this study, the significant decrease of fungal diversity in CP-Y and CP-M treated soils was likely related to a combined effect of CP fumigation and organic amendments, where the fungal community was initially shaped by CP fumigation followed by the predominance of fungi that survived CP fumigation and could utilize labile or recalcitrant C compounds. This was consistent with the similar β-diversity between CP-U, CP-Y, and CP-M treated soils from 3 to 16 days and the significantly dissimilar β-diversity between CP-U, CP-Y, and CP-M treated soils from 30 to 128 days of incubation. Nonetheless, CP fumigation of soil yielded the most dissimilar fungal community, regardless of substrate C availability, as indicated by the continual divergence of CP-treated soils from all other treatments throughout the incubation. Previous studies also reported that CP fumigation used alone significantly changed

fungal β-diversity compared to non-fumigated soil (Zhang et al., 2019a; Zhu et al., 2021). Similar to the bacterial β-diversity, this study also demonstrated that adding plant residues to CP-fumigated soil did not result in the recovery of the fungal community diversity.

5.4.3 Effect of chemical fumigation and substrate C availability on the relative abundance of bacterial and fungal phyla and species

The CT-M treatment was the only treatment that did not affect the relative abundance of bacterial phyla compared to the CT-U soil; however, the CT-M soil had a greater relative abundance of Ascomycota, which was evident both at the phyla and species level, likely related to most lignin and lignocellulose degrading fungal species belonging to this phylum (Brink et al., 2019). Conversely, the CT-Y treated soil had a greater relative abundance of Proteobacteria, likely related to the large number of fast-growing r-strategists belonging to this phylum (Cederlund et al., 2014; Lladó & Baldrian, 2017). In addition, the CT-Y soil was dominated by fungi belonging to the phylum Mortierellomycota, evident at both the phyla and species level, which likely reflects the large community of saprobic species belonging to this phylum responding to labile C additions (Bonafante & Venice, 2020).

Chemical fumigation with MS did not greatly affect the relative abundance of bacterial phyla, as only the MS-Y soil had a significantly greater abundance of Acidobacteria than the CT-U and CT-Y soils. Conversely, chemical fumigation with CP had the greatest effect on bacterial phyla relative abundance, regardless of soil amendment or substrate C availability, likely contributing to the most dissimilar bacterial β-diversity observed in CP-treated soils. In particular, all CP-treated soils increased the relative abundance of Firmicutes compared to the non-fumigated soils.

This is similar to previous studies that applied CP to unamended soils (Fang et al., 2018b; Li et al., 2017b) and may be related to a large number of bacteria capable of producing stress-resistant endospores within this phylum (Egan et al., 2021; Feld et al., 2015; Filippidou et al., 2016).

Interestingly, all chemical fumigant treatments significantly increased the relative abundance of Ascomycota compared to non-fumigated soils, which was also evident at the species level after 3 and 128 days of incubation. Along with a large community of saprotrophic fungi (Yao et al., 2017), Ascomycota also has a large number of pollutant degrading fungi (Galitskaya et al., 2021; Harms et al., 2011). Therefore, the increase of Ascomycota in chemically fumigated soil may be related to the proliferation of fungi responding to cell lysates released from microbial debris and the addition of organic amendments, along with the proliferation of fungi capable of degrading or resistant to MS and CP fumigation.

5.4.4 Effect of chemical fumigation and substrate C availability on pathogenic fungal species

In general, the addition of plant residues to chemically fumigated soil, regardless of C availability, increased the efficacy of the soil fumigants, as indicated by the greater fold-decrease in the relative abundances of *Verticillium spp*. and *Fusarium spp*. in the CP-Y, CP-M, MS-Y, and MS-M treated soils relative to the CT-U soil compared to CP-U or MS-U soil relative to the CT-U soil. However, the addition of plant residues to non-fumigated soil was also effective at managing fungal pathogens in this study, as indicated by the fold-decrease in the relative abundances of *Verticillium spp*. and *Fusarium spp* in the CT-Y and CT-M soils relative to the CT-U soil. Previous studies have also demonstrated that soil organic amendments reduced soil-borne diseases in

potato production systems (Hao & Ashley, 2021; Lazarovits et al., 2001; Ninh et al., 2015). Disease suppression in the CT-Y and CT-M soils may reflect the modification of the soil microbial community through microbial competition for nutrients and space and or through the production of secondary antagonistic metabolites (i.e., fungitoxins) (Bonanomi et al., 2018; Bonanomi et al., 2020). The findings in this study indicate that chemical fumigation is not the only option for reducing soil pathogens.

5.5 Conclusions

This study showed that chemical fumigation, either used alone or combined with soil organic amendments, altered at least one measure of soil bacterial and fungal diversity. In general, adding plant residues to chemically fumigated soil had a greater effect on microbial diversity and community composition than chemical fumigation used alone, subsequently promoting a selective community for both fumigation and organic C additions. These findings provided evidence that the addition of young and mature barley residues to chemically fumigated soil did not recover soil microbial diversity. Interestingly, although adding plant residues to non-fumigated soil transiently decreased microbial diversity indices and altered the microbial community composition, soil respiration and soil N cycling were increased (Chapter 4), indicating that these treatments increased microbial activity and that soil function was preserved. Conversely, the decrease of soil respiration and inhibition of nitrification (Chapter 4) in fumigated soil indicates that the decrease of microbial diversity indices and altered community composition resulted in a loss of soil function. Although the effect of chemical fumigation on soil respiration or N cycling was transient (Chapter 4), the changes in microbial community diversity were enduring, particularly in the CP-treated

soils. It is unknown how these changes to microbial community diversity and composition would affect soil function long-term.

CHAPTER 6: CONCLUSIONS

6.1 Thesis findings

Chemical fumigation, either used alone or combined with an organic amendment, and biofumigation impacted essential soil processes and the soil microbiome, as indicated by changes in soil N cycling, the abundance of N cycling microorganisms, soil respiration, and microbial community diversity. In general, biofumigation elicited an initial and transient effect, whereas chemical fumigation had a more marked and enduring impact on the soil N cycle, N cycling microorganisms, soil respiration, and microbial community diversity.

Chemical fumigation had a greater impact on the soil N cycle and N cycling microorganisms than biofumigation, as chemical fumigation with CP, MS, or MSBR significantly inhibited nitrification and decreased the abundance of soil nitrifiers compared to MR (Chapter 2). Chemical fumigation with CP, in particular, had the most marked effect on the soil N cycle and N cycling microorganisms, significantly inhibiting nitrification for nine weeks, increasing N_2O emissions, and decreasing the abundance of soil nitrifiers and denitrifiers (Chapter 2). Conversely, biofumigation did not affect net N mineralization, nitrification, or the abundance of N cycling microorganisms compared to the untreated soil and non-biofumigant barley residues (Chapter 2). Biofumigation increased denitrification and N_2O emissions; however, this was expected, as mustard plant residue addition likely increased available C and N supply.

Biofumigation and chemical fumigation affected soil bacterial diversity differently. Biofumigation had a greater impact on bacterial diversity at earlier time

points, transiently decreasing bacterial species evenness and yielding the most dissimilar community composition compared to chemical fumigation after 3 days of incubation (Chapter 3). Chemical fumigation with MS used alone did not affect bacterial diversity indices, yet MS fumigation combined with fresh young barley residues transiently decreased bacterial species evenness (Chapter 3). This was unexpected and provided evidence that ITC-based fumigation only affects bacterial diversity when combined with labile soil amendments. Conversely, CP fumigation lowered bacterial species evenness that could not recover to the state of the untreated soil (Chapter 3). Although all fumigated soils progressed towards an alternative community assemblage over time, CP fumigation yielded the most dissimilar bacterial community composition (Chapter 3).

Biofumigation had a greater effect on soil respiration than chemical fumigation used alone, as biofumigation increased respiration rate compared to the untreated soil, whereas chemical fumigation with CP or MS did not (Chapter 2). This was not unexpected and likely represented increased microbial activity responding to the addition of labile mustard plant residues. However, unexpectedly, soil fumigated with MS and amended with barley plant residues (MSBR) had a significantly lower respiration rate and a greater percentage of plant residues remaining after 160 days than the biofumigated soil or soil amended with barley residues (Chapter 2). These findings provided evidence that chemical fumigation affected the soil C cycle, plant residue decomposition, and soil microbial activity.

The findings of Chapter 4 further confirmed the inhibitory effect of chemical fumigation on plant residue decomposition and microbial activity. Chemical

fumigation with CP or MS reduced the decomposition of the added barley residues, regardless of substrate C availability (Chapter 4). In particular, CP fumigation had a more significant effect on plant residue decomposition than MS fumigation, as evidenced by the lower cumulative respiration and percentage of apparent plant residue decomposition in the CP-treated soils than the MS- and CT-treated soils (Chapter 4). Given that soil C dynamics are controlled by a diverse group of heterotrophic soil microorganisms (Spohn et al., 2016; Xiao et al., 2018), these findings raised the question of how chemical fumigation and substrate C ability affects the soil microbial community.

The impact of chemical fumigation and substrate C availability on the soil bacterial and fungal community diversity was further investigated in Chapter 5. Chemical fumigation with MS transiently decreased bacterial species evenness and richness only when combined with young barley residues, further validating the results obtained in Chapter 3. However, MS fumigation did not affect the fungal diversity indices, regardless of soil amendment or substrate C availability (Chapter 5). Chemical fumigation with CP had the most marked effect on bacterial and fungal diversity. CP fumigation decreased bacterial species evenness and richness, regardless of soil amendment or substrate availability, and could not recover to the similar level of diversity in the non-fumigated soil (Chapter 5). However, CP fumigation only decreased fungal species evenness and richness when combined with plant residues, which also could not recover to the similar level of diversity in the non-fumigated soil (Chapter 5).

6.2 Thesis limitations

Although the aim of this thesis was accomplished, there were experimental limitations. For example, soil microcosms were not sampled during the first two days of incubation in all experiments due to safety reasons associated with toxic chemical fumigant products at high concentrations. Therefore, it is unknown what occurred during the first 48 hours following chemical fumigation. In addition, important trends that further support the findings of this thesis could have been missed, such as the total flux of CO_2 from non-fumigated soil, which, if captured, would strengthen the evidence of apparent reduced plant residue decomposition in chemically fumigated soil (Chapter 4).

In addition, while the bacterial 16S gene and fungal ITS region of rDNA are widely used to infer phylogenic relationships among bacteria and fungi (Taberlet et al., 2012; Abdelfattah et al., 2018), these short molecular markers often lack sufficient variability to provide precise species identification (Piombo et al., 2021). Therefore, although biofumigation (Chapter 3) and chemical fumigation (Chapter 3; Chapter 5) altered soil microbial community composition, this information could not be used to distinguish between pathogenic or beneficial species (i.e., biocontrol agents), which would have provided more insight into how biofumigation or chemical fumigation shaped the community and if they effectively reduced soil-borne pathogens. Additionally, amplicon-based sequencing cannot be used to infer function, as phylogeny and function are rarely conserved (Alteio et al., 2021; Fierer, 2017); therefore, changes in microbial community composition could not be used to predict

how biofumigation and chemical fumigation influenced microbial function and roles in ecological processes.

Moreover, this thesis investigated the effects of chemical fumigation and biofumigation under controlled conditions. Variable soil water content and soil temperature have been shown to influence soil microbial activity, mobility, nutrient availability, and microhabitat connectivity (Davidson et al., 2006; Fierer et al., 2006; Wolf et al., 2013). Therefore, observations under controlled conditions may not entirely reflect the spatiotemporal dynamics of the natural soil environment. For example, Yan et al. (2013b) observed an inhibition of nitrification in CP-fumigated soil for 12 weeks under controlled conditions; however, in a field study, CP fumigation inhibited nitrification for only two weeks. Therefore, although an effort was made in this thesis to use conditions that closely mimic *in situ* conditions and the agricultural practice of chemical and biofumigation in eastern Canadian potato production systems, further field experimentation is needed to support and validate the results obtained.

6.3 Thesis contributions

Nonetheless, the studies presented in this thesis were the first to investigate the effects of chemical fumigation, either used alone or combined with an organic amendment, and biofumigation using high GSL-mustard plants on the soil N cycle, the abundance of N cycling microorganisms, soil respiration, and microbial diversity. The study of microbial diversity, abundance, and the metabolic activities of soil microorganisms (i.e., key N processes and soil respiration) has become more common, as these biochemical indicators are used to assess the soil health risks associated with agricultural practices due to their high sensitivity to management (Ferris & Tuomisto,

2015; Fierer et al., 2021; Hills et al., 2020). Therefore, investigating these experimental parameters under controlled conditions has undoubtedly provided insight into the potential impact of chemical fumigation and biofumigation on soil function and health. Overall, the findings of this thesis suggest that biofumigation was less detrimental to soil health indicators than chemical fumigation.

However, in addition to suppression of soil pathogens, there are aspects of chemical fumigation that may provide short-term benefits for soil productivity. For example, the eastern Canadian climate experiences high precipitation events (Lynch et al., 2012; Wilson et al., 2019); therefore, nitrification inhibition following chemical fumigation (Chapter 2; Chapter 4) in the fall season may reduce NO_3^--N leaching and soil N_2O emissions, while increasing plant-available N for the following potato growing season. Moreover, soils in eastern Canada have become depleted in soil organic matter (Carter, 2007; Nyiraneza et al., 2017); therefore, decreased soil organic matter decomposition in chemically fumigated soil (Chapter 2; Chapter 4) may present a significant increase in soil organic C storage.

Nevertheless, the most marked effect of chemical fumigation, particularly CP fumigation, was the significant decrease in soil microbial diversity and changes to microbial community composition (Chapter 3; Chapter 5). Loss in microbial diversity is often associated with decreased functional redundancy and nutrient turnover (Delgado-Baquerizo et al., 2020). Consequently, the nitrification inhibition in chemically fumigated soils was likely linked to the decrease of nitrifying bacteria and archaea (Chapter 2). Moreover, given that soil organic matter decomposition is controlled by a diverse group of heterotrophic soil microorganisms (Spohn et al., 2016;

Xiao et al., 2018), the reduced apparent plant residue decomposition in chemically fumigated soil (Chapter 2; Chapter 4) was likely associated with the decrease in fungal and bacterial diversity and alterations in microbial community composition (Chapter 5). In addition to lower levels of nutrient cycling, decreased microbial diversity is also associated with reduced protection against soil pathogens (Delgado-Baquerizo et al., 2020; van Elsas et al., 2012). The non-target effects of chemical fumigation on the soil microbial community have led to the phenomenon known as 'fumigation rebound', by which fumigation provides a short-term benefit, as disease pressure worsens after fumigation (Kinkel, 2008; Hills et al., 2020). Therefore, although chemical fumigation presents short-term benefits for soil productivity, it raises the question, but at what cost? Future studies are needed to investigate the long-term implications of continual chemical fumigation on soil nutrient turnover, microbial diversity, and resilience to re-occurring disease infection, along with solutions for diversity restoration in chemically fumigated soil.

Canadian potato production faces persistent and recurrent problems associated with soil-borne diseases (Larkin et al., 2017; Larkin & Lynch 2018). Chemical fumigation and biofumigation are currently two practices used for disease management in the Canadian potato industry. However, the findings of this thesis have demonstrated that chemical fumigation and biofumigation impact non-targeted microorganisms and essential soil processes. Overall, chemical fumigation had a more significant impact on the soil N cycle, the abundance of N cycling microorganisms, soil respiration, and microbial diversity compared to biofumigation. Therefore, the minimal effect of biofumigation on the N cycle, soil respiration, N cycling microorganisms, and bacterial

diversity, along with its expectation to increase soil organic matter, indicates that biofumigation may be a more sustainable option for disease management in potato production systems than chemical fumigation.

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APPENDIX

Table A. 1 Results of the analysis of variance (ANOVA) of soil bacterial Shannon Diversity (H'), Pielou's Evenness (J), and Chao1 Richness in Study 2, Chapter 3. ANOVAs were calculated based on a RCBD with treatment, time, and blocks (rep) as fixed factors. Data were transformed (Box Cox transformation) prior to analysis.

Shannon Diversity (H')				
Source of variation	**df**	**SS**	**F**	***P***
Treatment	5	5.0727	67.55	< 0.0001
Time	4	0.4831	8.04	< 0.0001
Treatment x Time	20	3.1530	10.50	< 0.0001
Rep	4	0.5914	9.85	< 0.0001
Residual	116	1.7421		
Total	149	11.0423		
Pielou's Evenness (J)				
Source of variation	**df**	**SS**	**F**	***P***
Treatment	5	0.27397	79.173	< 0.0001
Time	4	0.01573	5.681	0.0003
Treatment x Time	20	0.23577	17.034	< 0.0001
Rep	4	0.01361	4.917	0.001
Residual	116	0.08028		
Total	149	0.61936		
Chao1 Richness				
Source of variation	**df**	**SS**	**F**	***P***
Treatment	5	2.416×10^{-9}	8.650	< 0.0001
Time	4	6.500×10^{-9}	2.909	0.0246
Treatment x Time	20	1.885×10^{-9}	1.687	0.0452
Rep	4	1.965×10^{-9}	8.794	< 0.0001
Residual	116	6.481×10^{-9}		
Total	149	1.9246×10^{-8}		

Table A. 2 Results of the analysis of variance (ANOVA) of cumulative soil respiration (mg CO_2-C kg^{-1} dry soil) and nitrous oxide (N_2O) emissions (µg N_2O-N kg^{-1} soil) from 0-16 days of incubation in Study 3, Chapter 4. ANOVAs were calculated based on a 3 x 3 factorial arrangement in a RCBD with fumigant treatment, residue treatment, and blocks (rep) as fixed factors. Data were transformed (Box Cox transformation) prior to analysis.

Cumulative soil respiration				
Source of variation	**df**	**SS**	**F**	***P***
Fumigant	2	2.8058	18.22	< 0.0001
Residue	2	8.3807	54.43	< 0.0001
Fumigant x Residue	4	1.0616	3.45	0.02
Rep	4	0.8336	2.71	0.048
Residual	32	2.4636		
Total	44	15.5452		
Cumulative N_2O emissions				
Source of variation	**df**	**SS**	**F**	***P***
Fumigant	2	2.391	4.69	0.016
Residue	2	13.079	25.63	< 0.0001
Fumigant x Residue	4	4.267	4.18	0.008
Rep	4	1.878	1.84	0.145
Residual	32	8.163		
Total	44	29.77		

Table A. 3 Results of the analysis of variance (ANOVA) of cumulative soil respiration (mg CO_2-C kg^{-1} dry soil) and nitrous oxide (N_2O) emissions (μg N_2O-N kg^{-1} soil) from 0-128 days of incubation in Study 3, Chapter 4. ANOVAs were calculated based on a 3 x 3 factorial arrangement in a RCBD with fumigant treatment, residue treatment, and blocks (rep) as fixed factors. Data were transformed (Box Cox transformation) prior to analysis.

Cumulative soil respiration				
Source of variation	**df**	**SS**	**F**	***P***
Fumigant	2	0.000295	43.88	< 0.0001
Residue	2	0.000283	42.13	< 0.0001
Fumigant x Residue	4	0.000040	3.01	0.033
Rep	4	0.000036	2.65	0.051
Residual	32	0.000108		
Total	44	0.000762		
Cumulative N_2O emissions				
Source of variation	**df**	**SS**	**F**	***P***
Fumigant	2	0.000002	1.46	0.248
Residue	2	0.000012	11.65	< 0.0001
Fumigant x Residue	4	0.000013	5.87	0.001
Rep	4	0.000001	1.68	0.178
Residual	32	0.000017		
Total	44	0.000047		

Table A. 4 Results of the analysis of variance (ANOVA) of the apparent plant residue decomposition of the barley residues added to the fumigant treatments examined from 0-16 days of incubation in Study 3, Chapter 4. ANOVA was calculated based on a RCBD with fumigation treatment and blocks (rep) as fixed factors. Data were transformed (Box Cox transformation) prior to analysis.

Source of variation	**df**	**SS**	**F**	***P***
Fumigation	2	197.6	5.19	0.036
Rep	4	132.8	1.75	0.233
Residual	8	152.1		
Total	14	482.5		

Table A. 5 Results of the analysis of variance (ANOVA) of the apparent plant residue decomposition of the barley residues added to the fumigant treatments examined from 0-128 days of incubation in Study 3, Chapter 4. ANOVA was calculated based on a RCBD with fumigation treatment and blocks (rep) as fixed factors. Data were transformed (Box Cox transformation) prior to analysis.

Source of variation	**df**	**SS**	**F**	***P***
Fumigation	2	6.644	11.24	0.005
Rep	4	1.432	1.21	0.378
Residual	8	2.365		
Total	14	10.440		

Table A. 6 Results of a permutational multivariate analysis of variance (PERMANOVA) of the β-diversity of the bacterial community in Study 2, Chapter 3. PERMANOVA was calculated based on a RCBD with treatment, time, and blocks (rep) as fixed factors.

Source of variation	**df**	**SS**	**Pseudo-F**	***P***
Treatment	5	3.423	3.4457	0.0001
Time	4	1.923	2.4071	0.0001
Treatment x Time	20	5.381	1.3543	0.0001
Rep	4	1.537	1.9346	0.0001
Residual	116	23.046		
Total	149	35.301		

Table A. 7 Results of a permutational multivariate analysis of variance (PERMANOVA) of the β-diversity of the bacterial community in Study 4, Chapter 5. PERMANOVA was calculated based on a 3 x 3 x 6 factorial arrangement in a RCBD with fumigant treatment, residue treatment, time, and blocks (rep) as fixed factors.

Source of variation	df	SS	Pseudo-F	*P*
Fumigant	2	4.962	14.1671	0.0001
Residue	2	1.032	2.9457	0.0001
Time	5	3.113	3.5549	0.0001
Fumigant x Residue	4	1.103	1.5744	0.0001
Fumigant x Time	10	3.809	2.1748	0.0001
Residue x Time	10	1.990	1.1361	0.0167
Fumigant x Residue x Time	20		1.0515	0.0981
Rep	4	1.776	2.5351	0.0001
Residual	212	37.126		
Total	269	58.593		

Table A. 8 Results of a permutational multivariate analysis of variance (PERMANOVA) of the β-diversity of the fungal community in Study 4, Chapter 5. PERMANOVA was calculated based on a 3 x 3 x 6 factorial arrangement in a RCBD with fumigant treatment, residue treatment, time, and blocks (rep) as fixed factors.

Source of variation	df	SS	Pseudo-F	*P*
Fumigant	2	10.943	69.331	0.0001
Residue	2	3.769	23.879	0.0001
Time	5	2.994	7.588	0.0001
Fumigant x Residue	4	3.196	10.126	0.0001
Fumigant x Time	10	4.146	5.253	0.0001
Residue x Time	10	1.640	2.078	0.0002
Fumigant x Residue x Time	20	2.493	1.580	0.0004
Rep	4	1.242	3.935	
Residual	212	16.730		
Total	269	47.154		