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Abstract

Agricultural management influences 40% of Earth's land area, plays a key role in soil carbon (C) dynamics and storage. Yet its combined effect with global warming on soil organic C (SOC) storage in deep horizons remains uncertain. This knowledge gap stems from limited understanding of microbial processes underlying SOC dynamics in subsoils, while most research continues to focus on surface soils (0–30cm). As a result, the responses of subsoil microbial communities (>30cm) to warming, within the context of different agricultural management practices, remain largely understudied, despite their critical role in ecosystem functions and services.

This study examined the effects of whole-soil warming (+4°C) on C cycling, microbial resilience and soil functionality in cropland and grassland. Using a multidisciplinary approach, it explored interactions between agricultural practices, warming-induced ecosystem changes and mechanisms driving soil health and sustainability. To achieve these aims, an *in-situ* whole-soil warming (+4°C) experiment, started in 2021, provided data over 3 consecutive years to 1m depth. The study focused on critical soil processes, including CO_2 production, surface fluxes and water content across depths, to investigate C cycling dynamics. It also examined microbial biomass and richness to assess structure and resistance of microbial communities under varying management practices. Additionally, functional indicators such as enzymatic activity and eco-enzymatic stoichiometry (ES), were measured to explore microbial roles in nutrient cycling and soil health.

The results showed that warming reduced soil moisture in both grassland and cropland, impacting CO_2 production. The impacts on soil CO_2 efflux depend on season and management practices. Subsoil CO_2 production in grassland was more sensitive to warming, increasing by 42% compared to control plots, whereas in cropland, it increased by only 10%. In contrast, topsoil CO_2 production decreased by 4.2% in cropland but not in grassland. Despite these warming responses of CO_2 production and efflux, microbial biomass and richness remained unchanged across soil profile under both land management. However, warming disrupted microbial communities, with bacteria being more affected than fungi. This disruption reduced 53% of microbial network complexity in cropland, compared to 40% decline in grassland across soil profile. Grassland microbial networks exhibited greater resistance to warming than those in cropland. Notably in cropland, all microbial interactions were impacted by warming, while in grassland, the effects were limited to bacterial-bacterial interactions. These microbial

responses were mirrored by contrasting effects on SOC dynamics depending on land management. In grassland, SOC storage remained stable across depths, whereas in cropland, SOC storage declined by 18% in surface soil but increased by 86% in deep soil. These shifts aligned with changes in CN-acquiring enzyme activities, ES and bacterial communities' composition, suggesting a microbial-driven mechanism linked to SOC decomposition and/or C inputs. These findings highlight the depth-specific and management-mediated responses of microbial metabolism and soil C dynamics to warming emphasizing vital role of land management in shaping soil ecosystem resistance to climate change.

In conclusion, agricultural practices significantly influence warming induced responses of CO_2 production and efflux, microbial communities and SOC dynamics. The contrasting responses observed in grassland and cropland suggest that soil management strategies could play a critical role in either mitigating or exacerbating warming-driven changes in microbial metabolism and SOC storage. This study highlights the need for management practices that enhance microbial resistance, particularly in croplands, while suggesting long-term research to unravel microbial driven SOC persistence and develop depth-specific strategies to sustain SOC stocks, nutrient cycling and agroecosystem resistance under climate change.

Keywords: Soil warming; Subsoil CO_2 production; Soil respiration; Extracellular enzyme activity; Microbial community composition; Agricultural land management; Whole-soil profile; Carbon-climate feedback

Résumé

La gestion des pratiques agricoles, qui impacte environ 40 % de la superficie des terres émergées, joue un rôle clé dans la transformation des écosystèmes terrestres, en particulier dans les dynamiques du carbone des sols et leur capacité de stockage. Cependant, l'effet combiné de ces pratiques avec le réchauffement climatique sur le stockage du carbone organique du sol (COS) dans les horizons profonds reste peu compris. Cette lacune est en grande partie due à la connaissance encore limitée des processus microbiens responsables du COS dans les sous-sols, la majorité des recherches étant concentrées sur les sols superficiels (0–30 cm). En conséquence, les réponses des communautés microbiennes des horizons profonds (>30 cm) au réchauffement, et leur interaction avec diverses pratiques agricoles, demeurent largement inexplorées, bien que ces communautés soient essentielles au fonctionnement des sols et aux services écosystémiques.

Cette étude a évalué les effets d'un réchauffement sur l'ensemble du profil du sol (+4 °C) sur le cycle du carbone, la résilience microbienne et les fonctionnalités des sols en prairies et en terres cultivées. Une approche multidisciplinaire a été adoptée pour analyser les interactions complexes entre pratiques agricoles, impacts du réchauffement et mécanismes influençant la durabilité des sols. Un dispositif expérimental *in situ* de réchauffement (+4 °C), débuté en 2021, a permis de collecter des données sur trois années consécutives jusqu'à une profondeur de 1 mètre. L'étude a porté sur des processus critiques, tels que la production de CO_2, les flux en surface, et le contenu en eau à différentes profondeurs pour comprendre les dynamiques du cycle du carbone. Par ailleurs, la biomasse et la diversité microbiennes ont été examinées afin d'évaluer la structure et la résilience des communautés microbiennes sous différentes gestions agricoles. Les activités enzymatiques et la stœchiométrie éco-enzymatique ont également été mesurées pour explorer les rôles microbiens dans les cycles des nutriments et la santé des sols.

Les résultats montrent que le réchauffement a réduit l'humidité des sols dans les prairies et les terres cultivées, tout en affectant la production de CO_2. Les efflux de CO_2 ont varié selon la saison et les pratiques agricoles. Dans les horizons profonds des prairies, la production de CO_2 a augmenté de 42 % sous l'effet du réchauffement par rapport au traitement témoin (non chauffé), contre seulement 10 % dans les terres cultivées. En surface, la production de CO_2 a diminué de 4 % dans les terres cultivées, mais est restée stable dans les prairies. Bien que la biomasse et la diversité microbiennes soient restées globalement inchangées, le réchauffement a significativement perturbé les communautés microbiennes. Les bactéries ont été davantage affectées que les champignons, entraînant une réduction de 53 % de la complexité des réseaux microbiens dans les terres cultivées, contre 40 % dans les prairies. Les réseaux microbiens des prairies ont montré une plus grande résilience, avec des perturbations limitées aux interactions bactériennes, tandis que dans les terres cultivées, toutes les interactions microbiennes ont été affectées.

Ces perturbations microbiennes ont conduit à des dynamiques contrastées du COS selon les pratiques agricoles. Dans les prairies, le stockage de COS est resté stable sur l'ensemble du profil, tandis que dans les terres cultivées, une diminution de 18 % a été observée en surface, accompagnée d'une augmentation de 86 % dans les horizons profonds. Ces variations étaient liées aux activités des enzymes d'acquisition de l'azote, à la stœchiométrie éco-enzymatique, et à la composition des communautés bactériennes, suggérant un mécanisme microbien influençant la décomposition du COS et/ou les apports en carbone.

Ces résultats mettent en évidence des réponses spécifiques à la profondeur et modulées par la gestion agricole. Ils soulignent le rôle crucial des pratiques de gestion dans la résilience des sols face au changement climatique.

En conclusion, les pratiques agricoles influencent de manière significative les réponses des sols au réchauffement, notamment en ce qui concerne la production et les flux de CO_2, les communautés microbiennes et les dynamiques du COS. Les différences observées entre prairies et terres cultivées montrent que des stratégies de gestion adaptées peuvent atténuer ou, au contraire, accentuer les effets du réchauffement sur les sols. Cette étude met en avant l'importance de promouvoir des pratiques qui renforcent la résilience microbienne, particulièrement dans les terres cultivées, pour préserver les stocks de COS et les cycles des nutriments face aux scénarios climatiques futurs. Elle appelle également à des recherches à long terme pour mieux comprendre les mécanismes microbiens déterminants pour la persistance du COS et pour développer des stratégies adaptées, spécifiques à la profondeur, visant à améliorer la séquestration du carbone et la résilience des agroécosystèmes.

Mots-clés: Réchauffement des sols; Production de CO_2 dans le sous-sol; Respiration des sols; Activité des enzymes extracellulaires; Composition de la communauté microbienne; Gestion des terres agricoles; Hoizons profonds; Rétroaction carbone-climat.

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List of Acronyms

AP	Acid phosphatase
BG	β-1,4-glucosidase
C	Carbon
CEC	Cation exchange capacity
CO_2	Carbon dioxide
DOC	Dissolved organic carbon
EEA	Extracellular enzyme activities
EL-FAME	Ester-linked fatty acid methyl ester
ES	Enzyme stoichiometry
GMN	Gram negative bacteria
GMP	Gram positive bacteria
IPCC	Intergovernmental Panel on Climate Change
KOH	Potassium hydroxide
LAI	Leaf area index
LAP	Leucine aminopeptidase
MTE	Metabolic theory of ecology
N	Nitrogen
NAG	β-1,4-Nacetylglucosaminidase
NaOH	Sodium hydroxide
NPP	Net primary production
O_2	Oxygen
OM	Organic matter
OTUs	Operational taxonomic units
P	Phosphorus
PCA	Principal component analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational multivariate analysis of variance
PLFA	Phospholipid fatty acid
POC	Particulate organic carbon
POM	Particulate organic matter
RCP	Representative Concentration Pathway
SOC	Soil organic carbon
SOM	Soil organic matter
SSP	Shared Socioeconomic Pathway
TDN	Total dissolved nitrogen

List of Definition of Terms

Acid Phosphatase (AP) – An enzyme involved in the hydrolysis of mineral phosphorus in soil.

β-1,4-glucosidase (BG) – An enzyme that breaks down cellulose into glucose, playing a crucial role in the carbon cycle.

Cation Exchange Capacity (CEC) – The ability of soil to hold and exchange positively charged ions, affecting soil fertility.

Carbon Dioxide (CO_2) – A greenhouse gas released during respiration and decomposition processes.

Dissolved Organic Carbon (DOC) – The fraction of organic carbon present in soil water, available for microbial uptake.

Extracellular Enzyme Activities (EEA) – Enzymes secreted by microorganisms that break down organic material.

Ester-linked Fatty Acid Methyl Ester (EL-FAME) – A method used for analyzing microbial community composition in soils.

Enzyme Stoichiometry (ES) – The balance of enzymes related to carbon, nitrogen, and phosphorus cycling.

Leucine Aminopeptidase (LAP) – An enzyme involved in nitrogen cycling, breaking down peptides and proteins.

Metabolic Theory of Ecology (MTE) – A theory linking metabolic rates to ecological processes.

β-1,4-N-acetyl-glucosaminidase (NAG) – An enzyme that breaks down carbon (hydrolysis of SOC) along with nitrogen (chitin), aiding in nitrogen and carbon cycling.

Oxygen (O_2) – Essential for aerobic respiration in soil microbial communities.

Organic Matter (OM) – Decomposed plant and animal residues in the soil-system.

Operational Taxonomic Units (OTUs) – A classification method used in microbial ecology to group similar sequences.

Principal Component Analysis (PCA) – A statistical method used to identify patterns in soil microbial data.

Polymerase Chain Reaction (PCR) – A technique used to amplify DNA for microbial richness and major groups analysis.

Permutational Multivariate Analysis of Variance (PERMANOVA) – A statistical method used to assess differences in microbial communities.

Soil Organic Carbon (SOC) – The carbon component of soil organic matter, crucial for soil fertility and climate regulation.

Soil Organic Matter (SOM) – The organic fraction of soil, including plant and microbial residues.

Total Dissolved Nitrogen (TDN) – The total nitrogen content in soil water, affecting nutrient cycling.

Chapter 1. Introduction

1. General introduction

Globally, almost 3000 Pg C is stored as soil organic carbon (SOC; Köchy et al., 2015), which is the largest biologically active carbon (C) pool in terrestrial ecosystems, storing twice as much C as the Earth's atmosphere and vegetation combined (Friedlingstein et al., 2020). Soils have significant capacity for long-term SOC storage, with an average age of 3100 years down to a depth of 1.0-m (He et al., 2016). Storing SOC, could thus contribute as negative emission technology to the solutions to reduce atmospheric CO_2 concentrations (IPCC, 2018), while delivering many co-benefits (Minasny et al., 2023). Despite these potentials, the true extent to which this SOC storage will contribute to climate change mitigation remains uncertain because soil can be source in addition to sink of CO_2, and the key source of uncertainties stems from poor understanding of SOC turnover processes (Ren et al., 2024). One of the reasons is that research on SOC has predominantly focused on surface soils up to 30 cm (Yost and Hartemink, 2020), despite the fact that subsoils (30-200 cm) contain 68% of the global SOC pool (Jackson et al., 2017). Subsoil SOC cycles much more slowly than surface SOC and typically has longer turnover times (Shi et al., 2020), while still playing an active role in global C cycle (Hicks Pries et al., 2017; Rumpel and Kögel-Knabner, 2011).

A major factor influencing SOC is agriculture, which affects SOC storage through various land management techniques such as tillage, fertilization, irrigation, crop selection and residue management (Emde et al., 2021; Gocke et al., 2023). Indeed, over the past 200 years, agricultural systems have led to an estimated loss of 113 Pg C from top 2.0-m of soils (Sanderman et al., 2017), highlighting the potential for climate mitigation by restoring this ‘C debt’ through improved agricultural management practices that enhance SOC storage and

biogeochemical cycles supporting it (Ma et al., 2022; Rumpel et al., 2020; Soussana et al., 2019).

Given that agricultural land covers nearly 40% of the Earth's land area (FAO, 2020), improving SOC sequestration through improved management presents a critical opportunity to mitigate climate change (IPCC, 2022; Smith et al., 2016). However, such strategies often overlook soil warming as a significant disturbance (Y. Guo et al., 2023) that could interact with management practices, affecting SOC storage and dynamics across all depth of the soil profile.

The Earth's temperature is projected to increase by 4.5°C by 2100 under the shared socioeconomic pathway (SSP) 5-8.5 emissions scenario (Pörtner et al., 2022). Such warming can affect almost all aspects of terrestrial carbon cycling processes and are likely to increase ecosystem C fluxes, with potential feedbacks on the buildup of atmospheric CO_2 concentrations and climate change (IPCC, 2022, 2021; Ito et al., 2020). In soil, warming may affect biota activity as it could increase microbial activity (Conant et al., 2011), extracellular enzyme activity (German et al., 2012), and enzyme synthesis (Meng et al., 2020), while decrease in carbon use efficiency (CUE) was also observed (J. Li et al., 2019). Indeed, SOC dynamics under warming may influenced by substrate availability, soil microflora physiology, and physicochemical factors, all of which may affect SOC dynamics.

Moreover, increased soil temperature may impact soil biodiversity and food webs through alterations in microbial community structures and their stability (e.g., growth rates and species interactions as a network) (Barnes et al., 2024). These changes can disrupt key processes such as nutrient cycling and organic matter (OM) decomposition, given their direct involvement in enzyme production, substrate degradation, and interactions with other soil organisms (Maron et al., 2018; Pandey et al., 2018; Sokol et al., 2022). The stability and complexity of microbial networks determine the resilient of soil ecosystems to climate change and their capacity to maintain key functions that support plant growth and overall ecosystem health (Montoya et al.,

2006; Wagg et al., 2014). By understanding the organization and functioning of microbial communities, we may be able to better predict and manage the impacts of climate change on soil biodiversity and ecosystem stability.

The general objective of this Ph.D. thesis was to investigate the effects of agricultural land management practices, specifically cropland and grassland, on whole-soil warming (+4°C) and its effects on biogeochemical carbon-cycling and soil functioning. This understanding is crucial for assessing the role of soil in global C cycle and its implications for climate adaptation, aiming to enhance soil C sequestration and agroecosystem resilience by improved management in the face of global climate change. This study had several specific objectives. At first, I reviewed the literature on how agricultural management practices and soil warming affect soil C profiles to identify research gaps that laid the foundation for this study (**Chapter 1)**. To address these research gaps, I utilized an *in-situ* whole-soil warming (+4°C) experiment started in 2021, extended to 1.0-m depth under contrasting management practices: cropland and grassland. Initially, I examined the warming responses of CO_2 production at different soil depths and CO_2 efflux from topsoil during various seasons under both management practices. This investigation aimed to understand how warming interacts with management to affect seasonal soil CO_2 efflux and its origin within the soil profile (**Chapter 2**). Next, I studied how soil warming and management influence soil microbial communities and their network complexity in the whole-soil profile. This objective sought to assess the microbial resilience and soil functionality in agroecosystems (**Chapter 3**). Finally, I explored the responses of microbial communities and extracellular enzyme activities to warming under contrasting management. This investigation aimed to reveal how warming interacts with land management to affect microbial activity in relation to SOC dynamics and nutrient cycling (**Chapter 4**).

We tested the following hypotheses:

H1: CO_2 efflux will increase year-round in soils under continuous grassland but only seasonally in cropland soils, while CO_2 production will increase across soil depth in grassland but not cropland soils, because grasslands having greater root and microbial activity, as well as consistent root-derived C inputs throughout the year;

H2: Warming may affect the composition of microbial communities according to soil depth, and the magnitude of these effects may be management-specific due to different intensity of land management practices;

H3: The complexity of microbial network may collapse in response to warming, depending on management practices, as warming-induced reductions in soil water content may be land management-specific;

H4: The warming response of C-, N-, and P-acquiring enzyme activity in different soil depth may be depending on management due to the presence of microbial communities with contrasting functionality and resilience.

Literature review and syntheses

2. Agricultural land management practices that affect soil carbon profile

Plants are the primary contributors of carbon (C) to soils, whether through aboveground and belowground litter or via root exudates (Fan et al., 2023; H. Li et al., 2024). Agricultural land management practices can thus modify the distribution of C along the vertical soil profile through their effect on plant communities (Müller et al., 2016; Dal Ferro et al., 2020; Hu and Chabbi, 2022). For example, different agricultural practices, driven by crop species selection can influence both the quantity and quality of above- and below-ground net primary production (NPP), which serve as C inputs and SOM precursors (Dietzel et al., 2017; Hirte et al., 2021; Liang et al., 2022; Peixoto et al., 2022). Among these inputs, root C and rhizodeposition have been proposed as dominant source of SOC and root-derived C may contribute more to refractory SOC pools than aboveground residues (Berti et al., 2016; Cotrufo et al., 2015; Kätterer et al., 2011; Sierra et al., 2024). Indeed, dead root biomass, rhizodeposition, and exudates are more prone to microbial transformation and stabilization processes than aboveground plant litter (Prescott and Vesterdal, 2021; Rasse et al., 2005). These processes, including bioturbation, decomposition, humification, polymerization, and the immobilization of organic C (Jastrow et al., 2007; Müller et al., 2016; Paterson et al., 2008; Prescott and Vesterdal, 2021; Witzgall et al., 2024), leading to build-up of persistence microbial necromass and metabolites, which are key constituents of stable SOC accumulation within the soil matrix (Cagnarini et al., 2019; Cotrufo et al., 2013; Gleixner, 2013). Additionally, microbial C use efficiency and necromass production may be crucial for both the stabilization and destabilization of SOM, and have a significant influence on SOC dynamics along soil profiles (Rumpel and Kögel-Knabner, 2011; Xu et al., 2013; Dynarski et al., 2020; Prescott and Vesterdal, 2021; Witzgall et al., 2024). However, the effectiveness of these processes can be influenced by agricultural management

practices and site-specific factors such as climate, parent material, vegetation type, and soil characteristics (details in Table 1). All of these factors significantly influence (i) the nature of SOM, including its protection in soil aggregates, associated with minerals, or partially decomposed litter residues, (ii) the abundance, diversity and functional traits of the decomposer community, and (iii) the availability of oxygen, water and nutrients along soil profiles (details in Table 2).

Generally, the rate of C inputs to subsoils is much lower than surface soils, and the rate of C release back to the atmosphere is slower—evidenced by the older age of C in deeper soil profiles (Shi et al., 2020). However, evidence suggests that various processes, such as the leaching of dissolved and particulate organic matter, bioturbation, stratified root C inputs, and differing C turnover rates can significantly influence the distribution of SOC storage along soil profiles (Rumpel and Kögel-Knabner, 2011). These processes are often specifically altered by different agricultural management practices (details in Table 3). For instance, tillage-induced physical redistribution, common in annual cropland, tends to alter physical mixing of soil particles, water transport, advective movement of C, and vertical distribution of annual dead root C inputs and microbial activity (Sierra et al., 2024). This may act as a more significant mechanism for the formation of soil C profiles than no-tillage practices observed in perennial grassland (Hicks Pries et al., 2023; Sierra et al., 2024). Additionally, applying various rates of inorganic fertilizer, organic amendments (i.e., biochar, compost, biosolid), and different irrigation rates may significantly influence the regulatory mechanisms of SOC pathways along soil profiles due to their impacts on microbial activity, depending on land management practices and cropping systems (Table 3).

Table 1. Influence of agricultural practices and site-specific factors on various processes related to soil organic carbon (SOC) dynamics.

Process	Description	Influence of agricultural practices	Influence of site-specific factors	References
Plant C inputs	Plant contributes SOC through above- and below-ground litter, root exudates and rhizodeposition	Crop species selecting, type of vegetation, tillage, and cover cropping ↑/↓ quantity and quality of C inputs	Climate (temperature, rainfall), parent material, and soil texture ↑/↓ decomposition rates and microbial activity	(Kuzyakov and Domanski, 2000; Lal, 2004; Moinet et al., 2020; Panettieri et al., 2020; Rasse et al., 2005; P. Smith et al., 2015)
Root C and rhizodeposition	Root C and rhizodeposition are key source of SOC, with roots contributing to stable SOC pools	Root biomass and turnover rates ↑/↓ depending on crop type, root architecture, irrigation, and fertilization practices	Temperature, soil pH, nutrient availability, vegetation types and moisture ↑/↓ root growth and exudation rates	(Chandregowda et al., 2023; Fan et al., 2016; Guasconi et al., 2023; Poirier et al., 2018; Rasse et al., 2005; J. Wang et al., 2021; Zhao et al., 2023)
Microbial transformation and stabilization	Microbial processes decompose plant inputs, contributing to stable SOC formation through necromass build-up	Practices like organic amendments, reduced tillage, crop diversification and crop rotations ↑ microbial activity and SOC stabilization	Vegetation type, soil characteristics, and temperature ↑/↓ microbial community composition and CUE	(Butcher et al., 2020; Kallenbach et al., 2019; Kang et al., 2022; Y. Li et al., 2024; Luo et al., 2017; Spohn et al., 2016; Wang et al., 2018; Wu et al., 2021)
Bioturbation	Soil mixing by organisms (e.g., earthworms) redistribute OM, influencing SOC dynamics	No-till farming and maintaining vegetation cover ↑ soil biota activity and bioturbation; tillage ↓ long-term bioturbation due to mechanical disturbance	Climate, vegetation and soil type ↑/↓ soil organism activity and distribution, subsequently ↑/↓ bioturbation rate	(Arias et al., 2020; Bossuyt et al., 2005; Creamer et al., 2022; Kraus et al., 2023; L. Ma et al., 2022; Michel et al., 2022; Ponge et al., 2013; Vion-Guibert et al., 2024)
Humification and polymerization	Conversion of plant residues to humus and complex organic polymers that are stable in soil as SOC	Organic amendments (e.g., compost, manure, biosolid) and reduced tillage ↑ humification processes	Climate, parent materials and vegetation type ↑/↓ influence the composition of humic substances formed	(Jastrow et al., 2007; Lehmann and Kleber, 2015; Stevenson, 1994; Wagai et al., 2008; Wiesmeier et al., 2019; Xu and Tsang, 2024)
Immobilization of organic C	Temporary retention of C in microbial biomass or SOM	Fertilization (especially N addition) and organic amendments ↑/↓ microbial C immobilization	Temperature, soil nutrient status, texture, and moisture ↑/↓ the rate of C immobilization by microorganisms	(Barrett and Burke, 2000; Y. Cheng et al., 2017; Kaye and Hart, 1997; Kirschbaum, 1995; Manzoni and Cotrufo, 2024; Patel et al., 2010; Qiu et al., 2008)
Microbial carbon use efficiency (CUE)	Efficiency of microorganisms in converting C inputs to biomass rather than CO_2, affecting SOC dynamics	Practices that enhance SOM (e.g., cover cropping) ↑ microbial CUE and SOC stabilization	Temperature, soil texture, moisture, and soil nutrient levels ↑/↓ microbial metabolisms and CUE	(Adingo et al., 2021; Butcher et al., 2020; Cruz-Paredes and Rousk, 2024; Dang et al., 2024; Dong et al., 2022; Manzoni et al., 2012; Mganga et al., 2022; Spohn et al., 2016; Tao et al., 2023; Zheng et al., 2019)
Microbial necromass accumulation	The build-up of microbial cell residues, a key component of stable SOC	Practices like reduced tillage and organic amendments ↑ microbial necromass accumulation, contributing to SOC	Climate, soil type, and vegetation ↑/↓ microbial turnover rates and necromass stabilization	(Islam et al., 2022; Kallenbach et al., 2016; Kästner et al., 2021; Y. Li et al., 2024; Liang et al., 2019; Miltner et al., 2012; Ni et al., 2020; Prommer et al., 2020; Q. Wang et al., 2024)

Here, the signs ↑ indicate positive, ↓ indicate negative, and ↑/↓ indicate positive and negative influences.

Table 2. Key factors influencing the nature of SOM, decomposer community, micro-environmental conditions along soil profile.

Factors	Influence on (i) Nature of SOM	Influence on (ii) Decomposer community	Influence on (ii) Availability of oxygen, water and nutrients	Influence along the soil profile	References
Root-derived C (exudates, rhizodeposition)	↑ aggregation, leading to physical protection of SOM within soil aggregates; ↑ mineral associations	↑ microbial abundance and diversity; stimulates bacterial communities for decomposition	↑ nutrient cycling and availability due to increased microbial activity and exudate-driven nutrient release	Concentrated in topsoil (0-30 cm) with limited exudation below	(Cotrufo et al., 2015; Fan et al., 2023; W. Li et al., 2024; Six et al., 2002)
Aboveground litter	↑ substrates for POM formation; partially decomposed residues contribute to active SOM fractions	↑ fungal decomposer activity, particularly with high lignin residues; ↑ functional diversity due to varied substrates	Litter quality affect water retention; high lignin slows decomposition and nutrient release	Impacts primarily topsoil (0-10 cm) with negligible effect in deeper layers	(Ekschmitt et al., 2008; Von Lützow et al., 2008)
Carbon use efficiency (CUE), necromass production	↑ CUE promotes microbial biomass and necromass, contributing to stable SOM; ↑ organo-mineral complex formation	↑ efficient microbial community; increased necromass enhances the microbial contribution to SOM stabilization	↑ efficient nutrient use reduces nutrient leaching; ↑ necromass improves soil aggregation, affecting O_2 and H_2O diffusion	↓ with depth due to reduced C inputs and limited O_2, but necromass contributes to stabilization in subsoil	(Gleixner et al., 2002; Nannipieri et al., 2003; Q. Zhang et al., 2023)
Bioturbation, polymerization, humification	↑ SOM protection via deeper burial of; ↑ polymerization process forms stable humic substances	↑ abundance and activity of decomposer fauna (e.g., earthworm, ants); specialized microbes involved in humification	↑ soil structure and porosity, enhancing O_2 diffusion and water infiltration	Active in surface layers; slower reliance on decomposers in subsoil (>30 cm)	(Paterson et al., 2008; Prescott and Vesterdal, 2021)
Tillage, crop rotation, cover cropping (e.g., annual crop vs. perennial crop)	↓ tillage disrupts aggregates, exposing SOM to decomposition; ↑ crop rotation and cover cropping increase SOM inputs, enhancing stability	↓ tillage reduces microbial diversity; ↑ crop rotations promote a varied microbial community	↑ tillage increase O_2 availability but ↓ water retention; ↑ crop rotation improves nutrient cycling	Tillage impacts topsoil (0-30 cm); crop rotations affect SOM and nutrient profiles down to 1.0-m	(Berti et al., 2016; Dal Ferro et al., 2020; Hu and Chabbi, 2022)
Climate, soil texture, mineralogy	↑ soil texture (e.g., clay) stabilizes SOM via mineral association; ↑ mineral weathering influences decomposition rates	↑/↓ climate (temperature, moisture) impacts microbial activity; ↑/↓ texture (e.g., clay) influences microbial habitat availability	↑ clay content improves water retention; ↑/↓ climate affects O_2 diffusion and nutrient leaching	↑ clay content increased with depth; ↓ microbial activity and decomposition rates with depth	(Button et al., 2022; Chabbi et al., 2009; Davidson et al., 2012; de Sosa et al., 2018)
Fertilization (organic vs. inorganic), irrigation	↑ organic fertilization improves SOM quality; ↑/↓ irrigation influences SOM turnover	↑ organic inputs promote a diverse microbial community; ↑ inorganic fertilizers favor fast-growing microbes	↑ organic fertilizers enhance nutrient availability; ↑/↓ irrigation influences microbial processes	Organic amendments affect primarily in topsoil; limited effects below 50 cm	(Jangid et al., 2011; Kuramae et al., 2012; Wang et al., 2017)

Here, the signs ↑ indicate positive, ↓ indicate negative, and ↑/↓ indicate positive and negative influences.

Table 3. Influence of various processes with agricultural management practices on soil organic carbon (SOC) distribution along the soil profile.

Processes and factors	Description	Influence of SOC distribution	Alteration by agricultural practices	References
Stratified root C inputs	Variation in root biomass and exudate inputs along the soil profiles (shallow vs. deep-rooted plants)	↑ SOC near root zones, particularly in deeper layers for deep-rooted species	Annual crops (shallow roots) ↑ SOC in topsoil; deep-rooted perennials ↑ C inputs at greater depths	(Guan et al., 2016; Hicks Pries et al., 2023; Hirte et al., 2018; Hu and Chabbi, 2021; Samson et al., 2021; Sierra et al., 2024)
Physical redistribution by tillage	Redistribution of soil particles and OM due to mechanical disturbance	↑ mixing of SOC across topsoil; ↓ aggregates stability, exposing SOM to decomposition	Intensive tillage ↓ SOC stability by redistributing OM; no-tillage ↑ soil structure and limits OM redistribution	(Button et al., 2022; Haddaway et al., 2017; Hicks Pries et al., 2023; Yoo and Wander, 2008)
Carbon turnover rates	Rate at which organic C is decomposed and transformed into microbial biomass or released as CO_2	↑ turnover in upper layers due to higher microbial activity; ↓ turnover in deeper layers stabilizes SOC	Tillage ↑ turnover by increasing aeration and microbial exposure; no-tillage ↓ turnover, enhancing SOC stability in deeper layers	(Dove et al., 2021a; Eilers et al., 2012; Lal, 2018; Li et al., 2014; Rumpel and Kögel-Knabner, 2011; Salomé et al., 2010; Schmidt and Martínez, 2019)
Bioturbation	Physical mixing of soil through soil faunal activity (e.g., earthworms, ants)	↑ vertical mixing of OM, ↑ SOC in subsoil layers	Intensive tillage ↓ bioturbation by disrupting faunal activity; conservation agriculture ↑ soil fauna activity	(Kautz et al., 2013; Mueller et al., 2024; Schlüter et al., 2022; Schnoor et al., 2011; Sierra et al., 2024)
Leaching of dissolved and particulate organic matter	Downward movement of soluble organic compounds and particulate OM via water flow	↓ SOC in upper layers; ↑ SOC accumulation in deeper layers due to leaching	Intensive irrigation and high rainfall ↑ leaching; organic amendments (e.g., compost, biochar) ↓ leaching by ↑ C retention	(Button et al., 2022; Ota et al., 2013; Rumpel and Kögel-Knabner, 2011; Sierra et al., 2024; Yin et al., 2022)
Use of organic amendments (e.g., biochar, compost, biosolid)	Addition of organic materials to improve soil quality and increase C inputs	↑ SOC storage via stable SOM inputs; ↓ leaching and erosion	Biochar ↑ SOM stability; compost ↑ labile C promoting microbial activity; biosolid ↑ nutrient availability	(Li et al., 2014; Paustian et al., 2016; Peixoto et al., 2022; Rumpel et al., 2012; Six et al., 2002; Tautges et al., 2019)
Application of inorganic fertilizers	Use of chemical fertilizers (e.g., nitrogen, phosphorus) to enhance crop growth	↑SOC inputs through enhanced plant biomass; ↓ SOC stability with accelerated decomposition	High application rates ↑ microbial activity and ↑ C turnover; balanced use ↑ SOC sequestration via ↑ plant productivity	(Y. Li et al., 2022; Naasko et al., 2023; F. Yang et al., 2022)
Irrigation rates	Regulation of water application to crops demand	↑ SOC inputs via ↑ plant productivity; ↓ SOC stability with excessive leaching	Optimal irrigation ↑ microbial activity and plant growth, excessive irrigation ↓ microbial activity and ↑ leaching	(Al-Shammary et al., 2024; Denef et al., 2008; Naasko et al., 2023; Núñez and Schipanski, 2023; Sharma et al., 2011)

Here, the signs ↑ indicate positive, ↓ indicate negative, and ↑/↓ indicate positive and negative influences.

Recent studies suggest that under arable field conditions, biological activity such as root growth and soil fauna activity (e.g., earthworms) leads to the formation of biopores, which not only create a connection between surface and subsoil for C and nutrients but also significantly enhance microbial activity and accelerate SOM turnover in the subsoil (Athmann et al., 2017; Hoang et al., 2017). Perennial root systems are notably more effective than annual root systems for the creation of stable and continuous biopores due to differences in root morphology (deep vs. shallow rooting system) and soil management practices (no-tillage vs. tillage interventions) (Islam et al., 2024; Or et al., 2021; Wendel et al., 2022). Furthermore, these agricultural and soil management practices influence the input of labile C through leaching of dissolve organic C (DOC) and preferential inputs of particulate organic C (POC) along the soil profiles, both of which are affected by the microstructural properties of biopores (Athmann et al., 2017; Schmidt and Martínez, 2019; Si et al., 2024). For example, grassland soils often have larger macropore diameters due to the presence of large biopores, which are periodically destroyed by tillage practices in croplands (Schlüter et al., 2022; Schnoor et al., 2011; Yang et al., 2022). This may significantly influence the physicochemical protection mechanisms that regulate SOC turnover rates and microbial access to SOC, thereby affecting both the stabilization and destabilization of SOC along soil profiles (Kleber et al., 2011; Moinet et al., 2020). Another recent study evidenced that the application of lime in C-rich Andosol in temperate climate led to induced distinct shifts in microbial community composition and functional profiles in both surface and subsoil, and indicating that SOC mineralization in the topsoil relies on microbial composition and function, while in the subsoil it is more strongly tied to the quality and quantity of the DOC (Y. Li et al., 2022).

Based on the above discussion and recent literature reviews (e.g., Button et al., 2022; Sierra et al., 2024), it is evident that agricultural land management practices have a profound

impact on soil C profiles. The remaining question is how these soil C profiles will respond to global climate change, another key factor affecting SOC dynamics.

3. Soil warming: A key disturbance influencing SOC dynamics and soil health

Soil warming, driven by global climate change, is increasingly recognized as a critical factor shaping SOC dynamics, microbial activity, and overall soil functionality (Y. Guo et al., 2023). Rising temperature induce significant changes in biological and chemical processes within soil systems, affecting SOC storage and nutrient cycling. The effects are complex and multifaced, often leading potential feedbacks to the climate systems through alterations in soil C fluxes mediated by microbial processes (Ito et al., 2020; Ren et al., 2024).

The decomposition of SOM, a key process of SOC turnover, is highly temperature-dependent. Elevated temperatures accelerate the breakdown of organic substrate, releasing CO_2 into the atmosphere and potentially triggering a positive feedback loop that exacerbates climate change (Allison et al., 2010; Ito et al., 2020; Ren et al., 2024). Conversely, warming may also influence SOC stabilization through processes such as aggregation, mineralization, and the formation of stable organo-mineral complexes (Dijkstra et al., 2021; Liang et al., 2017). The net effect of warming on SOC—whether it leads to a C gain or loss—depends on the balance between these competing processes (Hicks Pries et al., 2023). As key regulator of SOM decomposition, microbial communities play a central role in SOC turnover and nutrient cycling through their direct involvement in enzyme production, substrate degradation, and interactions with other soil organisms (Maron et al., 2018; Pandey et al., 2018; Sokol et al., 2022). Soil warming can influence these microbial processes by increasing microbial activity (Conant et al., 2011), enhancing extracellular enzyme kinetics (German et al., 2012), and stimulating enzyme synthesis (Meng et al., 2020), although it may also reduce CUE (J. Li et al., 2019). These changes, in turn, interact with factors, such as substrate availability, soil microflora physiology, moisture content and physicochemical conditions, all of which may affect SOC

stabilization and turnover rates under warming scenarios (Hicks Pries et al., 2023; Guo et al., 2023). As a result, the response of microbial diversity and activity to warming has significant implications for soil fertility and ecosystem services that soil provides.

Warming also may affects aggregate stability by altering microbial composition and activity, the quality of organic matter, and interaction between organic materials and minerals particles (Wang et al., 2016; Zhou et al., 2023). These changes occur because warming accelerates microbial-driven decomposition processes, often reducing the organic compounds that bind soil particles together, thereby impacting soil architectures (Zhou et al., 2023). This reduction in aggregate stability could lead to increased erosion, compaction, and the degradation of soil macrostructural properties such as biopores (Islam et al., 2024), which are critical for maintaining soil porosity and water infiltration (Liu et al., 2023; Regelink et al., 2015). Ultimately, these changes could degrade soil health, diminishing its capacity to retain water and nutrients essential for plant growth and ecosystem functionality.

However, warming responses of SOC dynamics are likely to vary with soil depth, with subsoil potentially being less sensitive to warming than surface soil, as suggested by kinetic theory. Subsoil C fractions often consist of simpler, necromass-derived compounds compared to the more complex SOC found in surface soil, which may make then less prone to warming-induced changes (Davidson and Janssens, 2006). Additionally, subsoil may experience less impact from warming-induced declines in CUE, which predominantly affect more complex substrates (Frey et al., 2013). The warming response of extracellular enzyme activity typically remains consistent or decreases with depth (Steinweg et al., 2018; Zuo et al., 2021), and the higher proportion of mineral-associated and physically protected SOC in subsoil, compared to free particulate organic carbon (POC), could further reduce the response of subsoil SOC to warming (Benbi et al., 2014; Gillabel et al., 2010; Qin et al., 2019). Despite lower decomposition rates in subsoils than in surface soils, recent studies have shown that part of

subsoil SOC could be more responsive to warming than previously assumed (Hicks Pries et al., 2017; Nottingham et al., 2020; Ofiti et al., 2021; Zhang et al., 2016), indicating that subsoil SOC dynamics should not be overlooked in climate warming scenarios. Indeed, the representative concentration pathway (RCP) 8.5 scenario predicts a global increase in subsoil temperatures by 4.5°C by 2100, nearly in synchrony with surface soil and air temperatures (Soong et al., 2020). Thus, soil warming may affect SOC in subsoil in addition to agricultural management (discussed previous section) and their combined effect must be considered to evaluate soil responses to climate change and to adapt management to its effects.

In the following section, we will test the hypothesis that the scientific literature has predominantly emphasized surface soil research compared to subsoil research in studying SOC responses to global climate change and agricultural land management practices.

4. Historical trends of subsoil SOC research focusing on climate change and agricultural land management practices

Given that most SOC is stored in subsoils (>30 cm), which have a greater potential to store persistent SOC compared to surface soils due to reduced microbial activity, physical protection, chemical recalcitrant, lower disturbance, and slower C turnover rates (Jackson et al., 2017; Button et al., 2022), we hypothesized that the scientific literature has predominantly emphasized surface soil research compared to subsoil research in studying SOC responses to global climate change and agricultural land management practices. We therefore conducted a literature search (see Query 1 to 6) to quantify the level of interest in these topics over the last 30 years (from 1998 to 2023).

We searched peer-reviewed articles published up to 2023 using the Web of Science platform with the following search terms (**Query 1**): *TS= (soil* AND ("carbon"))* as topic, meaning that all articles mentioning the topic of soil carbon in their title, abstract and/or

keywords were displayed. We then performed a more focused search for articles dealing with "soil organic carbon (SOC)" (**Query 2**), "surface soil SOC" (**Query 3**), "subsoil SOC" (**Query 4**), "subsoil SOC and global climate change" (**Query 5**), and "subsoil SOC and agricultural land management" (**Query 6**). All the data search was performed in June 2024.

Query 1: Soil carbon research

TS= (soil AND ("carbon"))*

Query 2: Soil organic carbon (SOC) research

TS= ("soil organic carbon" OR "soil organic matter")

Query 3: SOC research focused on surface soil

TS= (soil organic carbon AND ("surface soil" OR topsoil$ OR "A horizon"))*

Query 4: SOC research focused on subsoil

TS= (soil organic carbon AND ("deep soil" OR subsoil$ OR "B horizon"))*

Query 5: SOC research focused on subsoil and global climate change

TS= (soil organic carbon AND ("deep soil" OR subsoil$ OR "B horizon") AND ("global change" OR "climate change"))*

Query 6: SOC research focused on subsoil and agricultural land management

TS= (soil organic carbon AND ("deep soil" OR subsoil$ OR "B horizon") AND ("land management" OR "agricultural management" OR "managed agroecosystem" OR "agroecosystem"))*

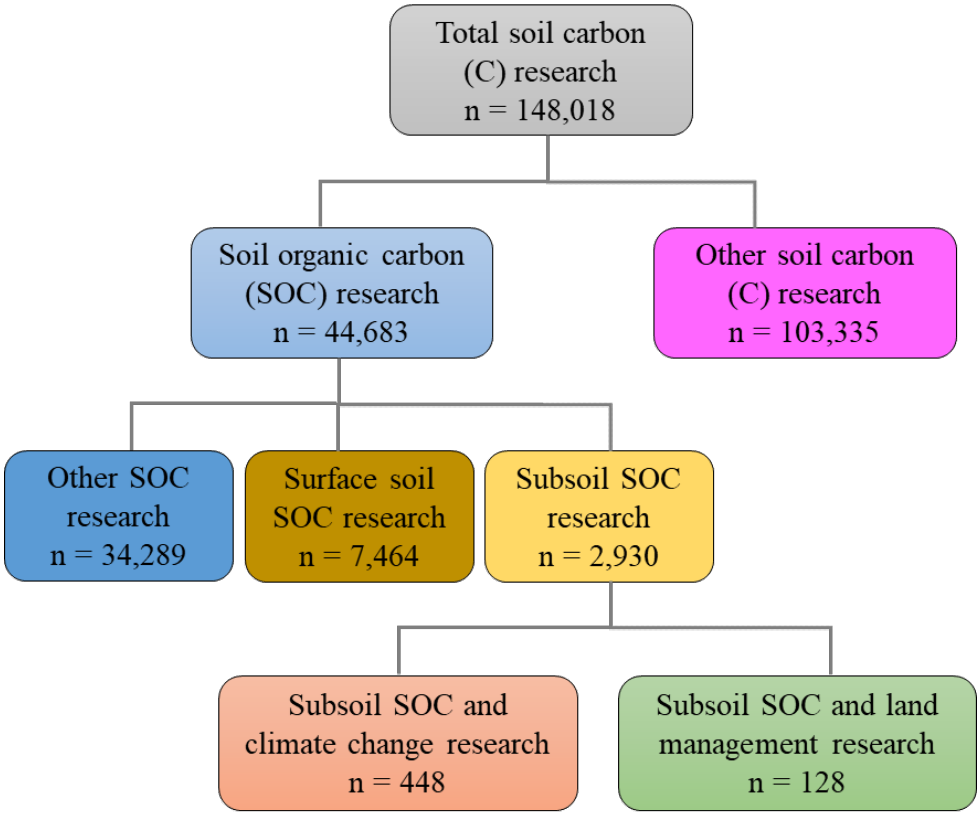


Fig. 1. Systematic scheme showing global soil C research articles addressing SOC and other soil C research. This study focuses only on global research on SOC pools, with a particular focus on subsoil SOC in response to global climate change and agricultural land management practices. Here, number of articles in the Web of Science from 1998 to 2023.

During the search query, we keep the peer-reviewed articles by specifying the filter elements: document types (article, review article, proceeding paper, data paper, early access, book chapters) and language (English). The results were then plotted as the cumulative number of articles published from 1998 to 2023. Results showed that 44,683 articles on SOC were published from 1998 to 2023, representing 30.2% of total soil C research (148,018). Of these, only 6.5% of studies focused on subsoil SOC (2,930), and within this 6.5%, only ≤1% focused on the effects of global climate change (448) and ≤0.3% focused on agricultural land management practices (128) (Fig. 1). However, over the last 30 years, the number of articles dealing with "soil organic carbon (SOC)" has increased, but the proportion of articles also addressing subsoil SOC in response to climate change and agricultural land management

practices has remained similar (Fig. 2). Thus, we conclude that despite the importance of subsoil SOC for climate mitigation and planetary health, the impact of global climate change and agricultural land management practices on subsoil SOC dynamics has not yet received the attention it deserves. These research gaps highlight the need for a deeper understanding of subsoil C dynamics and vulnerabilities, especially regarding land management and climate change, to develop strategies that enhance SOC storage, improve management, and safeguard food security and planetary health.

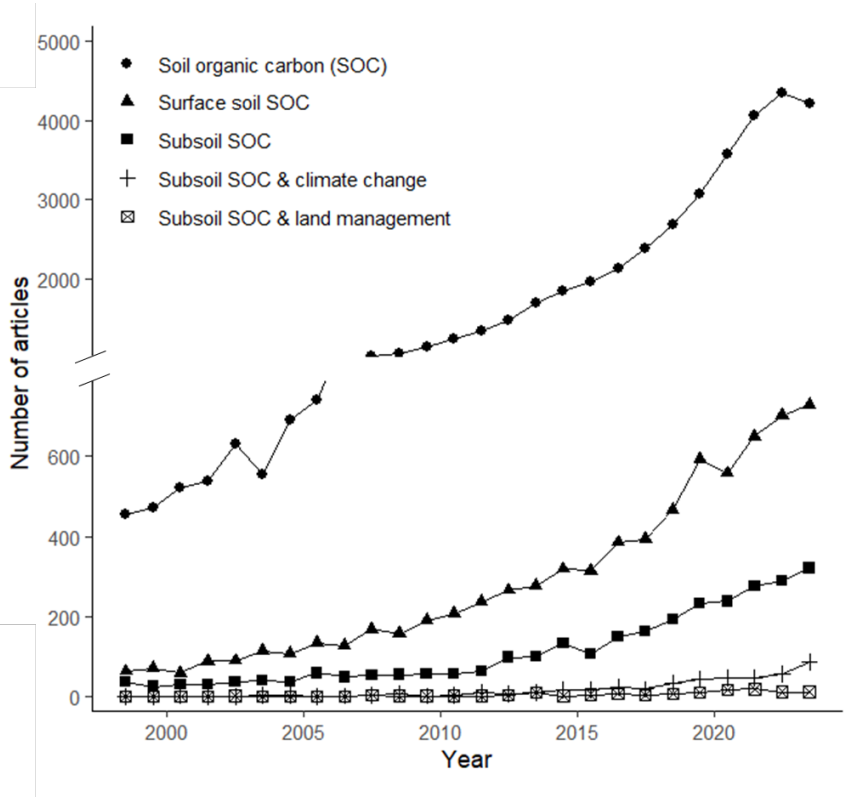


Fig. 2. Cumulative number of articles published on the topic of soil organic carbon (SOC) from 1998 to 2023. Literature search performed on the Web of Science platform in June 2024.

In the following, we will discuss those research gaps including the importance, source, stability, and responses of subsoil SOC to global change climate and agricultural management practices based on peer-reviewed articles. These climate change and management practices have been chosen to depict the most important drivers of SOC dynamics at the field and/or landscape levels.

5. Importance and source of subsoil carbon

Unlike surface soil C, which is more dynamic and susceptible to rapid decomposition, subsoil C is often more stable and can remain in the soil for longer periods, ranging from centuries to millennia (Rumpel et al., 2012; Shi et al., 2020). This stability is due to higher physicochemical protection, lower disturbance from agricultural practices, limited microbial activity, and slower C turnover rates (Jackson et al., 2017; Cagnarini et al., 2019; Button et al., 2022). As a result, subsoil C acts as a significant long-term C sink, helping to mitigate atmospheric CO_2 levels and combat climate change (Button et al., 2022; Rumpel and Kögel-Knabner, 2011). Moreover, subsoil C is crucial for maintaining soil health and fertility, as it supports root growth, improves water retention, and enhances nutrient availability, all of which are vital for ecosystem productivity (Hinzmann et al., 2021; Sierra et al., 2024). In agricultural systems, plant roots extend beyond 25 cm, where subsoil C becomes a critical source of nutrients for crop yield, especially as surface soils dry out rapidly in summer, limiting roots' ability to absorb water and nutrients from the upper soil layers (de Moraes et al., 2023; Hao et al., 2021). Therefore, recognizing the importance of subsoil C reservoir is pivotal for informed decision-making in agriculture, soil management, and climate change mitigation efforts, including food security for planetary health, making it a research priority.

Furthermore, emerging studies (Rumpel and Kögel-Knabner, 2011; Button et al., 2022) have shown that the primary inputs of C to subsoils include: (1) root-derived C (both dead roots and living root rhizodeposition); (2) leaching of DOC (dissolved organic C) from the surface soil; (3) transfer of POM (particulate organic matter) from surface soil through lixivation, faunal bioturbation, and/or preferential flow; and (4) microbially-derived C. However, the precursors of SOM within the soil profiles can vary depending on agricultural practices (Sierra et al., 2024; Cagnarini et al., 2019; Liang et al., 2022; Samson et al., 2021; Schmidt and Martínez, 2019). This variation arises because crop species selection influences both the quantity and quality of

aboveground and belowground net primary production (NPP), which serve as C inputs and SOM precursors (Dietzel et al., 2017; Hirte et al., 2021; Liang et al., 2022; Peixoto et al., 2022).

6. Stability and response of subsoil carbon to global change

It has long been assumed that SOC stored in subsoil is relatively stable and resistant to changes induced by agricultural management practices. This assumption was mainly based on radiocarbon dating studies, which suggest that subsoil C may be centuries to millennia old (Hobley et al., 2017; Wang et al., 1999). While decomposition rates in subsoils may be slower than those in surface soils, studies show that part of subsoil SOC may exhibit faster turnover rates due to preferential inputs and accumulation of labile OM in subsoil (Chabbi et al., 2009; Rumpel and Kögel-Knabner, 2011), which can be influenced by agricultural practices (Hicks Pries et al., 2023; Sierra et al., 2024). These inputs can induce positive priming effects, stimulating microbial activity and driving the decomposition of fresh OM and native SOC in subsoils (Fontaine et al., 2007). Additionally, subsoil SOC appears to be more responsive to warming than previously assumed, as increased temperatures stimulate microbial activity, accelerating subsoil SOC decomposition and undermining its long-term stability (Hicks Pries et al., 2017; Nottingham et al., 2020).

The stability of subsoil SOC was found to be related to its physical inaccessibility and lack of energy input when it is protected within the soils' architecture (Salomé et al., 2010). However, abiotic changes, such as warming and altered moisture regimes, can compromise this protection. For example, soil warming experiments, such as those conducted by Hicks Pries et al. (2017) and Soong et al. (2021), have demonstrated significant subsoil SOC losses under warming, attributing these outcomes to enhanced microbial activity. This microbial activity in subsoil could be highly temperature-sensitive, with warming alleviating resource limitations and enabling microbes to degrade both fresh and existing SOC (Bernard et al., 2022; Dove et al., 2021; Fontaine et al., 2007). Additionally, altered moisture conditions, such as increased

drying-rewetting cycles under warming, can exacerbate SOC mineralization and disrupt nutrient cycling, intensifying the loss of native subsoil SOC (S. Zhang et al., 2023). These cycles affect soil aggregate stability, reducing the physical protection of subsoil C and increasing its accessibility to microbial decomposition (Gao et al., 2024).

While deep-rooting plants are often promoted as a strategy to stabilize subsoil SOC (Button et al., 2022), their effects under warming may vary. Although their roots and exudates contribute to subsoil SOC inputs (Hicks Pries et al., 2018; Rasse et al., 2006; Suseela et al., 2017), elevated temperatures combined with high nutrient availability can intensify microbial processes that degrade both fresh and existing subsoil SOC, potentially offsetting the intended sequestration benefits (Siegwart et al., 2023). Furthermore, altered moisture regimes under climate change, such as drought stress, can compromise the efficiency of deep-rooting plants, reducing their potential to stabilize subsoil SOC pools.

In this context, agricultural practices may play a critical role in mediating subsoil SOC dynamics, especially under warming (Sierra et al., 2024). Practices such as deep plowing, which exposes buried SOC to surface soil, could amplify SOC losses under warming, further challenging efforts to enhance long-term subsoil SOC storage. Conversely, reduce tillage and cover cropping may promote SOC stabilization by minimizing soil disturbance and providing consistent C inputs (Button et al., 2022). However, warming can modulate these benefits. For instance, deep-rooting crops that enhance subsoil SOC inputs may become less effective under drought stress or prolonged warming periods, as these conditions alter root physiology and their associated microbial activity (Adkins et al., 2016; Poirier et al., 2018; Rossi et al., 2020). Additionally, management-induced changes interact with warming to modify subsoil microbial communities. For example, inorganic fertilization and the burial of exogenous OM inputs can alter microbial structure by increasing subsoil DOC availability via leachates from surface soils (Li et al., 2014; Major et al., 2010). These changes, coupled with warming, may disrupt

microbial communities adapted to nutrient-poor environments, favoring r-strategists that rapidly exploit labile C inputs, thereby destabilizing subsoil SOC pools (Cressey et al., 2018; de Sosa et al., 2018; Jones et al., 2018).

Furthermore, emerging studies have shown that subsoil SOC may respond differently to global climate change compared to surface soil, owing to distinct biogeographic patterns of microbial diversity and their functional responses to these changes (L. Cheng et al., 2017; Jia et al., 2017; Li et al., 2018; Liu et al., 2019). Consequently, agricultural practices influence the vertical gradient of abiotic and biotic factors throughout the soil profile, which affects the mechanisms controlling SOC inputs, losses, transformations and stabilization (Hicks Pries et al., 2023; Liu et al., 2019). If global climate change alters any of the factors as a function of agricultural land management practices, then SOC reservoirs in the subsoil may be affected depending on the specific management practices in response to global climate change.

Although a growing body of evidence suggest that enhancing subsoil C storage can contribute to reducing atmospheric CO_2 concentration, thereby supporting global climate mitigation efforts while simultaneously promoting soil resilience, fertility and ecosystem productivity (Button et al., 2022). Despite this potential, the true extent of these benefits remains uncertain due to the lack of *in-situ* field experiments assessing how subsoil SOC responds to climate change, such as warming and altered moisture conditions, under diverse agricultural land management practices (e.g., species selection, tillage, fertilizer uses and irrigation rates). Such studies are crucial to understanding the stability and dynamics of subsoil SOC in a changing global climate. Without *in-situ* empirical evidence, ensuring the reliability and effectiveness of subsoil SOC as a keystone of climate mitigation strategies is challenging. Advancing *in-situ* field-based research is imperative to unlock the full potential of subsoil SOC in addressing global climate challenges.

Moreover, it is urgent to explore how global change will impact the stability of subsoil microbial community networks, allowing us to quantify the effects of warming on the resistance and/or resilience of these microbes and their associated functions to further climate change-related disturbances (details below).

7. Soil microbial network stability and ecosystem health in responses to global change

Soil microbiomes are critical for maintaining the soil health and regulating ecosystem multifunctionality (Karimi et al., 2017; Wagg et al., 2014). Microbial communities exchange materials, energy, and information, forming intricate networks that contribute to ecosystem resilience and stability (Montoya et al., 2006a). These networks, influenced by various abiotic and biotic factors (Barnes et al., 2024; Yuan et al., 2021), support key functions such as nutrient cycling, C sequestration, SOM decomposition (Maron et al., 2018; Pandey et al., 2018; Sokol et al., 2022) and the formation of soil structure (Chenu and Cosentino, 2011; Eilers et al., 2012; Mueller et al., 2024). Agricultural management practices can significantly affect soil microbial diversity and networks through varying management practices, thereby influencing ecosystem health (Romdhane et al., 2022). Recent studies suggests that warming may either enhance or impair microbial functions, depending on the complexity of microbial networks in surface soils (Tian et al., 2022; Wu et al., 2022; Yuan et al., 2021). Yet, the response of subsoil microbial network complexity and stability to climate warming under different agricultural land management practices is still lacking and needs further *in-situ* investigation to better predict and manage the impacts of climate change on soil biodiversity and ecosystem stability.

Materials and Methods

1. Study area description

The experimental field site is part of the long-term Observatory for Environmental Research: Agro-ecosystems, Biogeochemical Cycles and Biodiversity (SOERE ACBB, http://www.soere-acbb.com/index.php/fr/), hosted by the INRAE (French National Institute of Agricultural Research and Environment), situated in Lusignan, Nouvelle-Aquitaine (46°25′12.91″ N, 0°07′29.35″ E; 128 m a.s.l.), western France. The climate of the experimental site is oceanic with a mean annual air temperature of 12.4°C and precipitation of 781 mm. Most of the precipitation occurred from November to March, and the monthly maximum and minimum temperatures are observed in August (27.5°C) and January (-2.9°C) (data from Météo France, 2007 to 2024). The site is flat with a slight slope gradient (0.5 percent). The soil is classified as a Cambisol (FAO soil group) with loamy texture in the upper horizons, and clayey texture at depth with a high content of oxides and kaolinite, classified as a Paleo-Ferralsol. Pedoclimatic characteristics are shown in Table 4.

Table 4. Soil properties by horizon of the experimental site (Chabbi et al., 2009; Moni et al., 2010). Mean and coefficient of variation expressed as a percentage of the mean. Both grassland and cropland treatments are on the same soil type.

Horizons	Soil depth	Bulk density	$pH_{(H2O)}$	Particle size distribution			CEC	Base saturation	Oxides-Hydroxides			
				Sand	Silt	Clay			Al_o	Fe_o	Fe_d	Al_d
	/cm	/g cm^{-3}		——— /g kg^{-1} soil ———			/$cmol_c$ kg^{-1}	/%	——— /g kg^{-1} soil ———			
Lp (n=6)	0-30	1.43±5%	6.3±3%	105±14%	727±3%	168±87%	7.08±11%	0.98±7%	1.20±19%	3.22±22%	20.97±25%	3.08±25%
S (n=6)	30-59	1.45±4%	6.6±4%	95±9%	594±7%	311±11%	8.6±22%	0.94±6%	1.65±14%	1.53±17%	27.19±27%	5.11±18%
S2 (n=5)	59-84	1.60±7%	6.9±5%	103±11%	520±9%	377±21%	10.8±27%	0.94±5%	2.01±9%	1.82±15%	45.27±22%	7.20±21%
C1 (n=5)	84-143	1.59±8%	6.6±4%	98±8%	330±11%	572±9%	11.6±16%	0.93±9%	1.95±19%	2.03±14%	62.79±36%	7.42±22%
C2 (n=6)	>143	1.61±6%	5.2±3%	110±10%	242±13%	642±12%	9.9±17%	0.82±10%	1.87±15%	2.27±11%	67.02±32%	6.16±27%

Here, CEC stands for cation exchange capacity; Fe_o and Al_o standas for amorphous forms of Fe and Al; Fe_d and Al_d stands for non-crystalline forms of Fe and Al.

1.1 Land management history

The long-term experiment (SOERE ACBB) started in 2005 on an area previously covered by forest and then devoted to conventional agroecosystem management (grassland, rotational cropping, or temporary (ley) grassland) for at least the next 100 years. The aim of the experiment is to elucidate the impact of temporary grassland introduction into the rotational cropping cycle (maize (*Zea mays*), winter wheat (*Triticum* spp.), and winter barley (*Hordeum vulgare*)) on biogeochemical cycling. The experiment is conducted with several treatments and two controls (continuous grassland and continuous cropland). Temporary (ley) grassland treatments differ in duration (3 or 6 years of ley), fertilization strategy (with or without N fertilization) and harvesting regime (cutting or pasture). Croplands are ploughed once a year to a depth of 30 cm using a mouldboard plough prior to crop planting, and the crop residues (straw and yield) were returned to the soil after harvesting. For grassland treatments, a mixture of perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), and tall fescue (*Festuca arundinacea*) is used. Grassland was hayed three to four times for annual forage production, depending on biomass productivity. Plant root biomass is greater in the continuous grassland than in the ley grassland and cropland down to 90 cm depth (Table 5).

Table 5. Plant root mass from the whole-soil profile (mean ± SE, *n*=5) of the experimental site in the control plots with two different land management practice.

Field	Year	Root mass (kg ha^{-1}) in different soil depth		
		0-30 cm	30-60 cm	60-90 cm
Cropland	2019*	7160±340	918±26	222±9
	2020	4250±150	643±8	314±6
	2021	3560±190	726±7	258±9
Grassland	2019	9850±260	1200±45	484±8
	2020	11600±1100	1250±65	496±10
	2021	12800±1600	2000±13	563±9

*Data collected from temporary grassland in 2019, after that was in its cropland phase after 6 years of ley grassland (see Table 3).

Table 6. Long-term land-use management history under an experimental site since 2005 in the Nouvelle-Aquitaine (Lusignan) region of western France.

Year:	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Cropland:	Ley Grassland (harvested 3 to 4 times/year)						Maize	Wheat	Barley	Ley Grassland (harvested 3 to 4 times/year)						Maize	Wheat	Barley	Wheat	Barley
Grassland:	Continuous Grassland (harvested 3 to 4 times/year) for annual forage production																			

2. *In-situ* experimental soil warming setup

In 2019, we established an *in-situ* whole-soil warming (+4°C) experiment in both the continuous grassland and the temporary (ley) grassland that began its rotational cropping cycle (2020-2024) after 6-year of ley grassland (2014-2019) (Table 6 and Fig. 3). The land management types are hereafter referred to as 'grassland' and 'cropland'.

The *in-situ* warming experiment heated the soil to +4°C above ambient temperature (°C) across 10 m^2 circular plots to a depth of 1.0 m with 2.0 m long resistance heating cables while retaining the natural temperature gradient within the soil profile based on Hicks Pries *et al.* (2017) and Hanson *et al*. (2011). The warming experiment consists of three (n=3) replicated plot pairs: control (ambient temperature) and heated (ambient temperature +4°C). The control and heated pair plots were approximately 10 m apart from each other and the 3 replicate plots were approximately 5 m apart from each other (Fig. 4b). Briefly, warming was maintained throughout the 3.5 m diameter circular plot and down to 1.0 m depth with twenty-two 2.0-m-long steel pipes, which were installed vertically around 0.50 m apart and 0.10 m beyond the edge of the plots (Fig. 4a). In warmed plots, self-regulating heating cables (TECHNITRACE (CAMT EA 35W/m 230V), France) were placed inside the pipes, which were then filled with sand, while control plots did not have that type of heating cable. We installed additional heating cables 5 cm below the soil surface in a horizontal zigzagging pattern across the circular plot, around 0.30 m apart, to compensate for surface heat loss (Fig. 4a). Similar unpowered heated cables were not installed in the control plots, modified from Hicks Pries *et al.* (2017). The heaters were powered by an electric power supply, which was controlled by a silicon-controlled rectifier (SCRs, Watlow, Missouri, USA) via a current-to-voltage converter with a unipolar multiplexer (1KOhms resistance). The amount of electricity (source of heating) supplied to the deep heaters was based on the temperature difference between control-heated paired thermistors at 50, 75, and 100 cm depths at 0.75 m radial distance from the center of the plot. Similarly, the temperature difference of thermistors at 15 and 30 cm depth at radial distances of 0 and 0.75 m from the center of the

plot was used to calculate the power supplied to the surface heaters of each paired plot. We installed this *in-situ* soil warming experiment in two phases. First, the initial installation in 2019 to 2020, when the warming system was turned on. Second, the conditioning phase in 2020 to mid-2021. The conditioning phase was due to low soil warming with respect to our +4°C target, and we reached our +4°C target after 1.5 years of turning on the warming system before beginning our field data collection started in July 2021 (Fig. 4c) and continued until September 2024.

2.1 Automated temperature and moisture data measurements

Portable dataloggers (CR1000, Campbell Scientific, Utah, USA) recorded continuous soil profile temperature and moisture data at 10-second intervals. In each plot, a PVC tube containing six temperature probes (OMEGA 44007 thermistor) was placed at different depths (5, 15, 30, 50, 75, and 100 cm) with five replicates at 0.75 m radial distance from each other to monitor temperature, modified from Hicks Pries *et al.* (2017). The sensors at 15 and 75 cm depth were also used to regulate the surface and depth temperatures in order to ensure that the +4°C relative to the control is maintained throughout the soil profile. Another tube including five capacitance sensors equipped with enviroSCAN probes (Sentek, Australia) measured soil moisture at different depths (15, 30, 50, 70, and 90 cm) at a radial distance of 0.75 m from the center of each plot. The soil moisture readings were calibrated by comparing the sensor values at each depth to the volumetric water content recorded in the nearest (within 0.30 m) soil samples. We repeated this soil sampling two times over one year, in both dry and wet periods, to minimize the calibration errors.

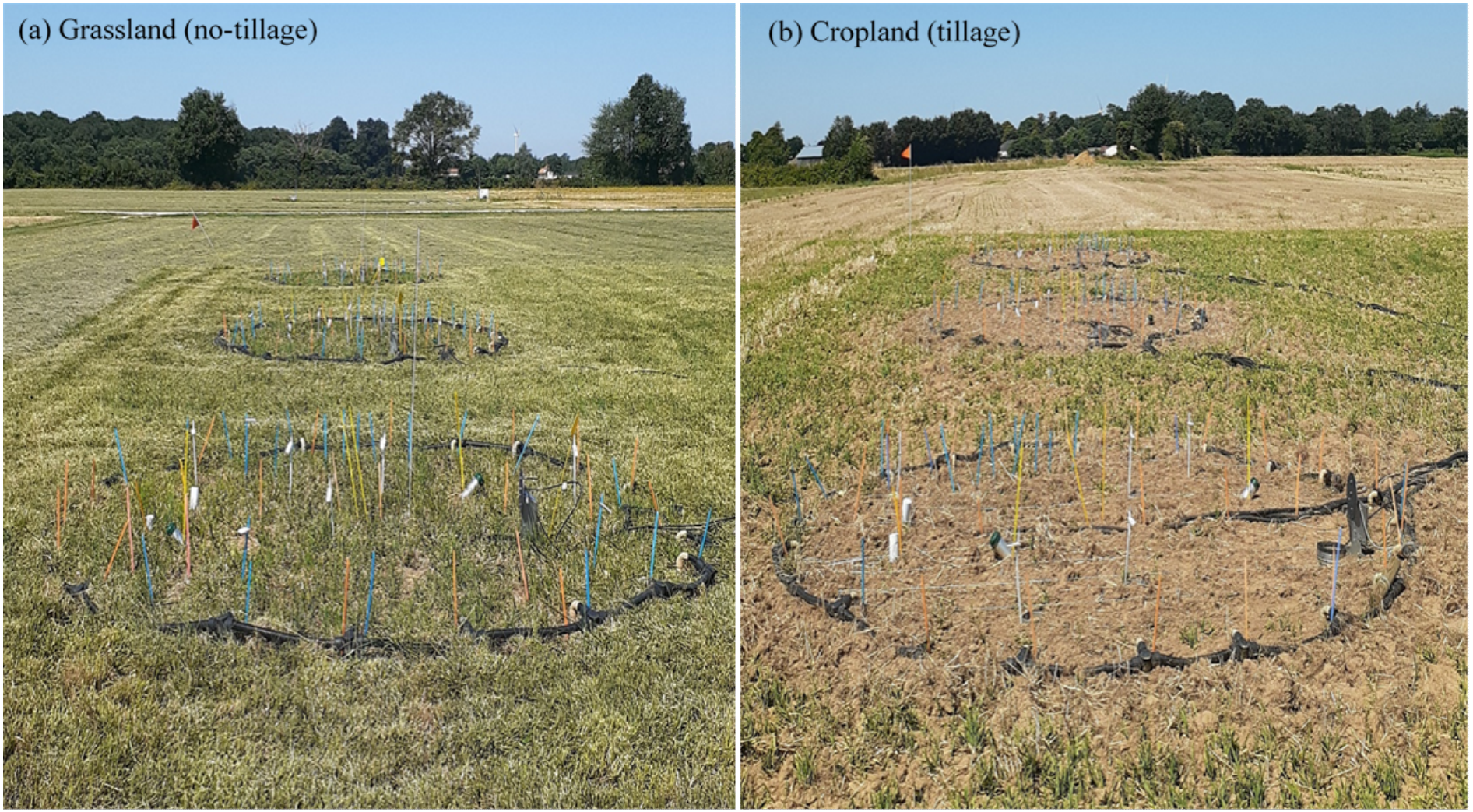


Fig. 3. *In-situ* whole-soil warming experiment under contrasting grassland management: (a) continuous grassland; and (b) cropland (cropland phase after 6 years of ley grassland) in a temperate region of western France.

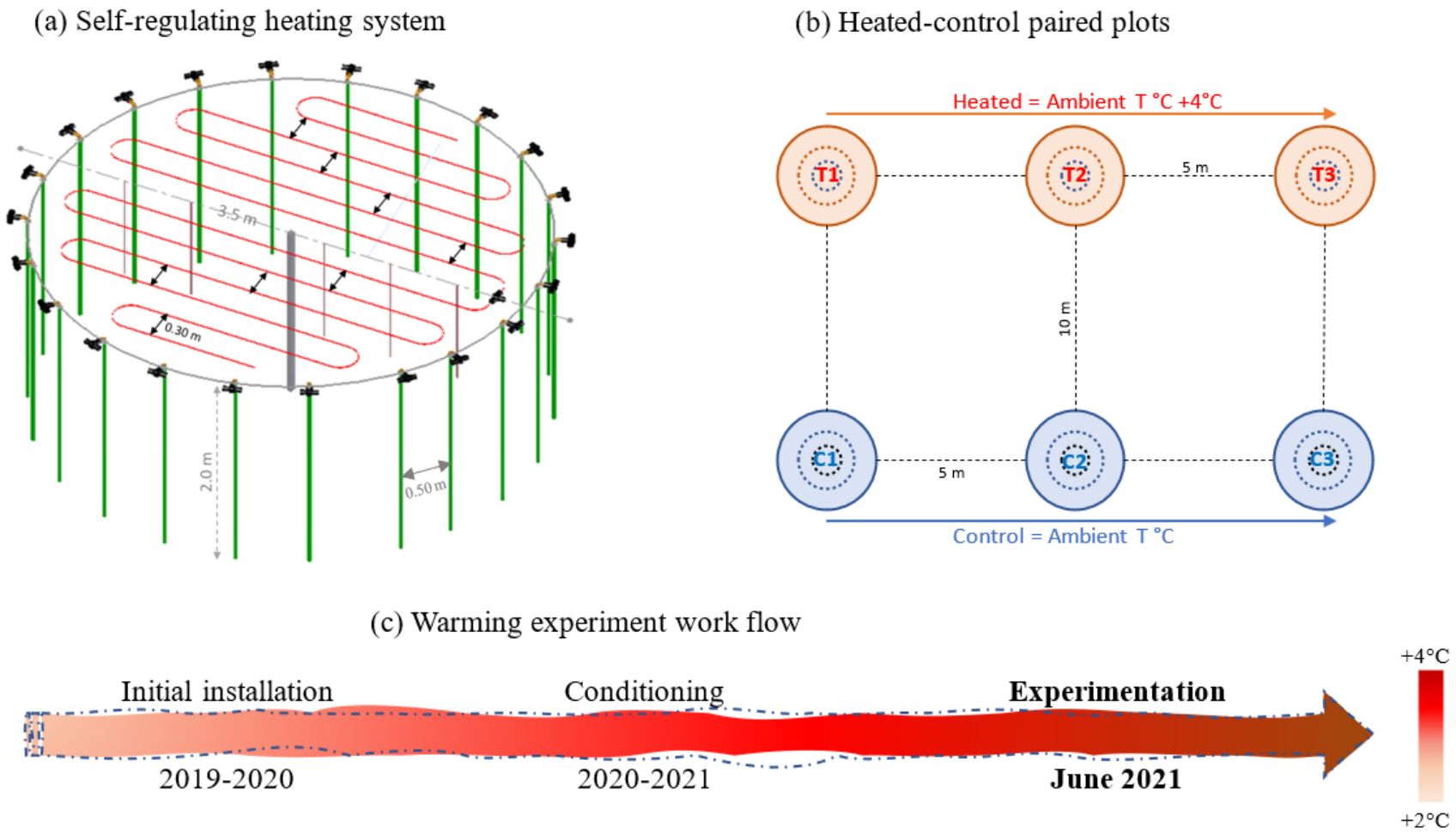
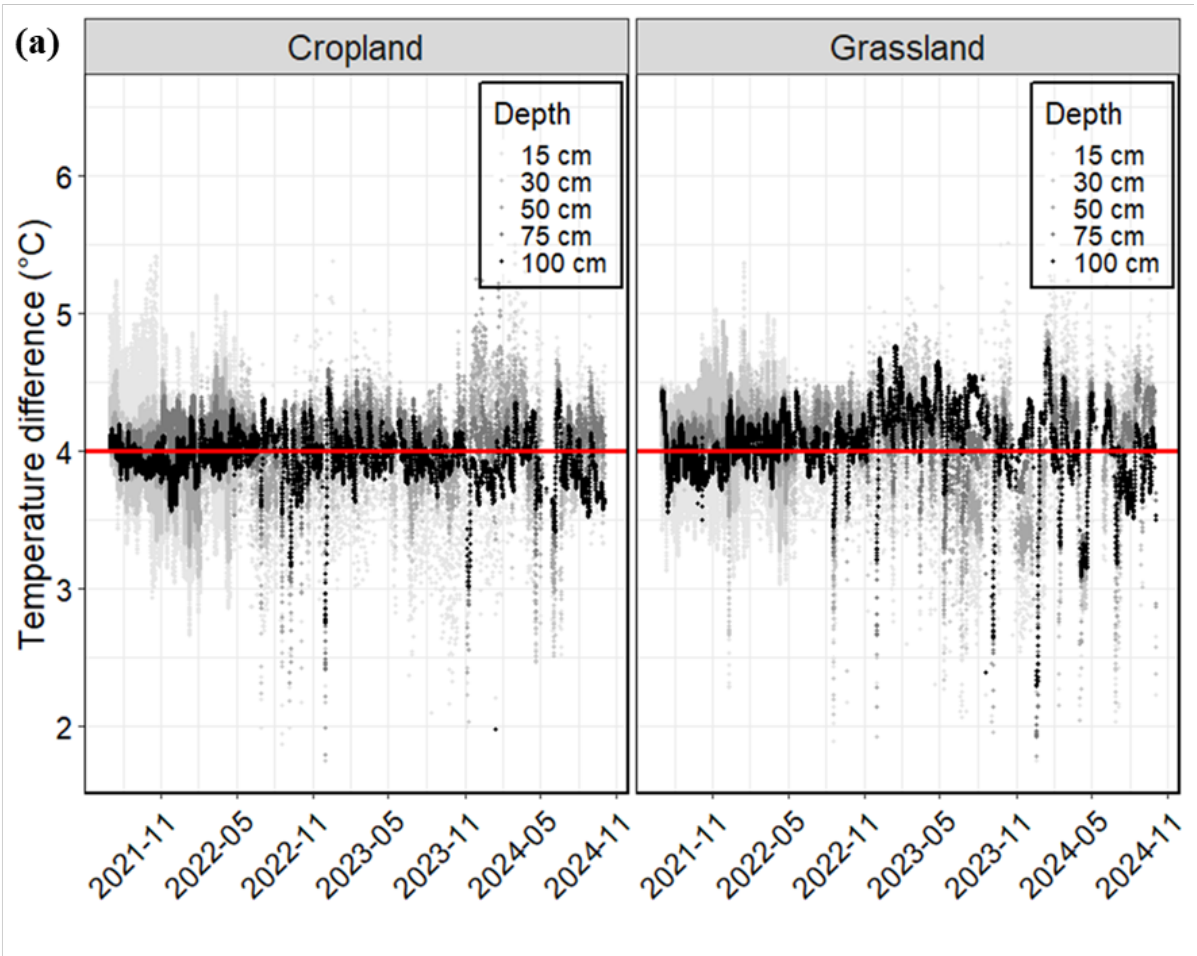


Fig. 4. *In-situ* soil warming experimental design under contrasting grassland management: (a) whole-soil warming methodology based on self-regulating heating cable down to 2.0 m soil profile (the red zigzag line represents the buried surface heating location at 5 cm depth while the green and gray bar represents the location of the deep heating system down to 2.0 m depth); (b) heated-control paired (replicates, *n*=3) plots; and (c) warming experiment work-flow (after 1.5 years, we have +4°C, which has continued) in a temperate region of western France.

2.2 Evaluation of *in-situ* soil warming (+4°C) performance

Our *in-situ* soil warming experiment shows a homogeneous temperature gradient, while the average soil temperature in the heated plots was elevated by +4±0.20°C for cropland and +4±0.23°C for grassland down to 1.0-m depth compared to the control plots, with the exception of 0-15 cm depth (+4±0.44°C for cropland and +4±0.40°C for grassland), throughout the entire study period (Fig. 5a-c) and did not change with weather (seasonal fluctuations) or soil moisture conditions. Indeed, the 0-15 cm depth showed a higher variability concerning our target (+4°C) because there is no surface heating to compensate for the heat loss due to close contact of ambient air temperature and radiation compared to deep soils.



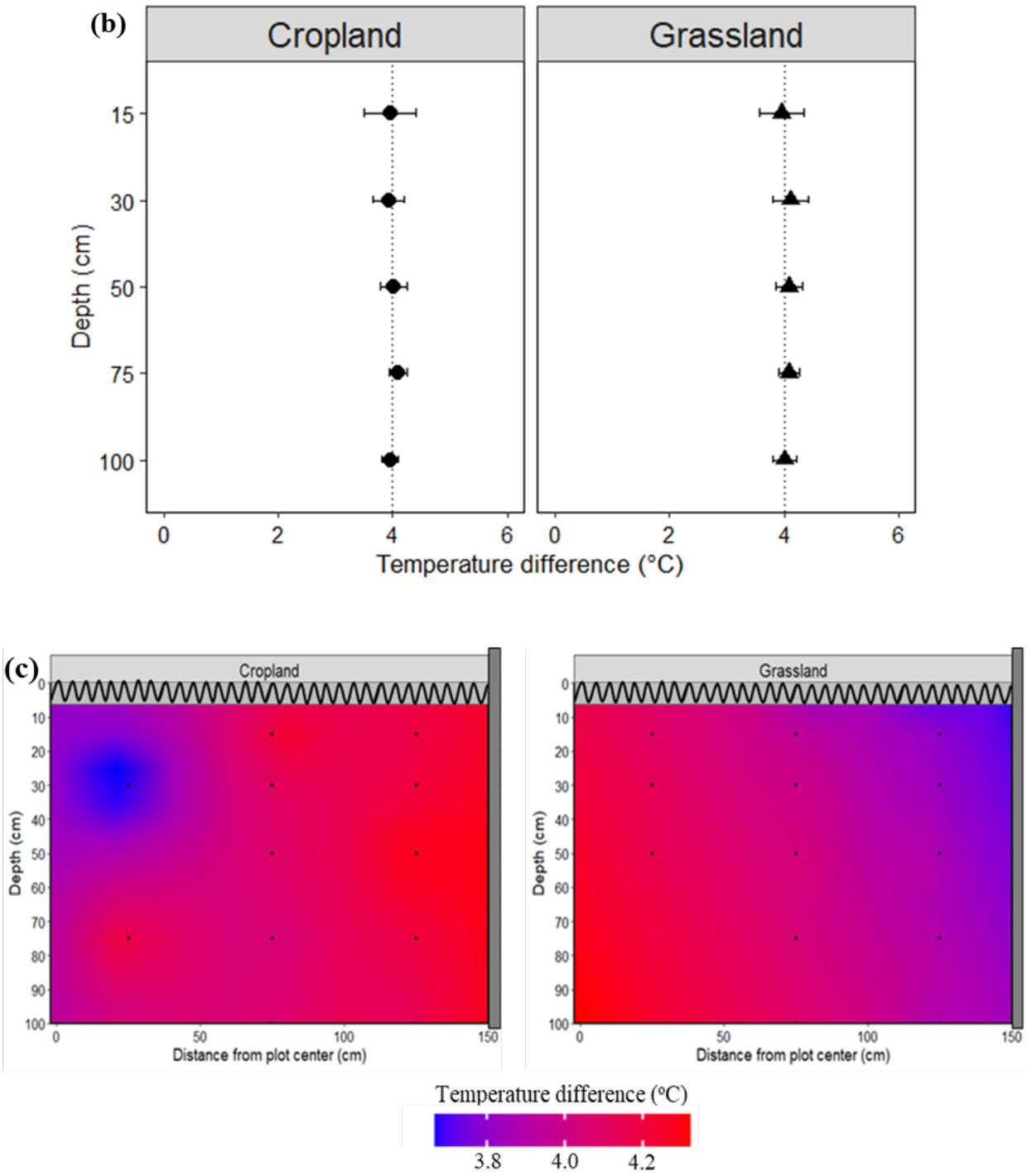


Fig. 5. Soil temperature: (a) the average temperature difference between the control-heated pair plots (*n*=3) at five depths over 3.3 years, measured continuously from July 2021 to September 2024, with the red line indicates +4°C target while the discontinuities in warming (drops followed by sharp increases) were due to electrical outages; (b) The mean temperature difference between the control-heated pair plots (*n*=3) at five depths over the study periods with error bars representing the standard deviation (SD) of the temporal variability; (c) A radial cross-section of

the study plot shows the average temperature difference (n=3 plot pairs) from August to October 2021. The black dots represent the location of the temperature sensors. The horizontal zigzag line (black) is the location of the surface heaters and the vertical grey bar represents the 2.0 m long deep heaters, which are located 25 cm from the edge of the plot at 175 cm from the plot center. The data were interpolated using the automap package in R.

3. Soil respiration field data collection and quantification

3.1 Soil CO_2 efflux

Measurements of surface soil respiration (CO_2 efflux) were performed from July 2021 to June 2022 using soil respiration auto-chambers (8100A, LI-COR, Lincoln, Nebraska), which continuously measured the soil respiration fluxes every 90 minutes in each plot. During each flux measurement, the air temperature was also recorded using a temperature probe (OMEGA 44007 thermistor), which was connected to the auto-chamber lid above the soil surface. The soil CO_2 efflux was calculated as follows:

$$f = \frac{dc}{dt} \times \frac{hMP}{RT} \text{ ---------- (1)}$$

Where f denotes the surface CO_2 efflux rates (μg m^{-2} s^{-1}), dc/dt is the rate of increase of the CO_2 concentration inside the chamber with time and is determined by the slope of linear regression; h is the height of the chamber; M is the molar mass of CO_2; P is the atmospheric pressure; R is the ideal gas constant; and T is the chamber air temperature in Kelvin. After that, the surface CO_2 efflux was converted to g C m^{-2} d^{-1} units.

To prevent CO_2 efflux from aboveground plant parts (leaves and stems), living plants inside the auto-chambers were manually eradicated once a week if they grew, and the removed plant material was left inside the chambers to die. Therefore, the CO_2 efflux measured in the soil did not include aboveground respiration from living plants (e.g., leaves and stems) but included belowground root respiration.

3.2 Soil profile CO_2 production

Gas measurements were carried out using a set of gas wells at 15, 30, 50, 70, and 90 cm depths of the soil profile, which had been installed at each plot at a distance of 0.20 m from the center. At 70 and 90 cm, the set up included two additional replicates to better account for deep soil heterogeneity. The gas wells were 6.35 mm (diameter) stainless steel tubes inserted at a 45° angle into the soil to the desired depth and topped with straight swage pipe-fittings with septa at the distal end (TECHNITRACE, France). We collected soil gas samples every fifteen days during six months (January 2022 to June 2022). Samples were collected from the gas wells with a 60 ml syringe, after clearing the headspace in each well. For gas analysis, 3, 20 ml samples were immediately injected into a portable infrared gas analyzer (EGM-5, PP Systems, USA) in the field and stored the gas concentration (ppm) reading with a nonvolatile semiconductor memory (TECHNITRACE, France). In addition, the actual CO_2 concentration from the EGM-5 data was calculated using a four-point standard calibration curve ranging from 0 to 20,000 ppm.

We modeled depth-resolved CO_2 production (μmol m^{-3} s^{-1}) from soil CO_2 concentrations (ppm) using Fick's first Law as a flux gradient approach (Davidson et al., 2006; Hicks Pries et al., 2017; Vargas et al., 2010):

$$F = -D\frac{dC}{dz} \qquad \text{---------- (2)}$$

Where F denotes the flux density of CO_2 across a horizontal surface on each depth of soil (μmol m^{-2} s^{-1}), D indicates the diffusion coefficient (mm^2 s^{-1}) of air into soil pores, and the change in CO_2 mol fraction at each depth is represented by dC/dz, which was calculated using the first derivative of a curve fit to the depth profile of CO_2 mol fraction. For each depth and time interval, the diffusion coefficient (D) was calculated as follows:

$$D = D_o * \xi \qquad \text{---------- (3)}$$

$$D_o = D_{ao}\left(\frac{T}{293.15}\right)^{1.75}\left(\frac{101.3}{P}\right) \qquad \text{---------- (4)}$$

Where D_o is the diffusion coefficient of CO_2 in the air at the soil temperature (T) and local air pressure (P), D_{ao} is a reference value for CO_2 in the air (15.7 mm^2 s^{-1}), and ξ is the dimensionless tortuosity factor. Tortuosity was estimated using six different equations (Jassal et al., 2005; Millington, 1959; Moldrup et al., 2001; Moyes and Bowling, 2013), and we picked the equation that resulted in the best match between the measured surface flux and the estimated surface flux from soil profile data. The most effective equation was determined by an empirical relationship (Hicks Pries et al., 2017; Moyes and Bowling, 2013):

$$\xi = 0.95\varepsilon^{1.93} \quad \text{---------- (5)}$$

Where ε is the calculated air-filled porosity (m^3 m^{-3}) from the total soil porosity (based on dry soil bulk density and solid particle density) and volumetric water content at each soil depth and measurement time. Finally, CO_2 production (μmol m^{-3} s^{-1}) was calculated from a given depth interval by subtracting the difference in flux densities (μmol m^{-2} s^{-1}) across that interval and dividing by the depth (m) increment. After that, the CO_2 production was converted to g C m^{-3} d^{-1} units.

4. Plant data collection and quantification

4.1 Leaf area index

During the growing season (March to June 2022), we measured leaf area (m^2) by scanning the leaf blades in a leaf area scanner (LI-2000, Li-Cor, Lincoln, Nebraska, USA) to estimate the leaf area index (LAI, m^2 m^{-2}) based on ground cover (m^2).

4.2 Above-ground biomass

Above-ground biomass was quantified by harvesting the vegetation cover at 5 cm above the soil surface in each plot (n=3), separately for cropland and grassland. Such harvests were carried out once per year in cropland after plant maturity in June 2022, July 2023, and July 2024 and twice or thrice per year in grassland during the growing season (March to October) from

2022 to 2024, within an area of 10 m^2 circular plots. After harvesting, the fresh plant biomass was oven-dried at 70°C for one week to quantify the dry biomass and converted to t ha^{-1} units.

5. Soil sampling, collection and laboratory analyses

In late May of 2022, 2023 and 2024, following one, two and three years of warming, soil samples were collected from control-heated pair plots (*n*=3) at five different depths (0-15, 15-30, 30-50, 50-70, and 70-90 cm) of the soil profile before harvesting (cropland and grassland). Briefly, soils were randomly sampled using a soil auger (8 cm diameter and 120 cm length) with five replicates (*n*=5) and after that, all replicates were pooled into one single composite sample for each plot and depth. Every time, the soil was kept at 4°C during transport and prior to processing, which occurred within 1 h after sampling. Then in the laboratory, fresh soil samples were immediately sieved through a 2-mm mesh and split into three subsamples: (i) a subsample stored at 4°C for potential extracellular enzymes activity (EEA) analyses, conducted within the same sampling week; (ii) a subsample freeze-dried and stored at -40°C for molecular microbial analyses and ester-linked fatty acid methyl ester (EL-FAME) analyses; and (iii) an air-dried subsample for physicochemical analyses.

In addition, soil core samples were collected for bulk density measurement in 2023 and 2024 at depths of 0-15, 15-30, 30-50, 50-70 and 70-90 cm, approximately 50 cm adjacent to the experimental plots (*n*=4) under both treatments and land management practices. Samples were collected using a steel core cylinder (18 mm diameter and 100 cm depths), and cores were sectioned into specific depth intervals. Sampling was conducted adjacent to, rather than within, the experimental plots to minimize disruption to these long-term experiments, as the use of mechanical instruments for soil core collection could interfere with ongoing research activities.

5.1 Soil moisture content and bulk density

The moisture content of all fresh soil samples was determined by weighing them before and after oven-drying at 105°C for 48 hours. Bulk density was calculated as the weight of the solid phase (oven-dried soil) divided by the total volume of the sampled soil in the core for each depth interval.

5.2 Soil organic carbon and total nitrogen stocks

Soil organic carbon (SOC) and total nitrogen (N) stocks were calculated for each depth of the profiles by multiplying soil C and N concentrations by the bulk density and the sampling depth using the following equation:

$$\boldsymbol{y = a \times b \times c} \quad \text{---------- (6)}$$

where $\boldsymbol{y}$ is the SOC and N stock per unit area (g m^{-2} soil), $\boldsymbol{a}$ is the C and N concentration in the soil sample (mg g^{-1} soil), $\boldsymbol{b}$ is the soil bulk density (g cm^{-3} soil), and $\boldsymbol{c}$ is the thickness of the soil layer (cm).

5.3 Physicochemical analysis

The pH of air-dried soil was measured using a glass electrode pH meter with a soil-water suspension ratio of 1:5 (w/v), after shaking for 30 minutes. The total carbon, total nitrogen contents of soil samples were determined using an elementary analyzer (Flash EA, Thermo Electron Corporation, Bremen, Germany). No carbonate was present in the soil; therefore, soil C is considered to be entirely organic.

5.4 Dissolved organic carbon and total dissolved nitrogen

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were quantified from soil solution samples collected using porous suction cup samplers placed at a depth of 90 cm. In March, April, October, November and December of 2023 and February and March of 2024, a vacuum pump generating a vacuum pressure of 0.6 bar was used to extract the soil solution from the samplers into pre-rinsed glass bottles. Thereafter, the soil solution was filtered

through a 0.45 µm polytetrafluoroethylene membrane filter, and the samples were stored in a cold chamber at 4°C until analysis. After that DOC and TDN contents of the soil solution samples were analyzed using a SHIMADZU V-SCD TOC analyzer.

5.5 Molecular analyses of soil microbial biomass and richness

Soil microbial DNA was extracted from each soil sample and quantified from 1g of freeze-dry soil using a single procedure standardized by the GenoSol platform (INRAe, Dijon, France; Terrat et al., 2012). Briefly, this protocol involves three specific steps: (i) microbial cell lysis by chemical and physical action; (ii) deproteination; and (iii) alcohol precipitation at -20°C and washing of the extracted nucleic acids. The crude DNA extracts were quantified through electrophoresis in a 1% agarose gel and ethidium bromide staining, using calf thymus DNA as a standard curve. The resulting quantified crude DNA was used to estimate the molecular soil microbial biomass (Dequiedt et al., 2011). Crude DNA was then purified using a Nucleospin Soil purification kit (Macherey Nagel, France). Subsequently, the purified DNA extracts were quantified using a Quantifluor staining kit (Promega, USA) with an Infinite 200 PRO plate reader (Tecan).

Bacterial and fungal richness were estimated in all soil samples by 16S rRNA gene and 18S rRNA gene metabarcoding, respectively. Briefly, a gene fragment targeting the V3 to V4 regions was initially amplified by PCR (Polymerase Chain Reaction) using primers F479 (5′CAG CMG CYG CNG TAA NAC3′) and R888 (5′CCG YCA ATT CMT TTR AGT3′) for bacterial richness (Terrat et al., 2015) and a gene fragment targeting the V7 to V8 regions was initially amplified by PCR using primers FR1 (5′ANC CAT TCA ATC GGT ANT3′) and FF390 (5′CGA TAA CGA ACG AGA CCT3′) for fungal richness (Chemidlin Prévost-Bouré et al., 2011). The PCR products were then purified using a Pronex purification kit (Promega, USA). Additionally, a second PCR was performed on the purified PCR products using 10-base pair multiplex identifiers (MID), which were added at the 5′ and 3′ ends of the primers for subsequent

sample identification. Meanwhile, for all libraries, equal amounts of all samples were pooled and purified using a Pronex purification kit for the removal of excess nucleotides, salts and enzymes. Finally, sequencing was performed on an Illumina MiSeq instrument (Illumina Inc., San Diego, USA).

5.6 Bioinformatic analyses for soil microbial communities

Bioinformatic analyses for the identification of bacterial and fungal taxonomic groups were performed using the BIOCOM-PIPE (https://forgemia.inra.fr/biocom/biocom-pipe), which was originally developed by the GenoSol platform (INRAe, Dijon, France; Djemiel et al., 2020). To compare the datasets efficiently and to avoid biased community comparisons, the sample reads were reduced by random selection (10000 reads for 16S rRNA and 18S rRNA gene sequences for each soil sample). The retained high-quality reads were used for (i) defining OTUs matrix using a post-clustering tool (ReClustOR; Terrat et al., 2020), which improves the reliability of OTU-based results and analyses, based on French RMQS biological reference databases (Terrat et al., 2017), and (ii) taxonomy-based analyses using similarity approaches against curated reference databases from SILVA R132 (Quast et al., 2012).

5.7 Extracellular enzymes activity analysis

The potential activities of four extracellular enzymes (EEA) involved in carbon, nitrogen, and phosphorus cycles were measured using the fluorometric assays according to the protocol suggested by Bell *et al.* (2013): β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP). Briefly, 2.75g of soil sample were suspended in 91 mL of 50 mM Tris buffer for 30 min with stirring. Buffer pH was adjusted to the soil pH of each sample using HCl or NaOH. After that, three technical replicates of 400 µL soil slurries were added to a 96-deepwell microplate. For each sample, calibration curves were prepared for the two standards (4-methylumbelliferone and 7-amino-4-methylcoumarin) and quench control for enzymes substrates and soil slurry were included. The microplates were

incubated with the targeted fluorometric substrates for 2 h at 25°C. After incubation, the microplates were centrifuged for 20 min at 3000 rpm, and 200 µL of supernatant from each well was transferred into a black flat-bottom fluorometric 96-well microplate. The enzyme-substrate catalytic reaction was then stopped at 3 h with 1M Tris buffer solution. Finally, fluorescence was measured using a fluorometric microplate (96-well) reader (Victor3, PerkinElmer, USA) with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. From the fluorescent values and standard calibration curves, each enzyme activity was calculated and converted to nmol g^{-1} soil min^{-1}. We also calculated enzymatic ratios of BG/AP, BG/(NAG+LAP) and (NAG+LAP)/AP to infer C vs. P, C vs. N, and N vs. P limitation according to the eco-enzymic stoichiometry (Sinsabaugh et al., 2008).

5.8 Ester-linked fatty acid methyl ester extraction for microbial communities' analysis

The abundance of microbial groups was quantified by the ester-linked fatty acid methyl ester (EL-FAME) extraction method (Schutter and Dick, 2000), which is less sensitive to a range of soil parameters (particularly pH), more cost-effective, and much faster than the phospholipid fatty acid (PLFA) extraction method but provides similar results (Li et al., 2020). Briefly, the FAME extraction involved a four-step process that was applied to freeze-dried soil samples (3 g). The first step involved releasing and methylating ester-linked fatty acids at 37°C with 15 ml of 0.2 M KOH in methanol for 60 minutes. The second step was the neutralization of the mixture with 1.0 M acetic acid. The third step involved the extraction of FAMEs with hexane containing the 19:0 internal quantification standard (methyl nonadecanoate acid). After that, the FAMEs were analyzed using a gas chromatograph (6890 Agilent) equipped with a flame ionization detector, a fused silica capillary column BPX70 (length 60m; inner diameter 0.32mm; film thickness 0.25µm), and ultra-high purity helium as a carrier gas. The column was kept at 80°C for 1 min, then heated to 160°C at a rate of 25°C/min, to 180°C at a rate of 2°C/min and finally heated to 220°C at a rate of 3°C/min and kept at 220°C for 10 min. The injector was kept at

280°C and the detector at 350°C. A volume of 1 µl was injected at a split ratio 1:10. Peaks were identified using FAME mix and Bacterial FA mix standards. Several selected fatty acids (FAMEs) were used as microbial biomarkers to distinguish different types of microorganisms (Joergensen, 2022; Li et al., 2020; Olsson et al., 1995; Ruess and Chamberlain, 2010; Willers et al., 2015; Zelles, 1999): a15:0, i15:0, i16:0, and i17:0 for gram positive (GMP) bacteria; 10:2-OH, 12:2-OH, 14:0-OH, 16:2-OH, and cyc19:0 for gram negative (GMN) bacteria; and 18:1ω9c, 18:2ω6t, 18:2ω9t, 18:3ω6, and 18:3ω3 for fungi. The sum of the EL-FAME was calculated as an indicator of active microbial biomass in soil. To compare differences in microbial community composition, absolute EL-FAME abundances were calculated to concentration (µg g^{-1} soil), then converted to relative abundances (mole percentage, mol%) by dividing the absolute abundance by the total EL-FAME abundance and multiplying by 100. We also calculated total bacteria by combining GMP and GMN bacteria. After that, the fungi/bacteria ratio was calculated by dividing total fungi by total bacteria.

6. Statistical analysis

To evaluate the impacts of the warming treatment on various measured responses, we ran mixed effects models in RStudio (R Core Team, 2022) using the *nlme* package (Pinheiro et al., 2017) and restricted maximum likelihood with blocks (n=3) as a random effect. Using Q-Q plots and residual histograms, we visually examined all models for homoscedasticity and normality of distribution, and we used a log transformation to meet model assumptions when necessary. Afterwards, we used slightly different statistical model structures owing to the varied data structures of different Chapters.

In Chapter 2: To test the fixed effects of treatment, depth, and their interaction on soil water content, we used plots crossed with the sampling date as random effects (to account for violations of independence), separately for cropland and grassland. For surface soil CO_2 efflux, we used a different model that included treatment, seasons, and their interaction as fixed effects,

and plots crossed with sampling date as random effects, to test the treatment and seasonal effects on surface soil CO_2 efflux, separately for cropland and grassland. To test the fixed effects of treatment, depth, and their interaction on soil CO_2 production from within the soil profile, we used plots crossed with the sampling date as random effects, separately for cropland and grassland. For SOC content, plots as random effects to examine the fixed effects of treatment, depth, and their interaction within the soil profile, separately for cropland and grassland. Besides that, we used treatment, field, and their interaction as fixed effects, and plots crossed with sampling date as random effects, to test the treatment and management effects on leaf area index. Also, a pairwise mean comparison test was performed using the *emmeans* package in RStudio (Searle et al., 2023) when significant differences ($p \leq 0.05$) were observed for any response variables from mixed effect models.

We also calculated the warming response for each block at each sampling date, to test whether warming affected whole-soil profile CO_2 production and surface CO_2 efflux. The warming response was expressed as a percentage difference between warmed and control conditions, which was normalized by the control values of soil CO_2 production and surface CO_2 efflux. A similar warming response was also tested for SOC concentrations.

$$Warming\ response\ (\%) = \frac{Warm - Control}{Control} \times 100 \qquad \text{---------- (7)}$$

In Chapter 3: The fixed effects of warming treatment, depth, and their interaction on soil microbial biomass and richness were tested separately on cropland and grassland. Plots were used as random effects to account for violations of independence, separately for cropland and grassland. Pairwise mean comparison tests were performed using the *emmeans* package in RStudio (Searle et al., 2023) when significant effects were observed from mixed effect models. Statistical significance was declared at $p \leq 0.05$. Moreover, principal component analysis (PCA) and PERMANOVA was performed to assess the effects of warming on the whole-soil profile bacterial and fungal community structure, separately for cropland and grassland. The PCA and

permutational multivariate analysis of variance (PERMANOVA) tests were performed using the *vegan* R packages (Oksanen et al., 2012).

To compute microbial co-occurrence networks (bacteria and fungi separately), we used the methodological analysis described in Karimi et al. (2019) using the *Statnet* packages in R-software. To compare the microbial networks of the different modalities, we used a leave-one-out approach to (i) standardize the number of samples used to compute the network and (ii) to calculate network replicates within each modality. Consequently, the networks were calculated using 12 replicates, each consisting of 11 samples per modality. For each replicate, network computation was based on calculation of Spearman's correlations between the abundance of OUT pairs directly derived from the OTUs matrix. Significant correlations at the 6% threshold were interpreted as co-occurrence (facilitation) relationships for positive correlations or co-exclusion (antagonistic) relationships for negative correlations. A set of metrics was calculated to assess the topology of the networks. Following this, we run mixed effect models using warming treatment as a fixed effect and plots as random effects to test the warming effects on microbial interactions separately for cropland and grassland.

In Chapter 4: The fixed effects of warming treatment, depth, and their interaction on soil physicochemical, biological and microbial properties were tested separately on cropland and grassland. For plot-scale variables (above-ground biomass, DOC and TDN), we used warming treatment as a fixed effect, separately for cropland and grassland. In all mixed effect models, plots and years were used as random effects to account for violations of independence, separately for cropland and grassland. Pairwise mean comparison tests were performed using the *emmeans* package in RStudio (Searle et al., 2023) when significant effects were observed from mixed effect models. The statistical significance threshold was adjusted at $p \leq 0.05$. Additionally, principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA, permutation=999) was performed to assess the effects of warming treatment,

depth, and their interaction on microbial community composition, separately for cropland and grassland. The plot-pair was used as strata argument in the PERMANOVA to account for spatial dependence. The PCA and PERMANOVA tests were performed using the *factoextra* and *vegan* R packages (Oksanen et al., 2012; R Core Team, 2022).

Chapter 2

Management impacts on whole soil warming responses of CO_2 production and efflux in temperate climate

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Abstract

Although global warming has the potential to increase soil CO_2 efflux, the magnitude of these changes are uncertain, as CO_2 production rates in deep soil are poorly constrained. In particular, management effects on the warming responses at depth are unknown. Here, we conducted an *in-situ* soil warming experiment down to 1.0-m depth in an agricultural Cambisol to study the warming responses of (1) CO_2 production across different depths and (2) CO_2 efflux from topsoil in different seasons under two management practices. To this end, we measured whole-soil profile water content, CO_2 production and CO_2 efflux under continuous grassland and cropland in response to elevated temperature (+4°C).

Warming decreased soil water content for both management practices. We found contrasting warming effects on surface CO_2 efflux, depending on season and land management practices. Subsoil CO_2 production was more sensitive to warming than topsoil CO_2 production with grassland subsoil showing a greater warming response than cropland subsoil. Topsoil CO_2 production decreased in response to warming in the cropland but not the grassland. We concluded that warming responses of CO_2 production and efflux are affected by soil management practices. Their effect on biological processes (roots and microbial activity) and factors affecting gas diffusivity, such as soil water availability and soil physical organization need to be assessed to model warming effects on carbon exchange between soil and the atmosphere in agricultural systems.

Keywords: Soil warming, Subsoil, CO_2 production, Soil respiration, Grassland, Carbon-climate feedback

1. Introduction

According to the Representative Concentration Pathways (RCPs) 8.5 scenario, the global soil temperature is expected to rise to +4.5±1.1°C above pre-industrial levels down to 1.0 m depth by the end of the 21st century (worst IPCC scenario) (Soong et al., 2020). These increasing temperatures will affect carbon (C) cycling in terrestrial ecosystems and possibly cause positive or negative feedbacks in terms of CO_2 exchange between the soil and atmospheric reservoirs (Carey et al., 2016; Davidson and Janssens, 2006). One of the most important mechanisms driving feedbacks to climate change is the increase in microbial activity in response to warming, which can increase the decomposition of soil organic carbon (SOC) and the soil CO_2 efflux to the atmosphere (Crowther et al., 2016). SOC is the largest biologically active C pool in terrestrial ecosystems, storing twice as much C as the earth's atmosphere and vegetation combined (up to 2500 Pg C) (Friedlingstein et al., 2020; IPCC, 2013). But it is still uncertain how this reservoir will respond to warming because the mechanisms controlling SOC turnover and microbial access to SOC are not fully understood (Bradford et al., 2016; Schmidt et al., 2011; Gestel et al., 2018).

In view of the international efforts to increase soil organic C (SOC) stocks in agricultural systems through the development of sustainable agricultural practices (Rumpel et al., 2020; Pete Smith et al., 2015), warming effects on C fluxes in managed agricultural systems are of crucial importance. Warming may affect the CO_2 soil efflux through its impact on the production and transport of CO_2 within and between individual soil depths. This was evidenced in recent *in-situ* whole soil warming (+4°C) experiments, in temperate and tropical forests, which found that warming caused substantial increases in soil CO_2 efflux due to the stimulation of soil profile CO_2 production (Hicks Pries et al., 2017; Nottingham et al., 2020). This increase was induced by the heterotrophic decomposition of SOC pools rather than autotrophic sources (Nottingham et al., 2020; Soong et al., 2021).

Rising temperatures are expected to stimulate CO_2 efflux by their effect on the plant-soil system (Bond-Lamberty and Thomson, 2010; Jochheim et al., 2022; Nottingham et al., 2020) and the magnitude of these responses are likely controlled by water availability (Védère et al., 2022). Water regulates plant and microbial activity, substrate availability, and soil gas diffusivity (Chen et al., 2016; McGrath et al., 2022; Wood et al., 2013). Also, seasonal climate variations affect soil temperature, soil water content, and plant phenology in temperate ecosystems (Vincent et al., 2006), leading to variable soil CO_2 efflux across time (Vanhala, 2002; Vincent et al., 2006). The controlling factors of CO_2 production and efflux, such as soil water content, microbial activity, root abundance and activity, which control substrate supply, and soil tortuosity are all influenced by soil management practices (Kuzyakov, 2006; Oertel et al., 2016; Pagenkemper et al., 2014; Vargas and Allen, 2008). Insight into how soil warming interacts with management to affect seasonal soil CO_2 efflux and its origin within the soil profile will enhance our ability to predict terrestrial C fluxes and C sink potential under future projected climate change.

The majority of publications addressing the effects of agricultural management on SOC turnover have focused on topsoil, ignoring the amount and climate vulnerability of SOC in deep soil layers (Voort et al. 2019; Yost & Hartemink, 2020; Moreland et al., 2021). It has been assumed that SOC stored in deep soil is relatively stable and unresponsive to agricultural practices. This assumption was mainly based on radiocarbon dating studies showing that subsurface C could be hundreds to thousands of years old (Hobley et al., 2017; Wang et al., 1999). Although decomposition rates in deeper soils are slower than in surface soils, recent studies have shown that part of subsoil C could turn over much faster due to preferential input of labile organic matter which could induce positive priming effects in subsoil (Chabbi et al., 2009; Min et al., 2020; Rumpel & Kögel-Knabner, 2011) and also be more responsive to changing abiotic conditions (Hicks Pries et al., 2017; Nottingham et al., 2020; Ofiti et al., 2021;

Zhang et al., 2016) and agricultural management practices (Harrison et al., 2011; Osanai et al., 2020; Samson et al., 2021; Shahbaz et al., 2017) than previously assumed. Moreover, the microbial communities in subsoils are also quite different from those in surface soils, owing to lower SOC availability and more favorable conditions for oligotrophs due to nutrient-poor conditions (Ekelund et al., 2001; Fierer et al., 2003; Oertel et al., 2016; Scheffers et al., 2016; Stone et al., 2014; Zifcakova, 2020). Because of the soil depth profile with gradients of many abiotic and biotic factors affecting the mechanisms that control SOC input, loss, transformation, and stabilization, deep SOC is likely to respond differently to soil warming than surface SOC (Hicks Pries et al., 2023; Von Lützow and Kögel-Knabner, 2009). However, up to now it is unknown how deep soil C under different agricultural practices will respond to warming.

In this study, we thus aimed to provide *in-situ* experimental evidence on the SOC response to warming in managed agricultural soils down to 1.0 m depth. We used a whole-soil warming experiment established on an agricultural Cambisol to investigate how soil water content, CO_2 production, and CO_2 efflux respond to +4°C of warming under continuous grassland and temporary grassland in its cropland phase. In particular, we had the following study questions: (1) How does warming affect soil water content under contrasting land management?; (2) How does whole-soil warming affect the CO_2 efflux under contrasting management practices and seasons?; (3) How does the CO_2 production among differ depths respond to warming under contrasting land management? We hypothesized that in response to *in-situ* warming (H1) water content will decrease across all soil depths, because warming induces higher evapotranspiration, (H2) CO_2 efflux will increase year-round in soils under continuous grassland but only seasonally in cropland soils, and (H3) CO_2 production will increase across the whole soil profile in grassland but not cropland soils, because grasslands show more root activity and root-derived C inputs throughout the year.

2. Results

2.1 Warming effects on soil profile water content

In control plots, annual average soil water contents across the different soil depths ranged from 23.7 to 26.4% in cropland and 24.3 to 28.5% in grassland (Fig. 6). The soil water content significantly ($p \leq 0.05$) decreased in most depths with *in-situ* warming for grassland, and a decreasing trend was noted for cropland, which was only statistically significant at 50 cm ($p \leq 0.05$). In addition, we observed a significant interaction between warming treatment and depth ($p < 0.001$) for both cropland and grassland. Indeed, grassland showed a stronger warming response than cropland, as warming reduced the water content under grassland by 9.7 to 22.9% and by 0.35 to 13.2% under cropland compared to control plots. The greatest differences between the two management practices were found at 30 cm depth with warming, where soil water content was strongly decreased for grassland and slightly decreased for cropland (Fig. 6).

The depth-wise soil water content also depended on the season (significant, $p < 0.001$) for both land management practices (Supp. Fig. S1). During the winter season, topsoil (0-15 cm) showed higher water content, while during other seasons deeper soils had a higher water content for both cropland and grassland. Despite the seasonal variations, *in-situ* warming reduced soil water content throughout the year for both land management practices.

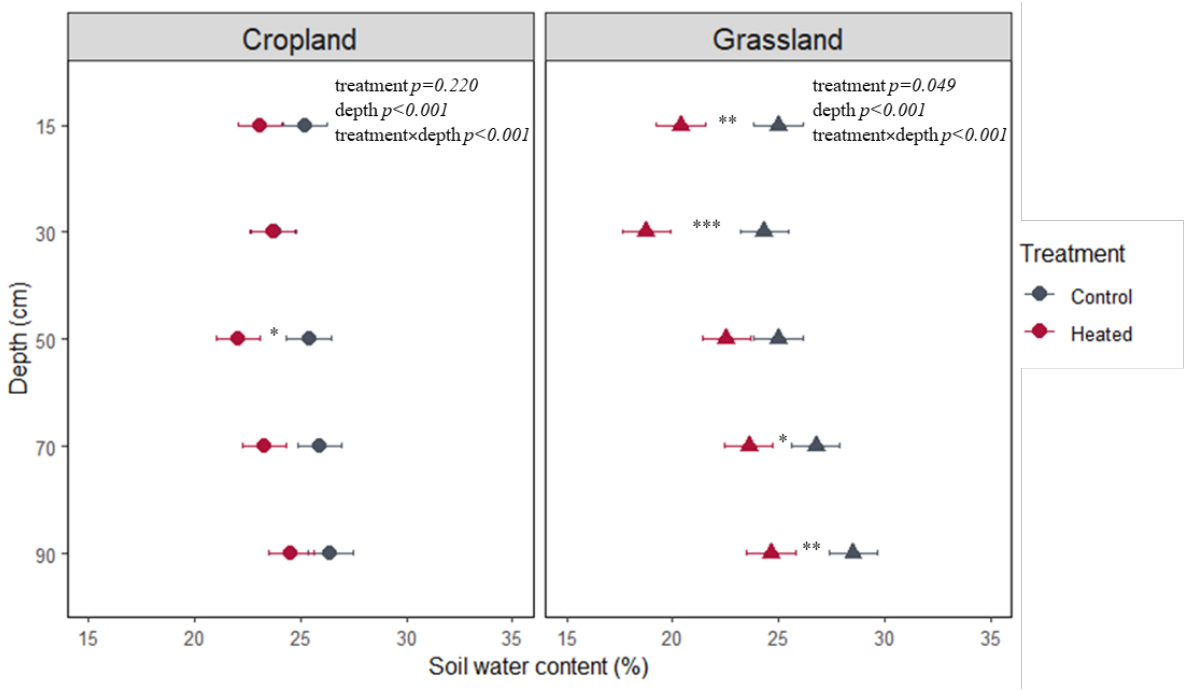


Fig. 6. Mean soil water content (%) in the control and heated plots (mean ± SE, *n*=3) throughout the 1.0 m depth of soil profile over the study period (July 2021 to June 2022). In the figure, the *p*-values denote the statistically significant effects of treatment, depth, and their interaction (treatment×depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

2.2 Warming effects on seasonal CO_2 efflux

The mean surface CO_2 efflux (g C m^{-2} d^{-1}) in the four seasons is presented in Fig. 7. In control plots, CO_2 efflux ranged from 0.52 to 1.35 g C m^{-2} d^{-1} under cropland and 0.50 to 2.47 g C m^{-2} d^{-1} under grassland (Fig. 2.5). The response of CO_2 efflux to warming depended on the season (Fig. 7). Comparing the different seasons for the grassland, our results show that *in-situ* warming only increased (non-significantly) surface CO_2 efflux in winter, while warming significantly ($p \leq 0.05$) reduced CO_2 efflux in other seasons (Fig. 7). In contrast, *in-situ* warming had no effect on surface CO_2 efflux in cropland during the summer and winter, while in autumn and spring warming reduced CO_2 efflux (non-significantly). Overall, the whole-soil warming response of annual surface CO_2 efflux was reduced by 21.8% under cropland (from 399 g C m^{-2}

y^{-1} in control plots to 312 g C m^{-2} y^{-1} in heated plots) and by 20.6% under grassland (from 528 g C m^{-2} y^{-1} in control plots to 419 g C m^{-2} y^{-1} in heated plots).

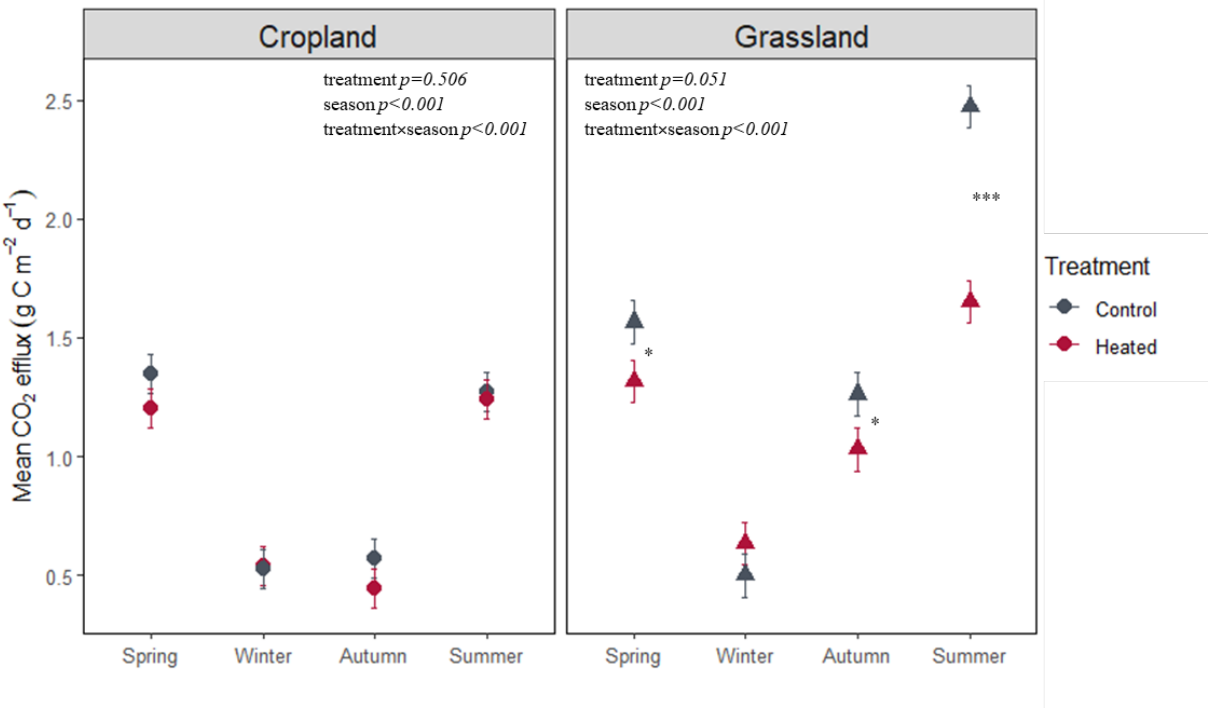


Fig. 7. Mean seasonal variations of soil CO_2 efflux (g C m^{-2} d^{-1}) in the control and heated plots (mean ± SE, *n*=3) from the whole-soil profile over the study period (July 2021 to June 2022). In the figure, the *p*-values denote the statistically significant effects of treatment, season, and their interaction (treatment×season). The asterisk indicates any significant treatment effect within season as determined by post hoc analysis (*$p \leq 0.05$; ***$p \leq 0.001$).

2.3 Warming effects on soil profile CO_2 production

The CO_2 production in the different soil depths, which was measured during six months, is presented in Fig. 2.6. In control plots, CO_2 production ranged from 0.54 to 4.68 g C m^{-3} d^{-1} under cropland and 0.84 to 5.21 g C m^{-3} d^{-1} under grassland (Fig. 8). In grassland, the CO_2 production significantly ($p \leq 0.05$) increased with *in-situ* warming in subsoil (below 30 cm), while statistically non-significant differences were observed between the two treatments in topsoil (0-30 cm) (Fig. 8). In contrast, in cropland, the CO_2 production significantly ($p \leq 0.05$) decreased with *in-situ* warming at the 0-15 cm and 30-50 cm depths, but an increasing trend was also noted

below 50 cm (Fig. 8). The interaction between treatment and depth was significant ($p<0.001$) for cropland but not for grassland ($p=0.084$).

Overall, we found that warming-induced CO_2 production in subsoil was higher for both grassland and cropland as compared to their controls, with a larger increase in grassland (42%) than in cropland (10%). In contrast, the warming-induced CO_2 production rate in topsoil (0-30 cm) differed between grassland and cropland: warming increased CO_2 production by 2.4% in grassland but decreased CO_2 production by 4.2% in cropland. However, it should be noted that the depth-wise CO_2 production measurements occurred during the winter and spring seasons, while we do not have data for the summer and autumn seasons. The warming response on cumulative CO_2 production from the whole-soil profile during six months was increased by 1.2% under cropland (from 84 g C m^{-3} half.y^{-1} in control plots to 85 g C m^{-3} half.y^{-1} in heated plots) and by 16.3% under grassland (from 98 g C m^{-3} half.y^{-1} in control plots to 114 g C m^{-3} half.y^{-1} in heated plots).

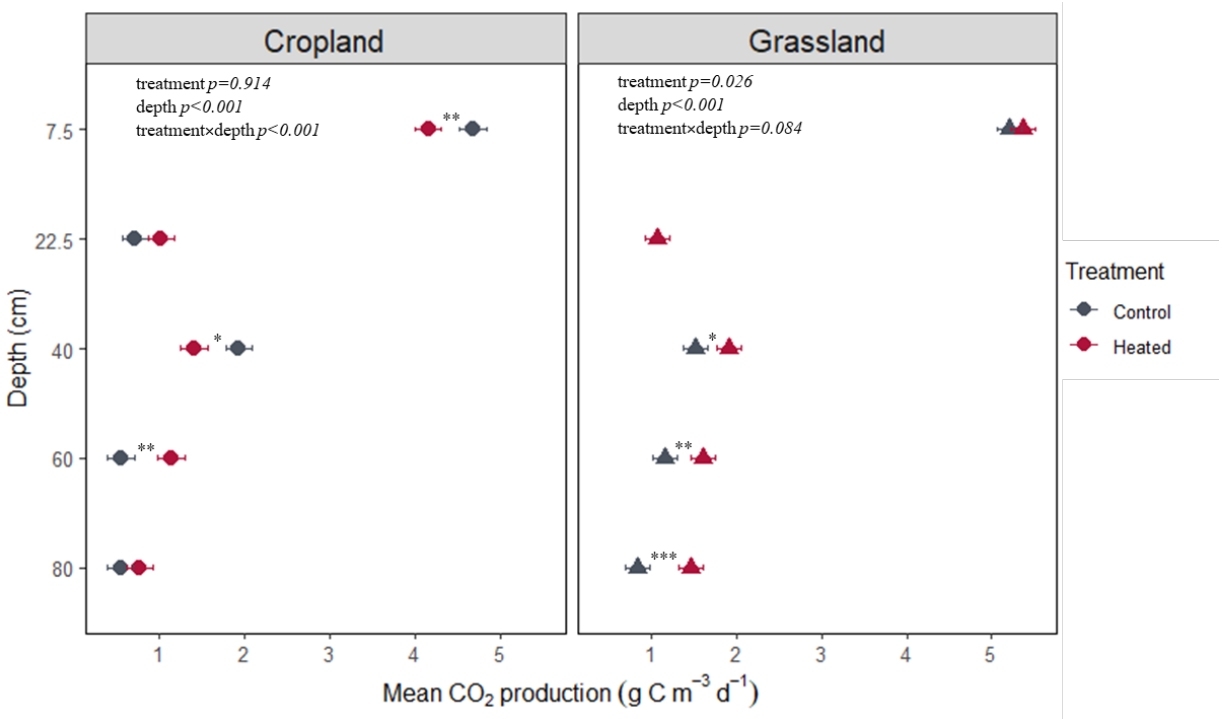


Fig. 8. Mean CO_2 production (g C m^{-3} d^{-1}) in the control and heated plots (mean ± SE, n=3) with depth increment (0-15, 15-30, 30-50, 50-70, and 70-90 cm) over the study period (January 2022 to June 2022). In the figure, the p-values denote the statistically significant effects of treatment,

depth, and their interaction (treatment×depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

2.4 Warming effects on soil profile carbon content

In control plots, soil organic carbon (SOC) concentrations along the different soil depths ranged from 20.24 to 5.42 kg C m^{-3} soil in cropland and 22.59 to 5.20 kg C m^{-3} soil in grassland (Fig. 9). *In-situ* warming had no effect on topsoil SOC content under both land management practices, whereas subsoil (>30 cm) showed a contrasting response to warming: it significantly increased under cropland but not under grassland (Fig. 9). The interaction between treatment and depth was significant ($p<0.001$) for cropland but not for grassland ($p=0.484$).

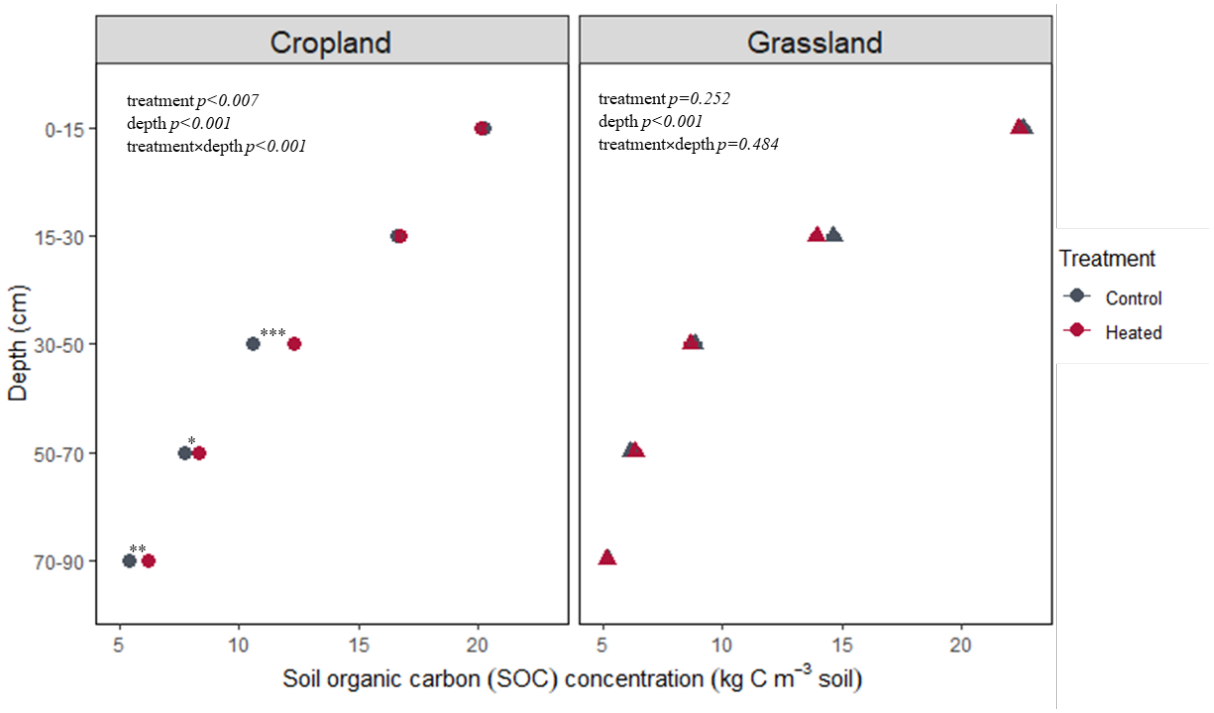


Fig. 9. Soil organic carbon (SOC) concentration (kg C m^{-3} soil) in the control and heated plots (mean ± SE, n=3) with depth increment (0-15, 15-30, 30-50, 50-70, and 70-90 cm), sampled in May 2022, one year after the onset of *in-situ* warming. In the figure, the p-values denote the statistically significant effects of treatment, depth, and their interaction (treatment×depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

Overall, we found that warming-induced SOC content in subsoil was higher in cropland (12.7%) but lower in grassland (0.09%). In contrast, the warming-induced SOC content in topsoil was lower for both cropland (0.05%) and grassland (2.42%) as compared to their controls. Over one year, the warming response on SOC content from the whole-soil profile was increased by 4.9% under cropland (from 60.6 kg C m^{-3} in control plots to 63.6 kg C m^{-3} in heated plots) and decreased by 1.6% under grassland (from 57.4 kg C m^{-3} in control plots to 56.5 kg C m^{-3} in heated plots).

2.5 Warming effects on leaf area index

In control plots, the leaf area index was 3.2 under cropland and 3.4 under grassland (Table 7). It significantly increased with *in-situ* warming for grassland ($p<0.01$) and also showed an increasing, but non-significant trend for cropland (Table 7).

Table 7. Warming response of leaf area index (mean ± SE, n=3) under the two different land management practices during the plant growing season (March to June 2022) with different treatments (control: heated).

	Leaf area index (m^2 m^{-2})	
Field	Control	Heated
Cropland	3.2 ± 0.2^{b}	3.6 ± 0.2^{ab}
Grassland	3.4 ± 0.2^{b}	4.6 ± 0.2^{a}

Here, different letters indicate the statistically significant effects of treatment and field interaction, as determined by post hoc analysis ($p<0.01$).

3. Discussion

3.1 Negative warming effects on whole-soil profile water content under the two land management practices

Our findings revealed that *in-situ* warming had a negative effect on soil water content in the whole-soil profile for both land management practices (Fig. 6). These results are in agreement with a recent meta-analysis by Xu *et al.* (2013), which showed that warming generally leads to soil drying in agricultural ecosystems. The two main processes of water loss from the soil reservoir include evaporation from the soil surface and transpiration by plants (Chapin et al., 2002). Both may be affected by land management practices (Liancourt et al., 2012). In this study, we showed that grassland soil was more vulnerable to soil water loss under *in-situ* warming compared to cropland. Despite the lack of air warming, warming-induced reduction in soil water content could be attributed to increased plant growth and transpiration processes with a higher leaf area index (Table 7), as also found by other authors (Chen et al., 2021; Wan et al., 2002; Xia et al., 2010). However, the plants' effects on soil water might be species- or management-and/or site-specific (Wang et al., 2012). In particular, in grassland with a complex root system exploring deeper soil layers it might be stronger than croplands. Indeed in grasslands, perennial plants could respire throughout the year involving (i) transpiration by plant leaves and (ii) water consumption by plant roots for their physiological and metabolic activities (Du et al., 2011). In contrast, cropland soil is characterized by periods of bare soil, plant regrowth and harvest, involving less root production (Table 5) (Hu & Chabbi, 2021) and consequently probably much less water consumption by plant roots and transpiration. As soil water becomes depleted under *in-situ* warming, this could alter the plants' C allocation strategies depending on land management practices, thereby affecting the C inputs and turnover in the soil profile (Li et al., 2019; Mokany et al., 2006; Vedere et al., 2022).

3.2 Contrasting warming response of seasonal CO_2 efflux under the two land management practices

Our data indicated that *in-situ* warming suppressed surface CO_2 efflux for both land management practices in the spring and autumn, whereas the other seasons (winter and summer) showed a contrasting warming response of surface CO_2 efflux (Fig. 7). *In-situ* warming could be a constraint for the spring and autumn seasons by slowing the processes that lead to both autotrophic and heterotrophic respirations that are controlled by precipitation and soil moisture determining plant growth (Miao et al., 2020; Quansah et al., 2015). In contrast, the different warming responses of soil CO_2 efflux in the winter and summer for both land management practices might be related to the continuous rhizodeposition supplied by the perennial root systems in grassland while cropland soil is bare during these periods (Hu & Chabbi, 2022; McGowan et al., 2019). In grassland, the warming response was negative in summer but positive in winter, which could be due to the warming-induced lower soil water content and seasonal fluctuation of plant growth and their root activity (Du et al., 2019; Hu et al., 2017). For example, the combination of high soil temperature and low soil water content might constrain root and microbial activity under summer warming, while in winter adequate moisture fuels greater microbial activity (Supp. Fig. S1) (Davidson et al., 1998; Vincent et al., 2006). In cropland, *in-situ* warming did not affect surface CO_2 efflux during summer or winter, which could be because the plants are only actively growing in the spring, providing evidence for plant activity as a control (Chen et al., 2021).

The contrasting warming response of seasonal CO_2 efflux leads to an overall reduction in whole-soil warming annual surface CO_2 efflux, depending on land management practices. Our findings differ from those found in various terrestrial ecosystems where warming stimulated soil CO_2 efflux (Hicks Pries et al., 2017; Lin et al., 2011; Luo et al., 2010; Ofiti et al., 2021; Schindlbacher et al., 2009; Yan et al., 2022). Nevertheless, our data are consistent with studies reporting reduced soil and microbial respiration under warming in an old-field grasslands (Wan

et al., 2007), in some seasons in tallgrass prairie (Verburg et al., 2005), and in an alpine meadow (Saleska et al., 1999). However, one recent meta-analysis demonstrated that on average, global soil will be a source of C to the atmosphere under a future warmer climate (Wang et al., 2022), supporting the expectation of positive SOC loss as a warming feedback (García-Palacios et al., 2021). In our study, warming-induced reductions in soil water content may be strongly influencing surface CO_2 efflux, through its influence on microbial communities and their functionalities, which are temperature- and moisture-dependent (Curiel Yuste et al., 2007; Davidson and Janssens, 2006). Water availability is a critical factor that can affect plant and microbial respiration. When water is limited, plants slow down photosynthesis to conserve water (Wang et al., 2018), leading to a decrease in plant contributions to soil respiration (Ribas-Carbo et al., 2005). Similarly, water limitation can reduce microbial activity and their contribution to respiration as insufficient water can impede substrate diffusion to microbial cells (Lehmann et al., 2020; Moyano et al., 2013; Védère et al., 2022). Moreover, *in-situ* warming may reduce the contribution of seasonal fine root respiration as a source of surface CO_2 efflux. In warmer climates, grassland ecosystems may increase their total root system length but lose a proportion of fine roots growing in the superficial layer of soil (Pilon et al., 2013).

3.3 Warming alters soil CO_2 production along soil depths under the two land management practices

Our results showed greater positive effects of warming on subsoil CO_2 production than in topsoil for both agricultural practices (Fig. 8). This is consistent with previous whole-soil warming observations in a temperate forest (Hicks Pries et al., 2017), but not in a tropical forest (McGrath et al., 2022). The stronger warming effect on CO_2 production in subsoil than in topsoil could be due to a contrasting response of microbial metabolisms and/or root respiration in both parts of the soil profile. In subsoil, microbial activity is generally much lower than in topsoil (Rumpel and Kögel-Knabner, 2011), which was explained by the absence of fresh organic matter input (Fontaine et al., 2007). Under warming, energy input into subsoil through root growth and

dissolved organic matter could be increased and stimulate copiotrophic populations at greater depths, leading to CO_2 production through decomposition of fresh root litter (Müller et al., 2016; Sanaullah et al., 2016; Soong et al., 2021). Root growth deeper in the soil profile could be stimulated by lower soil water content in warmed soil, which forces plants to explore deeper soil horizons (Joslin et al., 2000; Sotta et al., 2007).

Warming effects on CO_2 production were contrasting in the two management systems. Subsoil CO_2 production was more pronounced under warming of grassland soil compared to cropland. This might be explained by greater root production and activity under grassland than cropland, as indicated by the greater standing root biomass in this agroecosystem (Table 5). Conversely, we observed that warming-induced subsoil SOC content was significantly higher in cropland but slightly lower in grassland. These findings suggest that the turnover or accumulation of subsoil SOC, as well as subsequent mechanisms for CO_2 production under warming condition, may vary based on different management practices. For instance, if warming leads to an increase in above-ground biomass, soil disturbance caused by tillage might be advantageous. This disturbance helps in transferring crop residues, dead roots and topsoil C into subsoil layers where C accumulates, as shown in study by Yang et al. (2022). Consequently, this process results in higher subsoil SOC content in cropland. Additionally, the organic materials added through this process could serve as a food source for microorganisms, potentially leading to increased CO_2 production in the short-term.

In contrast, warming might cause a greater allocation of below-ground C in grassland compared to cropland. This allocation is primarily derived from rhizodeposition provided by the perennial root system, as indicated in Table 5 (Hu & Chabbi, 2022; McGowan et al., 2019). Consequently, continuous interaction between living root systems and subsoil microbes could have contributed to higher CO_2 production in grassland. This is supported by the negligible SOC loss observed under warming conditions.

In topsoil, warming-induced CO_2 production was differently affected by management. Under grassland, increased CO_2 production in topsoil could be attributed to a greater contribution of plant roots and rhizosphere microbes or saprotrophic microorganisms under warming conditions, which are the primary contributors to SOC decomposition (Schindlbacher et al., 2009; Zhou et al., 2007), as evidenced by lower SOC content under warming conditions. While decreasing CO_2 production in response to warming in cropland may be attributed to a double effect of both warming and tillage-induced soil disturbance (ploughing horizon at 30 cm depth), which can lead to shifts in soil microbial community and function due to lower plant root biomass (Table 5), resulting in lower CO_2 production and less sensitivity to warming-induced SOC decomposition, as evidenced by negligible SOC loss.

3.4 Future prospects

Although an increase in CO_2 production in subsoil is well documented in this study (Fig. 8) and supported by another whole-soil warming study in a temperate forest (Hicks Pries et al., 2017), the biotic fixation and movement of CO_2 in the context of projected global warming under different agricultural management practices are still unknown. Despite a few recent studies confirming that soil microbes can fix CO_2 in temperate soils (Nel and Cramer, 2019; Spohn et al., 2020). Moreover, warming might change the microbial community composition and in particular increase fungal contribution, leading to the formation of more stable pore structures (Miller and Jastrow, 2000). These effects may depending on soil management, as there is lower fungal contribution in cropland than in grassland due to ploughing events (Schnoor et al., 2011). In order to get a complete picture of soil responses, heterotropic respiration should in future studies be distinguished from autotropic respiration.

Additionally, CO_2 flux within the soil profile may also be influenced by its microstructural properties. Indeed, a recent study found that grassland soil has a larger macropore diameter due to the presence of large biopores that are periodically destroyed in cropland (Schlüter et al., 2022). The processes affecting soil structure may have an impact on soil gas

diffusivity, which determines whether CO_2 produced at depth is lost to the atmosphere or fixed in situ (Liao et al., 2023). Thus, we propose that for understanding and modeling the effects of soil warming on CO_2 efflux under different agricultural management practices, the parameters influencing CO_2 diffusivity, such as soil architecture need to be taken into account in addition to factors affecting CO_2 production such as root activity and microbial community composition.

4. Conclusion

In this study, we investigated seasonality of whole soil warming responses of CO_2 efflux. Moreover, we compared CO_2 production across soil depths down to 1m. We conclude that *in-situ* soil warming led to soil drying, the extent of which depended on soil management. Soil drying was more pronounced in grassland compared to cropland, supporting our initial hypothesis (H1). We also found contrasting seasonal dynamics of surface CO_2 efflux. While our findings indicate that *in-situ* warming suppressed surface CO_2 efflux for both land management practices in the spring and autumn, winter and summer showed greater increases and decreases in response to warming, respectively, under grassland compared to cropland. These findings reject our second hypothesis (H2), which states that CO_2 efflux would increase year-round in response to warming under continuous grassland but only seasonally in cropland soils.

Subsoil (>30 cm) CO_2 production was more vulnerable to warming than in the topsoil, and this increase due to warming was more pronounced in grassland compared to cropland, confirming our last hypothesis (H3). In topsoil (0-30 cm), warming decreased CO_2 production in cropland but not in the grassland. Overall, land management had a significant impact on CO_2 production throughout the soil profile, which may driven by both autotrophic and heterotrophic sources. Thus, we suggest that management influences the warming responses of CO_2 production and efflux by its effects on soil physical organization in addition to plant and microbial activity with root carbon input.

Chapter 3

How does warming affect microbial communities in whole-soil under contrasting management?

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Abstract

Deciphering the impact of anthropogenic warming on soil microbial community is of major importance considering their strong involvement in many of ecosystem functions and services that soil provides for human well-being. While most research has focused on the topsoil, the effects of warming on microbial communities in deep soil layers (>30 cm) remain largely unexplored. Here, to explore the effects of warming, we conducted a year-long *in-situ* whole-soil profile warming (+4°C) experiment down to 0.70-m depth in an agricultural Cambisol under both cropland and grassland. Our aim was to examine how this short-term warming affects soil microbial communities (bacteria and fungi) in different soil depths under differ land management practices. We carried out soil DNA extraction and metabarcoding to determine the response of the soil microbial communities to warming.

Our results revealed that warming did not impact microbial biomass and diversity (i.e., richness) across soil depths under both land management practices. However, it shaped the structure of microbial communities, with bacteria being more reactive than fungi regardless of the land management practices. Moreover, microbial networks were notably more complex in grassland soils than in cropland. Warming, however, led to a significant reduction in microbial network complexity throughout the whole-soil profile regardless of the land management. This reduction was more pronounced in cropland, where microbial links decreased by 53.4%, compared to a 40.3% decrease in grassland. Interestingly, all microbial interactions were significantly affected by warming in cropland, while in grassland, only bacterial-bacterial interactions were significantly affected. These findings suggest that climate warming may alter the structure and complexity of soil microbial communities depending on land management practices, potentially influencing soil function and agroecosystem health.

Keywords: Soil warming; Microbial community composition; Microbial network complexity; Agricultural land management; Whole-soil profile

1. Introduction

Soil, the Earth's living terrestrial skin, plays a central role in supporting life and hosts a vast array of biodiversity, including microorganisms and fauna, representing roughly one-quarter of all species on Earth (Guerra et al., 2021; Tecon and Or, 2017). With several thousand species *per* gram of soil, microorganisms (i.e., bacteria and fungi) dominate this biodiversity and are key players in soil ecosystem functioning (Allison and Martiny, 2008; Karimi et al., 2017). These microorganisms are crucial for the cycling of organic matter and nutrients (Maron et al., 2018; Pandey et al., 2018; Sokol et al., 2022), the formation of soil structure (Chenu and Cosentino, 2011; Eilers et al., 2012; Mueller et al., 2024), and the supply of essential ecosystem services such as carbon sequestration, water quality maintenance, pollutant mitigation, pathogen regulation and plant growth promotion (P. Smith et al., 2015; Wall et al., 2012). Within soil ecosystems, microorganisms coexist and form complex ecological interaction through mechanisms such as mutualism, commensalism, competition, predation, and parasitism (Faust and Raes, 2012; Karimi et al., 2017; Ma et al., 2020; Montoya et al., 2006b). These complex ecological relationships can be represented as networks (Pržulj and Malod-Dognin, 2016). The characterisation of these networks may be used to provide simple and operational indicators of ecosystem quality and functioning in response to global changes like climate warming or disturbances like agricultural management practices (Karimi et al., 2017; Pechlivanis et al., 2024; Yuan et al., 2021).

Recent studies on the topsoil have revealed that microbial functions can either be enhance or collapse under warming, driven by changes in microbial network complexity (Tian et al., 2022; Wu et al., 2022; Yuan et al., 2021). Yet, the response of subsoil (>30 cm) microbial communities to climate warming, particularly under different agricultural land management practices remains largely unexplored. Research considering the whole-soil profile has reported declines in microbial biomass (Blume et al., 2002; Fierer et al., 2003; Kramer et al., 2013),

reduced microbial diversity (Fierer et al., 2003; Manoharan et al., 2019; Will et al., 2010), and shifts in community structures (Eilers et al., 2012) in deeper soil horizons, likely due to limited nutrient and energy fluxes (Dove et al., 2021; B. Li et al., 2022). These findings suggest a distinct ecological dynamic for microbial communities in deeper soil layers, complicating the extrapolation of topsoil warming effects to subsoil microbial communities. Notably, Earth system models project that surface and subsoils will warm at nearly the same rate throughout the 21st century, with soil temperatures down to 1.0-m depth expected to rise by nearly 4.5°C by 2100 under the Representative Concentration Pathway 8.5 scenario (Soong et al., 2020). Focusing exclusively on surface, risks drawing incomplete or inaccurate conclusions about the effects of global change on soil functions, particularly given the substantial carbon reservoirs stored in deeper horizons. This highlights the crucial need to study both surface and subsoil layers to fully capture the impacts of climate change on soil dynamics and the ecosystem services they underpin.

According to metabolic theory of ecology (MTE), rising temperatures are expected to enhance microbial interactions by accelerating individual metabolic processes and promoting faster growth rates at higher temperatures (Brown et al., 2004; Clarke, 2006). Consequently, soil warming is anticipated to increase the complexity of microbial associations (i.e., the number of interaction links), resulting in more intricate ecological networks (Tian et al., 2022; Yuan et al., 2021). However, elevated temperatures, coupled with associated environmental stressors such as reduced soil moisture (Khan et al., 2023), could impose strong filtering effects on microbial communities, potentially disrupting these interactions and altering networks (Wu et al., 2022). This contradicts the expectations of MTE, suggesting that under warming conditions, microbial network complexity might decrease or even collapse (Ullah et al., 2018). In addition to these uncertainties, it remains unknown how climate warming and varying agricultural management practices might interact to influence the complexity of soil microbial

networks throughout the whole-soil profile. While surface soil microbial communities are often studied (Tian et al., 2022; Wu et al., 2022; Yuan et al., 2021), the impact of whole-soil warming—extending to deeper layers—on microbial network structure and interactions is still poorly understood. This knowledge gap highlights the need for comprehensive research that considers both surface and subsoil dynamics to fully capture the effects of warming and management practices on ecosystem stability and function.

In this study, we investigated bacterial and fungal communities and their networks in whole-soil profiles after one year warming (+4°C) under contrasting agricultural management (grassland and cropland). The aim of our study was to determine if warming and agricultural land management practices interact to affect microbial biomass, richness and network complexity down to 0.70-m depth in an agricultural Cambisol soil. We tested the following hypotheses: (H1) microbial biomass and richness may respond differently to warming according to soil depth, and the magnitude of these effects may be management-specific due to different intensity of land management practices; and (H2) the complexity of microbial network may collapse in response to warming, depending on land management practices, as warming-induced reductions in soil water content were found to be land management-specific (Khan et al., 2023).

2. Results

2.1 Warming effects on molecular microbial biomass across the soil profile

In the control soils without warming, the molecular microbial biomass varied across the soil profile ranged from 37.5 to 11.9 µg DNA g^{-1} soil in cropland and from 45.4 to 2.2 µg DNA g^{-1} soil in grassland (Fig. 10). It decreased significantly (p<0.001) with depth under both land management practices (Fig. 10). Warming had non-significant (p≥0.110) effect on microbial biomass across soil depths under both land management practices (Fig. 10). Additionally, the interaction between warming treatment and depth was also non-significant (p≥0.145) (Fig. 10).

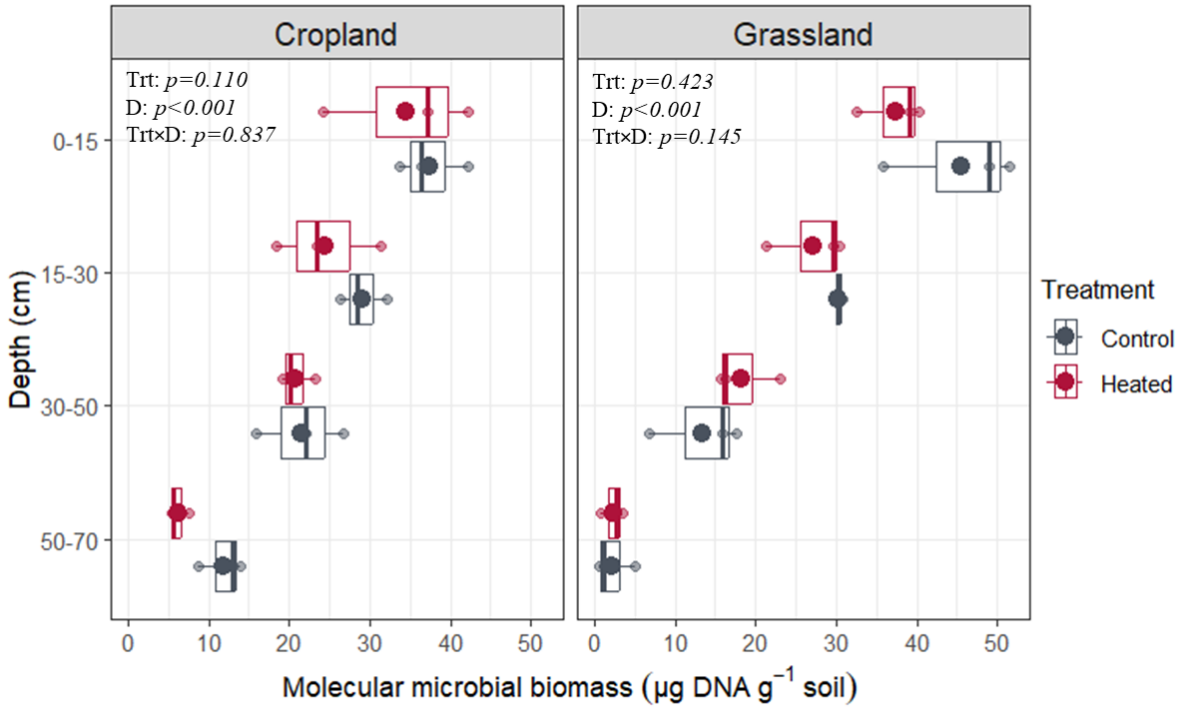
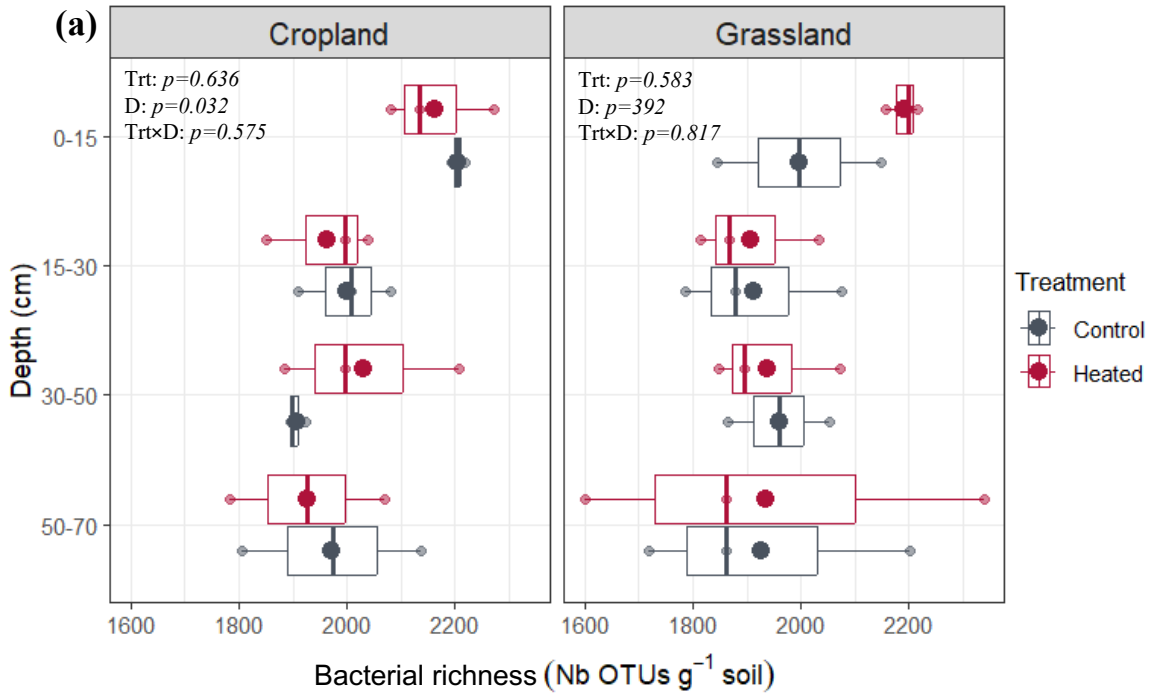


Fig. 10. Soil molecular microbial biomass in the control and heated plots (n=3), sampled in May 2022, one year after the onset of *in-situ* warming, throughout the 0-70 cm depth of soil profile under cropland and grassland. In the figure, p-values denote the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D) as determined by post-hoc analysis.

2.2 Warming effects on molecular microbial richness across the soil profile

In the control soils without warming, the bacterial richness across the soil profile ranged from 2170 to 1971 OTUs under cropland and from 1997 to 1927 OTUs under grassland (Fig. 11a). The fungal richness ranged from 1266 to 1781 OTUs under cropland and from 1069 to 1474 OTUs under grassland (Fig. 11b). Bacterial richness showed a significant (p=0.032) decreasing trend with depth only under cropland (Fig. 11a). Conversely, fungal richness showed a significant (p=0.038) increasing trend with depth only under grassland (Fig. 11b). Warming had no significant (p≥0.243) effect on bacterial or fungal richness under both land management practices (Fig. 11). The interaction between warming treatment and depth was also non-significant (p≥0.167) for both land management practices (Fig. 11).



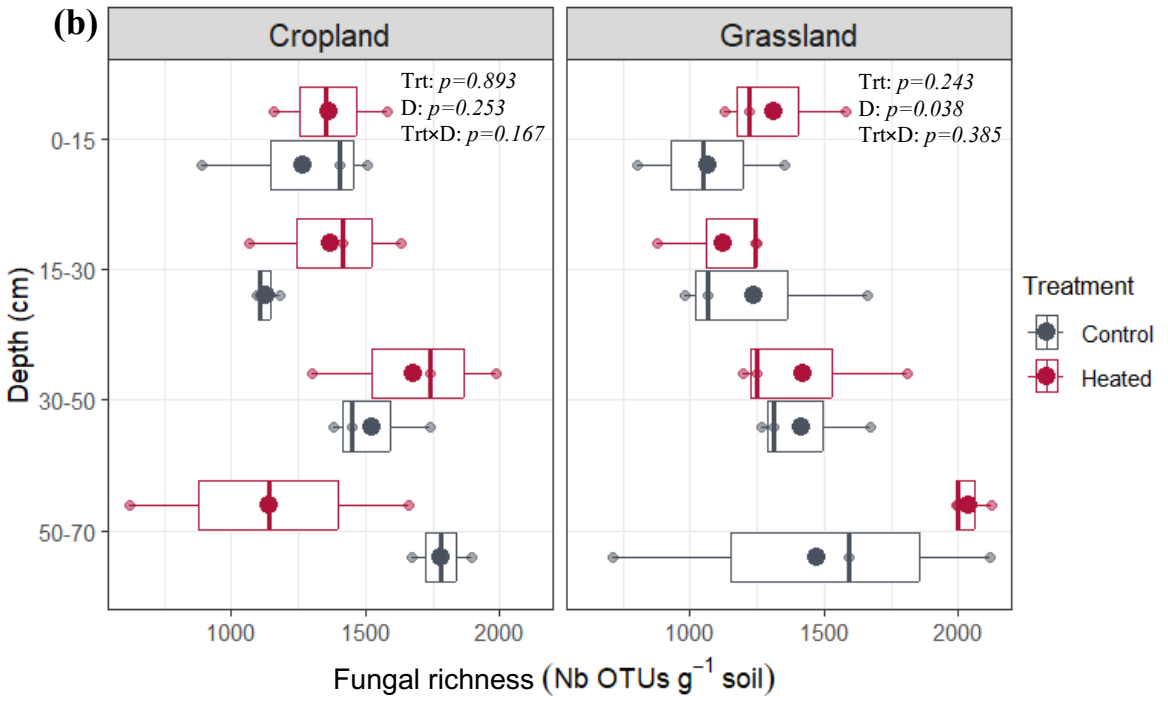


Fig. 11. Bacterial (a) and fungal (b) richness in the control and heated plots (n=3), sampled in May 2022, one year after the onset of *in-situ* warming, throughout the 0-70 cm depth of soil profile under cropland and grassland. In the figure, p-values denote the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D) as determined by post-hoc analysis.

2.3 Warming effects on whole-soil profile microbial community structure

The bacterial and fungal community structures and their responses to warming were visualized using PCA analysis (Fig. 12, Supp. Fig. S2). The first two axes explain more than 86% and 96% of the variation in bacterial and fungal community structures under both land management practices (Fig. 12). The bacterial community structure differed significantly (p≤0.01) with depth under both management practices (Supp. Fig. S2). In contrast, the fungal community structure did not show significant differences with depth regardless of land management. Warming shifted the whole-soil bacterial community structure, with significant changes observed in cropland (p=0.044) and to a lesser extent in grassland (p=0.078), but did

not affect the fungal community structure under both land management practices (Fig. 12, Supp. Fig. S2).

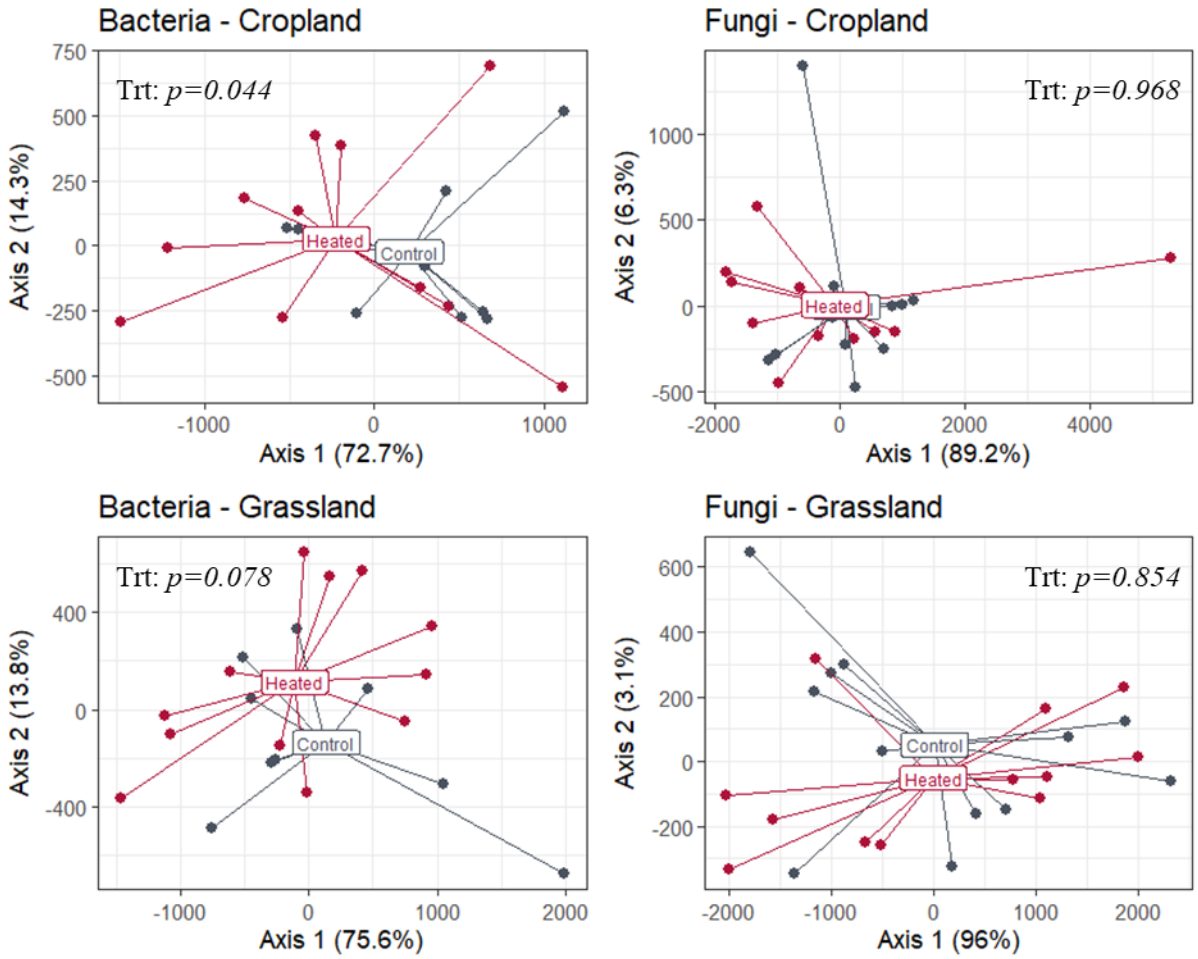


Fig. 12. Principal component analysis of the composition of bacterial and fungal structural communities in the control and heated plots (n=12), sampled in May 2022, one year after the onset of *in-situ* warming, in the whole-soil profile (0-70 cm) under cropland and grassland. In the figure, p-values denote the statistically significant effects of treatment (Trt) as determined by PERMANOVA test.

2.4 Warming effects on whole-soil profile microbial network complexity

Microbial network complexity was analyzed using co-occurrence network modularity analysis to observe the number of links and differentiate between bacteria-bacteria/bacteria-fungi/fungi-fungi interactions (Fig. 13). In control soils at ambient temperature, grassland had

a significantly ($p \leq 0.05$) higher number of links compared to cropland (Fig. 13). Bacterial-bacterial interactions were dominant under both land management practices, followed by bacterial-fungal and fungal-fungal interactions. Warming significantly ($p \leq 0.05$) reduced the number of links in the whole-soil profile by 53.4% and 40.3% under cropland and grassland management practices, respectively (Fig. 13). In cropland, all microbial interactions were significantly ($p < 0.01$) affected by warming. In contrast, in grassland, only bacterial-bacterial interactions showed a significant ($p < 0.01$) response to warming (Fig. 13).

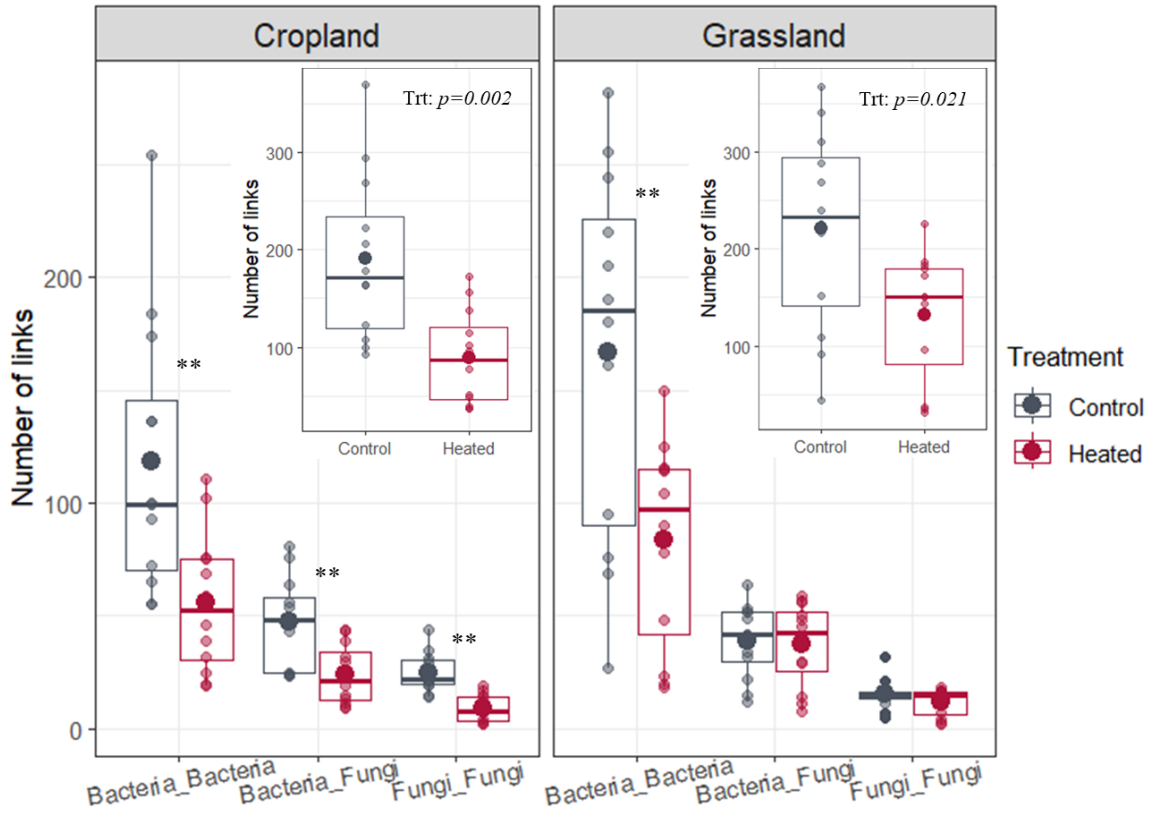


Fig. 13. Microbial network complexity as a number of links and nature of links in the control and heated plots (n=12), sampled in May 2022, one year after the onset of *in-situ* warming, throughout the whole-soil profile (0-70 cm) under cropland and grassland. In the figure, *p*-values denote the statistically significant effects of treatment (Trt) *as determined by post-hoc analysis.* The asterisk indicates any significant treatment effect within nature of links as determined by post hoc analysis (***p<0.01*).

3. Discussions

Since soil microbes (i.e., bacteria and fungi) may be the driving force behind soil functions (Maron et al., 2018), understanding the stability (i.e., resistance and/or resilience) of soil microbes in response to anthropogenic warming is crucial (Allison and Martiny, 2008; Karimi et al., 2017). Despite being limited to a one-year period, this study emphasizes the importance of detailed measurements across the entire soil profile. We present one of the few studies investigating how soil microbial biomass, diversity, community structure and network complexity responds to warming under both cropland and grassland management practices.

We found no significant effects of warming on microbial biomass and richness across soil depths whatever the land management practices, which contradicts our first hypothesis (H1) but is consistent with findings from other studies (Q. Zhang et al., 2023; Zosso et al., 2021). These results may be due to the ability of microbial communities to acclimate to warming, especially in ecosystems with seasonal temperature fluctuations, allowing them to maintain biomass and richness despite increased temperatures (D'Alò et al., 2024; Liu et al., 2024; Wang et al., 2024).

Despite the stability of communities in terms of biomass and richness, warming altered the bacterial community structure in the whole-soil profile, while fungal community structure remained relatively stable under both land management practices. These findings are consistent with several studies showing that bacterial communities tend to be more sensitive to warming than fungal communities (Ochoa-Hueso et al., 2018; Wan et al., 2023; Yang et al., 2021; Yuste et al., 2011). This difference is likely because warming often reduces soil moisture availability, which disproportionately affects bacterial communities, as they rely more heavily on moisture for growth and survival (Li et al., 2024; Wang et al., 2024). Reduced moisture can impact soil oxygen levels, slow microbial metabolism, and limit resource availability, all of which may disrupt the structure of bacterial community (Cui et al., 2020; Schimel, 2018; F. Yang et al.,

2022). Conversely, the fungal communities may be more resistant to warming and moisture changes, as many fungi are better acclimated to withstand drought by translocating water from moist to arid areas, and can utilize resources from more recalcitrant organic matter (Herman and Bleichrodt, 2022; D'Alò et al., 2024; Li et al., 2024; Wang et al., 2024). This physiological distinction between bacteria and fungi may explain the contrasting responses observed in our study. However, our findings contrast from a recent study on whole-soil profile warming in an alpine grassland, which found no significant impact of warming on bacterial or fungal community structure (Zhang et al., 2023). These discrepancies may be attributed to differences in ecosystem types, environmental conditions, soil properties, and the duration of warming experiment. For instance, the cold temperatures (1.1°C), low C availability, and high elevation (3500 m a.s.l.) of alpine grasslands may lead to different microbial responses compared to the oceanic climate conditions in our study (12°C; 128 m a.s.l.), which may have enabled bacterial communities to respond more readily (D'Alò et al., 2024; Liu et al., 2024; Wang et al., 2024).

The stability of microbial communities and their functional potential is primarily influenced by community composition and the complex interactions among microbial members, which form microbial networks (Faust and Raes, 2012; Karimi et al., 2017; Ma et al., 2020). Although these networks are based on mathematical algorithms derived from species co-occurrence, they offer valuable insights into how microbes coexist and functionally interact (Faust and Raes, 2012; Karimi et al., 2017; Ma et al., 2020). Increased network complexity marked by more interconnected species typically suggests a more resilient system capable of maintaining community stability (F. Yang et al., 2022; Yuan et al., 2021). Complex networks often exhibit microbial functional redundancy, where multiple species fulfill similar roles, ensuring the continuity of vital biogeochemical processes such as nutrient cycling, even if certain species are lost or impaired (Hu et al., 2024; Maron et al., 2018; Pan et al., 2024; Stamou et al., 2024; Sokol et al., 2022). In this study, we observed greater microbial network complexity

in grassland compared to cropland throughout the whole-soil profile. This finding suggests that agricultural management practices can strongly influence microbial interactions, with tillage in cropland disrupting microbial networks and continuous plant cover in grassland favoring these interactions (Romdhane et al., 2022). Although direct observation of these interactions is not possible, the higher network complexity in grassland implies that these microbial communities may be more resilient and better able to maintain soil functions—such as nutrient cycling—owing to stronger and more diverse microbial interactions supporting key biogeochemical processes (Li et al., 2024; Pan et al., 2024; Stamou et al., 2024). Warming significantly reduced microbial network complexity, as indicated by a decreased number of microbial links, supporting our second hypothesis (H2) and are consistent with studies in semiarid grassland (Wang et al., 2023) and subtropical forest ecosystems (Wang et al., 2024). These findings suggest that the stability and interactions of microbes were impaired by warming (Rillig et al., 2023; Wang et al., 2023).

Interestingly, we found that the effect of warming on microbial network complexity was more pronounced in cropland (53.4% reduction) than in grassland (40.3% reduction). This suggests that the response of network complexity and stability to warming may be influenced by land management practices, supporting our second hypothesis (H2). This is likely due to the initial network complexity, which was lower in cropland than in grassland, indicating that less complex networks are more vulnerable to warming because they have limited microbial interactions to buffer against environmental stresses (Rillig et al., 2023; Yang et al., 2021). Further supporting this, microbial interaction analysis revealed that in cropland, all interactions were affected by warming, whereas in grassland, only bacterial-bacterial interactions were affected. This implies that the more complex network in grasslands can maintain some of its stability despite warming. Indeed, reduced soil moisture conditions under warming observed in this study (Khan et al., 2023), may act as a strong filtering factor against the existing bacterial

community structure (Wu et al., 2022) and profoundly affect bacterial-bacterial interactions in both land management practices. Conversely, bacterial-fungal and fungal-fungal interactions in cropland may be more severely affected due to the combined effect of warming and tillage-induced soil disturbance (Shi and Thakur, 2023; Siles et al., 2023). This could lead to disrupt soil functioning and their associated ecosystem services more in cropland than in grassland ecosystem.

4. Conclusion

In summary, this study reveals how whole-soil warming interacts with agricultural land management practices to affect microbial biomass, diversity, community structure and network complexity down to 0.70-m soil depth. We found that warming had no effect on microbial biomass and alpha-diversity across soil depths. Instead, warming significantly altered bacterial community structure but did not affect fungal community structure throughout the soil profile, regardless of land management practices. Grassland soils supported more intricate microbial networks compared to cropland, yet warming reduced network complexity in both systems. The impact was more severe in cropland, where all microbial interactions were disrupted, compared to grassland, where only bacterial-bacterial interactions were affected. These findings suggest that climate warming may destabilize microbial community networks, particularly in systems with lower initial network complexity, potentially impairing soil functioning and the health of agroecosystems. This insight highlights the need for adaptive management strategies to mitigate warming impacts and safeguard soil health.

Chapter 4

Whole soil warming promotes surface soil carbon loss but deep soil carbon gain, depending on land management practices in temperate climate

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Abstract

The aim of this study was to investigate the effects of agricultural land management and soil warming on (1) the direction and magnitude of soil organic carbon (SOC) sequestration in whole-soil profiles and (2) the soil microbial metabolism involved. To this end, we conducted an *in-situ* warming (+4°C) experiment down to 1.0-m in an agricultural Cambisol. Soil microbial communities' composition (ester-linked fatty acid methyl ester profile) and extracellular enzyme activities were measured, related to SOC and nutrients under two different land management practices: cropland and grassland.

Our results indicated that 3-years of soil warming had no effect on SOC and nitrogen (N) stocks across the grassland profile, while cropland soil showed a decrease of SOC and N stocks by 18.1% and 15.0% in surface soil (0-15 cm) and an increase by 86.7% and 68.8% in deep soil layers (70-90 cm). These observed shifts in SOC and N stocks were aligned with corresponding changes in extracellular (CN-acquisition) enzyme activities, eco-enzymatic stoichiometry, and the composition of bacterial communities. Additionally, warming led to a slight decrease in aboveground-biomass production for cropland. Furthermore, we found that microbial biomass and its composition showed management-specific responses to warming in surface soil. Overall, these findings suggest that the warming effect on SOC stocks may depend on soil depth and land management. Therefore, agricultural land management could regulate the SOC response to warming by altering carbon inputs into the soil system with consequences for microbial community composition and activity.

Keywords: Soil warming; Extracellular enzyme activity; Microbial community composition; Agricultural land management; Whole-soil profile; Carbon-climate feedback

1. Introduction

Increases in atmospheric CO_2 concentrations and the resulting global warming can cause strong alterations in the biogeochemical cycles of terrestrial ecosystems and agricultural production (Grimm et al., 2013; IPCC, 2021; Scheffers et al., 2016). Specifically, agricultural land management and warming can lead to complex feedbacks in the soil-climate system (Field et al., 2007; IPCC, 2013) through their effects on soil organic matter (SOM) dynamics. In laboratory and modelling studies, it was shown that warming may stimulate soil microbial metabolism and enzyme activities (Davidson and Janssens, 2006; Conant et al., 2011), which may promote soil organic carbon (SOC) loss and accelerate nutrient mineralization processes in the soil profile (Wang et al., 2022). However, it has been pointed out that these processes are regulated by microbial access to carbon and nutrient sources (Moinet et al., 2020; Von Lützow and Kögel-Knabner, 2009). Additionally, under field conditions, the warming effects on SOC turnover may be controlled by plant responses to warming, in particular plant phenology, roots activity, and substrate supply (Pugnaire et al., 2019). Therefore, SOC stability in warmed soils may vary under different land management conditions with different vegetation types, which may have contrasting effects on microbial communities and their activity (Berg and Smalla, 2009; Chao et al., 2014). To foster climate change mitigation and adaptation by the introduction of sustainable agricultural management practices (Rumpel et al., 2020; Soussana et al., 2019), it becomes imperative to understand the warming impacts on SOC and nutrient cycling in managed agricultural systems.

Owing to their key role in SOC turnover and their sensitivity to abiotic conditions, soil extracellular enzyme activities (EEA) may be key parameters to understand the warming effects on microbial metabolism (Bell et al., 2010; Henry, 2012; Luo et al., 2017; Zuo et al., 2018). The EEA are mainly produced by microbial communities and control the depolymerization of labile and complex SOM that can be the rate-limiting step of soil nutrient recycling (Burns et

al., 2013; Jing et al., 2016; Sinsabaugh et al., 2009). Numerous extracellular enzymes with different functions co-exist in the soil-system. Some of them, the β-1,4-glucosidase (BG), the β-1,4-Nacetylglucosaminidase (NAG), the leucine aminopeptidase (LAP) and the acid phosphatase (AP) have been more extensively studied (Daunoras et al., 2024; Sinsabaugh et al., 2009). These enzymes catalyze the production of bioavailable monomers from dominant organic compounds and are considered indicative of carbon (C), nitrogen (N), and phosphorus (P) acquisition by soil microbial communities (Allison and Jastrow, 2006). While C-acquisition enzyme (BG), facilitate the hydrolysis of SOC, CN-acquisition enzymes (LAP and NAG) target substrates that contain C along with N (Daunoras et al., 2024; Mori et al., 2023). P-acquiring enzyme (AP), on the other hand, release mineral P through hydrolysis (Turner and Joseph Wright, 2014), and their effect on SOC is less direct, but may occur indirectly by promoting microbial metabolism in P-limited conditions (Mori et al., 2023). Altogether, these enzymes may influence the overall microbial activity and SOC dynamics. The ratio of these C-, N-, and P-acquiring enzymes, i.e., the enzyme stoichiometry, can provide insights into the long-term adjustments of microbial resource use and limitations that underlie biogeochemical response of soils to global changes like climate warming (Sinsabaugh et al., 2009). Indeed, warming has the potential to stimulate enzyme synthesis and accelerate SOM turnover (Conant et al., 2011; Zuccarini et al., 2020) but the magnitude of these responses are uncertain, as their reaction rates could depend on intricate interactions between microbial ecology and physiology (Fanin et al., 2022).

Until now, the effects of *in-situ* warming on SOC, microbial communities and EEA dynamics have been mainly investigated for topsoil layers (Yost and Hartemink, 2020; Dove et al., 2021), although more than 50% of global SOC are stored in subsoil horizons (Jobbágy and Jackson, 2000; Rumpel et al., 2012), which are characterised by longer turnover times (Rumpel and Kögel-Knabner, 2011; Hou et al., 2019). Despite the high proportions of stabilised

compounds occurring in subsoil, recent whole-soil warming studies suggest that part of the subsoil C may be responsive to changing abiotic conditions (Hicks Pries et al., 2017; Nottingham et al., 2020; Ofiti et al., 2021). In addition, subsoil C was found to be more affected by agricultural management practices than previously thought (Osanai et al., 2020; Samson et al., 2021; Shahbaz et al., 2017). The microbial community structures and microenvironments in subsoils are quite different from those of topsoil (Eilers et al., 2012; Stone et al., 2014) and may consequently be differently impacted by warming, both directly and indirectly through altered plant C inputs (Morrison et al., 2019). Land management could also modulate the microbial response to warming through change in the composition and traits of plants and soil microbes (De Vries et al., 2013; Piton et al., 2021). It has been suggested that soil microbial communities might be more sensitive to climate change in nutrient rich cropland than grassland soils, the later selecting for K-strategist like fungi that are more stress tolerant (De Vries et al., 2012). Thus, the microbial factors affecting biogeochemical response to warming may differ across depths under different agricultural land management.

In this study, we aimed to assess how microbial responses to 3 years of *in-situ* whole-soil-profile warming (+4°C) are modulated by land management practices. We investigated plant aboveground-biomass production, soil physicochemical properties, extracellular enzyme activities, microbial biomass and community composition down to 1.0-m depth in an agricultural Cambisol under cropland and grassland. We tested the following hypotheses: (H1) warming leads to SOC loss across all soil depths because it stimulates the activity of C-, N-, and P-acquiring enzymes; (H2) the warming response of enzyme activities is associated with a shift in enzyme stoichiometry because warming changes microbial communities and their nutrient requirements; and (H3) the warming effect on SOC is land management-specific because soil microbial communities and their functioning may be more sensitive to warming under cropland with annual vegetation than perennial grassland.

2. Results

2.1 Warming effects on above-ground biomass

In control soils under ambient temperature, the mean above-ground biomass was 2.8 t ha^{-1} in cropland and 2.2 t ha^{-1} in grassland (Table 8). It showed a non-significant ($p=0.530$) change with warming for grassland, while a marginally significant ($p=0.070$) decreasing trend was observed for cropland (Table 8).

Table 8. Warming effects on above-ground dry biomass (mean ± SE, n=6) from 2022 to 2024 under the two different land management practices with different treatments (control: heated).

	Above-ground biomass (t ha^{-1})		
Field	Control	Heated	p-value
Cropland	2.8±0.6	2.3±0.6	0.070
Grassland	2.2±0.3	2.4±0.3	0.530

Here, p-value represent the statistically significant effects of warming treatment as determined by post hoc analysis.

2.2 Warming effects on physicochemical properties along the soil profiles

In control soils under ambient temperature, pH ranged from 6.83 to 7.69 across depths under cropland and from 6.66 to 7.57 under grassland (Fig. 14a). It increased significantly ($p<0.001$) with depth but did not exhibit any significant warming effects for both land management practices (Fig. 14a). Similarly, soil moisture availability increased significantly ($p<0.001$) with depth but showed non-significant warming effects for both land management practices (Fig. 14b).

In control soils under ambient temperature, soil organic carbon (SOC) stocks along the soil profile ranged from 30.3 to 12.9 g C m^{-2} soil under cropland and from 32.0 to 13.1 g C m^{-2} soil under grassland. Nitrogen (N) stocks ranged from 3.0 to 1.6 g N m^{-2} soil under cropland and from 3.1 to 1.8 g N m^{-2} soil under grassland (Fig. 15a & 15b). Both SOC and N stocks significantly ($p<0.001$) decreased with depth for both land management practices. Warming

had no effect on SOC and N stocks along the soil profile under grassland (Fig. 15a & 15b). In contrast, under cropland, warming significantly ($p \leq 0.05$) decreased SOC stocks by 18.1% and N stocks by 15.0% at 0-15 cm, whereas it increased SOC stocks by 86.7% and N stocks by 68.8% at 70-90 cm depth (Fig. 15a & 15b). The interaction between warming treatment and depth was also significant ($p \leq 0.05$) only for cropland (Fig. 15a & 15b). Similarly, the C:N ratio decreased at 0-15 cm but increased at 70-90 cm with warming only for cropland (Fig. 15c).

In control soils under ambient temperature, dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in soil solution collected at 90 cm depth were 3.28 and 16.7 mg L^{-1} in cropland, and 1.79 and 3.8 mg L^{-1} in grassland (Table 9). DOC concentrations showed a non-significant ($p \geq 0.62$) change with warming for both land management, while a significant ($p \leq 0.05$) increasing trend in TDN concentrations was observed for both cropland and grassland (Table 9).

Table 9. Warming effects on dissolved organic carbon and total dissolved nitrogen (mean ± SE, n=6) from 2023 to 2024 under the two different land management practices with different treatments (control: heated).

	Dissolved organic carbon (mg L^{-1})		
Field	Control	Heated	p-value
Cropland	3.28±1.9	3.83±1.9	0.621
Grassland	1.79±0.1	1.77±0.1	0.912
	Total dissolved nitrogen (mg L^{-1})		
Field	Control	Heated	p-value
Cropland	16.7±6.9	43.6±6.6	0.001
Grassland	3.8±0.8	6.0±0.9	0.037

Here, p-value represent the statistically significant effects of warming treatment as determined by post hoc analysis.

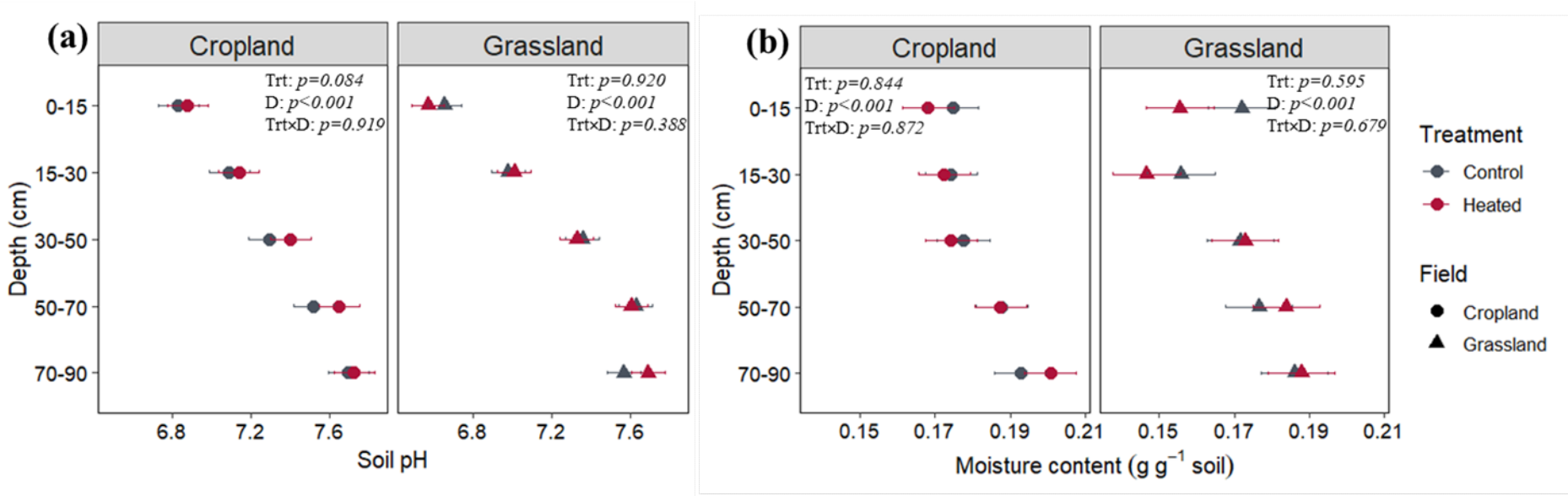


Fig. 14. Soil physicochemical properties: (a) soil pH, and (b) moisture content, in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

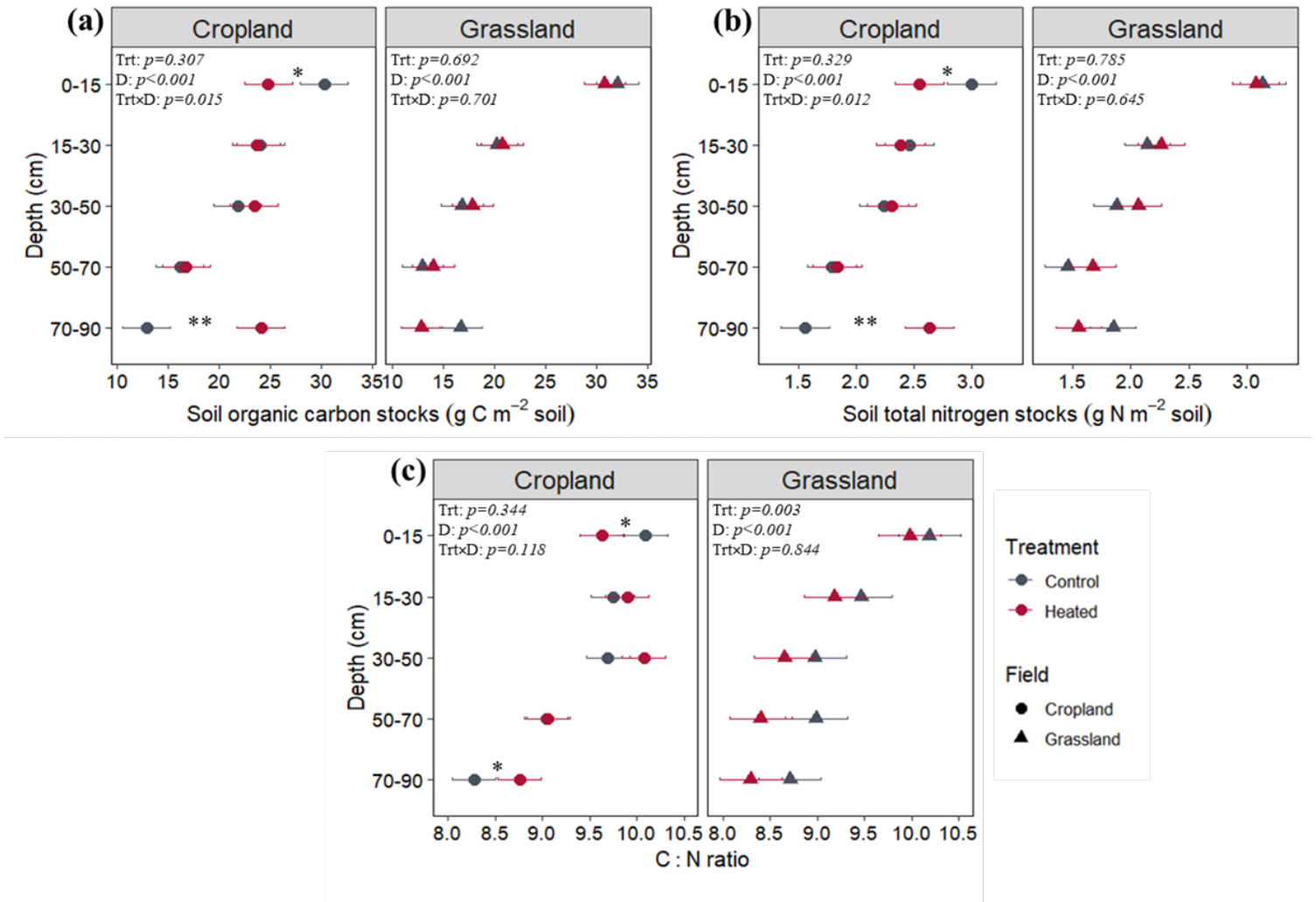


Fig. 15. Soil chemical properties: (a) soil organic carbon stocks, (b) soil total nitrogen stocks, and (c) C:N ratio, in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$).

2.3 Warming effects on extracellular enzyme activities and eco-enzymatic stoichiometry along the soil profiles

The potential activities of BG, AP, NAG and LAP enzymes decreased significantly ($p<0.001$) with depth for both land management practices (Fig. 16). The BG and AP activities did not show any significant warming response along the soil profile for both land management practices (Fig. 16a & 16b). In contrast, warming led to significantly ($p\leq0.05$) higher NAG activity at 0-15 cm under grassland, while under cropland lower activities were recorded at this depth (Fig. 16c). The LAP activity showed a significant ($p\leq0.05$) but contrasting warming response under both land management practices. Indeed, the LAP activity of warmed soil was significantly higher under cropland at 70-90 cm and lower under grassland at depths of 0-15 cm and 30-50 cm (Fig. 16d).

The ratios of BG/AP, (NAG+LAP)/AP and BG/(NAG+LAP) decreased significantly ($p<0.001$) with depth for both land management practices (Fig. 17). Warming had no effect on the BG/AP ratio for both land management practices (Fig. 17a) but the other two ratios showed contrasting warming response. Under grassland, warming significantly ($p\leq0.05$) increased the BG/(NAG+LAP) ratio but decreased the (NAG+LAP)/AP ratio at 30-50 cm depth (Fig. 17b & 17c). Conversely, under cropland, warming significantly ($p<0.05$) decreased the BG/(NAG+LAP) ratio at 0-15 cm depth (Fig. 17b) and increased the (NAG+LAP)/AP ratio at 70-90 cm depth (Fig. 17c).

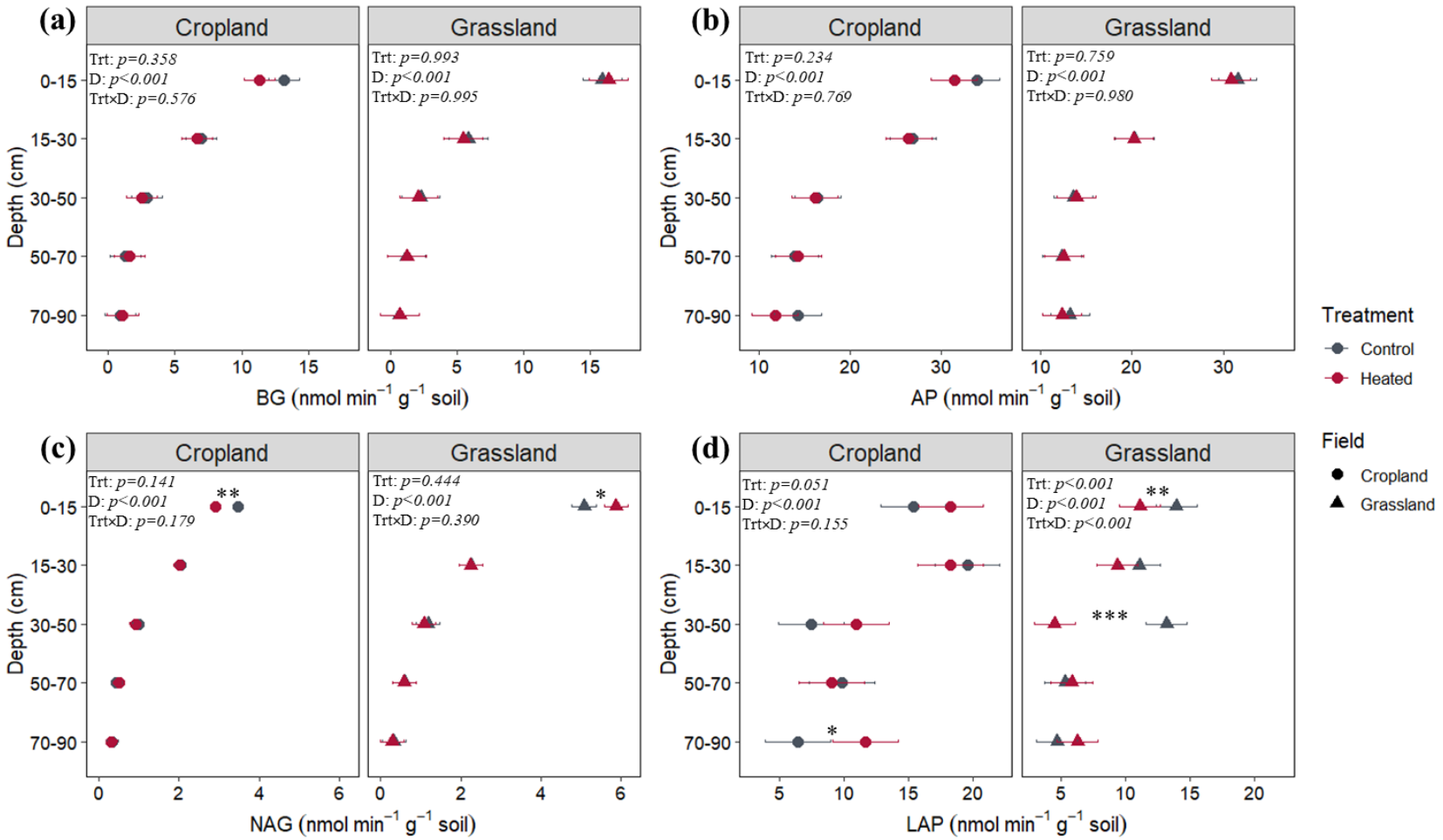


Fig. 16. Soil extra-cellular enzyme activity: (a) β-1,4-glucosidase (BG), (b) acid phosphatase (AP), (c) β-1,4-N-acetyl-glucosaminidase (NAG), and (d) leucine aminopeptidase (LAP), in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

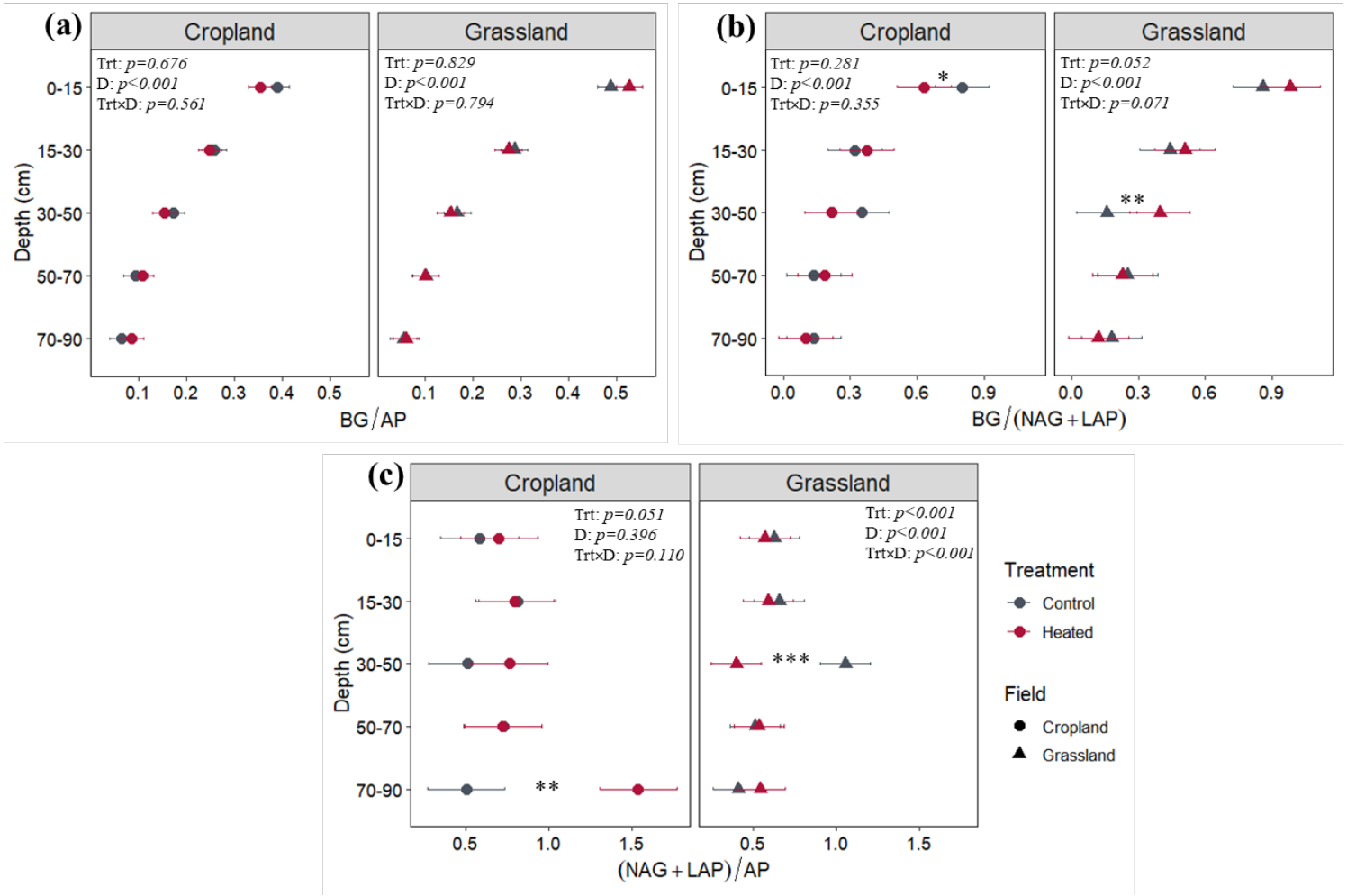


Fig. 17. Eco-enzymatic stoichiometry: (a) BG/AP, (b) BG/(NAG+LAP), and (c) (NAG+LAP)/AP, in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

2.4 Warming effects on microbial biomass and community composition along the soil profiles

The microbial biomass determined by EL-FAME extraction along the soil profile ranged from 17.8 to 6.6 µg-FAME g^{-1} soil under cropland and from 17.2 to 5.2 µg-FAME g^{-1} soil under grassland (Fig. 18a). It decreased significantly ($p<0.001$) with depth for both land management practices. Warming significantly ($p\leq0.05$) decreased the microbial biomass only at 0-15 cm depth under cropland but had no effect under grassland (Fig. 18a).

The bacterial and fungal community composition (mol%) and their ratio were significantly ($p\leq0.05$) affected by soil depth under both land management practices (Fig. 18). In addition, under cropland, warming significantly ($p\leq0.05$) decreased bacterial relative abundance at 0-15 cm depth (Fig. 18b), hence increasing the fungi/bacteria ratio at the same depth (Fig. 18d). Contrastingly, it decreased both bacterial and fungal relative abundance at 50-70 cm depth (Fig. 18b & 18c). Under grassland, significant warming effects ($p\leq0.05$) were recorded for bacterial relative abundance and the fungi/bacteria ratio (Fig. 18b & 18d).

In terms of the bacterial community composition, the relative contribution of gram-positive (GMP) bacteria and gram-negative (GMN) bacteria significantly varied ($p<0.001$) with depth for both land management practices (Fig. 19a & 19b). Under cropland, warming significantly ($p\leq0.05$) decreased both GMP and GMN bacteria at 0-15 cm and 50-70 cm depths but increased GMP bacteria at 70-90 cm depth. Conversely, under grassland, warming showed a significant ($p\leq0.05$) overall effect on both GMP and GMN bacteria, whereas a significant increasing effect was observed at 50-70 cm depth only for GMP bacteria (Fig. 19a).

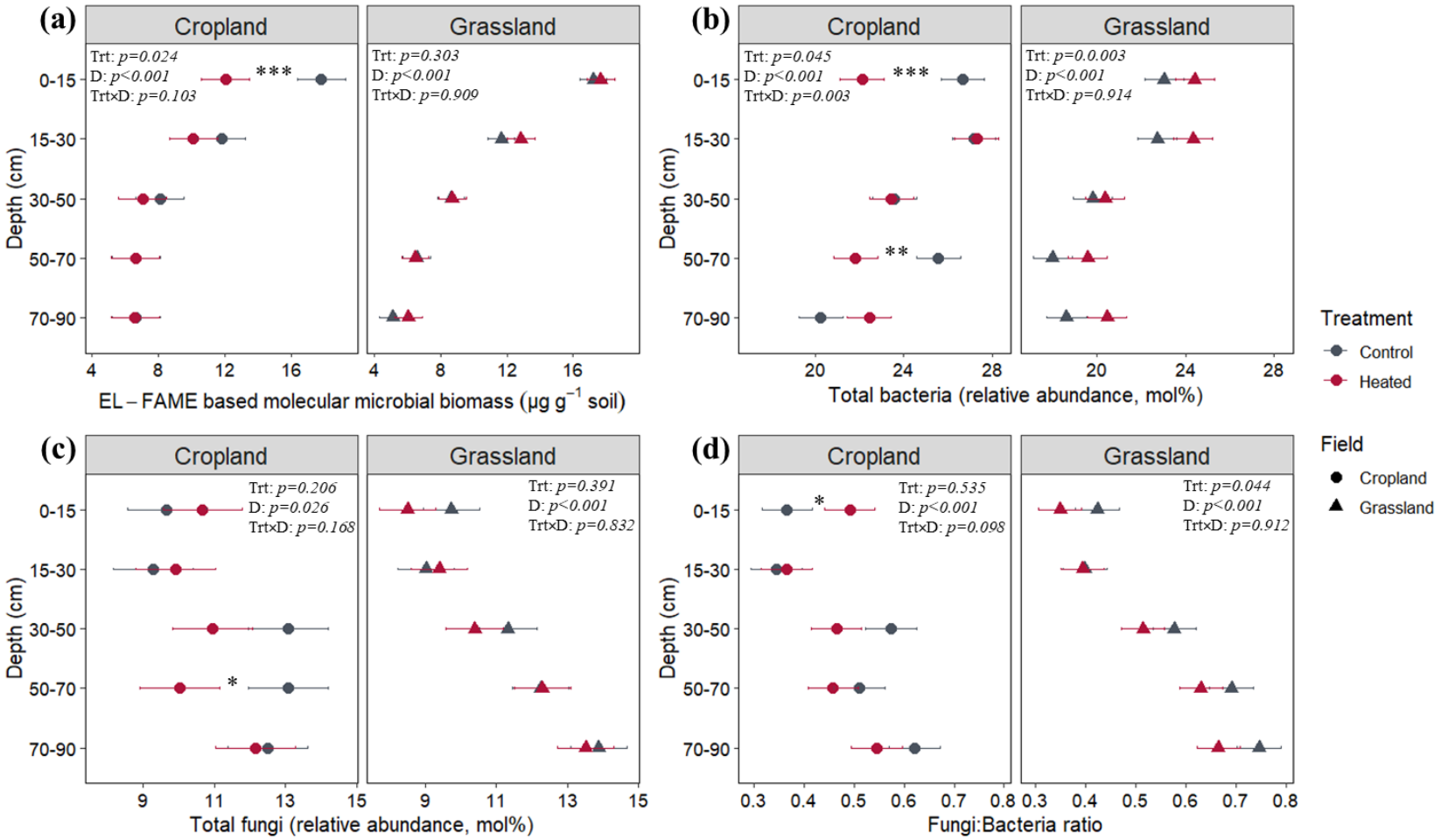


Fig. 18. Soil microbial community composition: (a) El-FAME based microbial biomass, (b) total bacteria, (c) total fungi, and (d) fungi/bacteria ratio, in the control and heated plots (mean ± SE, n=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, p-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

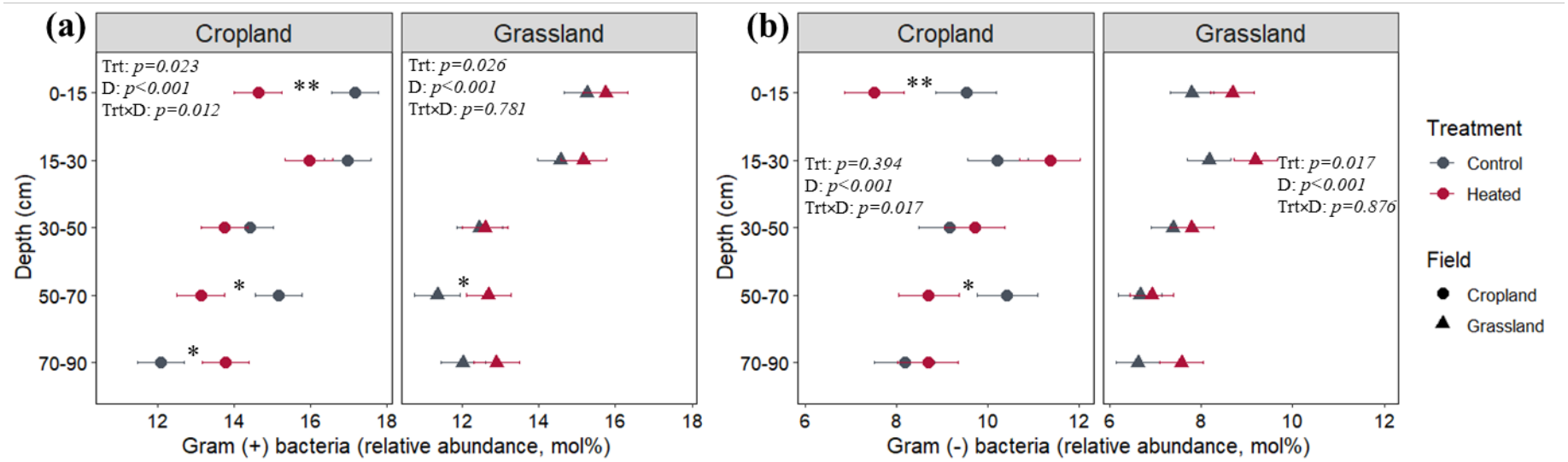


Fig. 19. Soil bacterial community composition: (a) Gram (+) bacteria, and (b) Gram (-) bacteria, in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

2.5 Principal component analysis and PERMANOVA

Shifts in microbial community composition profiles were explored using PCA to discriminate between *in-situ* treatments (control vs. heated) across soil depths for both land management practices. Our PCA results showed that the first two principal components (PCs) accounted for approximately 98.1% of data variance for cropland, and 99.2% for grassland (Fig. 20). PERMANOVA showed a significant depth effect ($p<0.001$) for both land management practices. Warming had a significant ($p<0.05$) effect on microbial community composition under cropland, which was appeared significant only at 0-15 cm depth. In contrast, warming had non-significant ($p\geq0.142$) effect on microbial community composition under grassland across soil depths (Fig. 20).

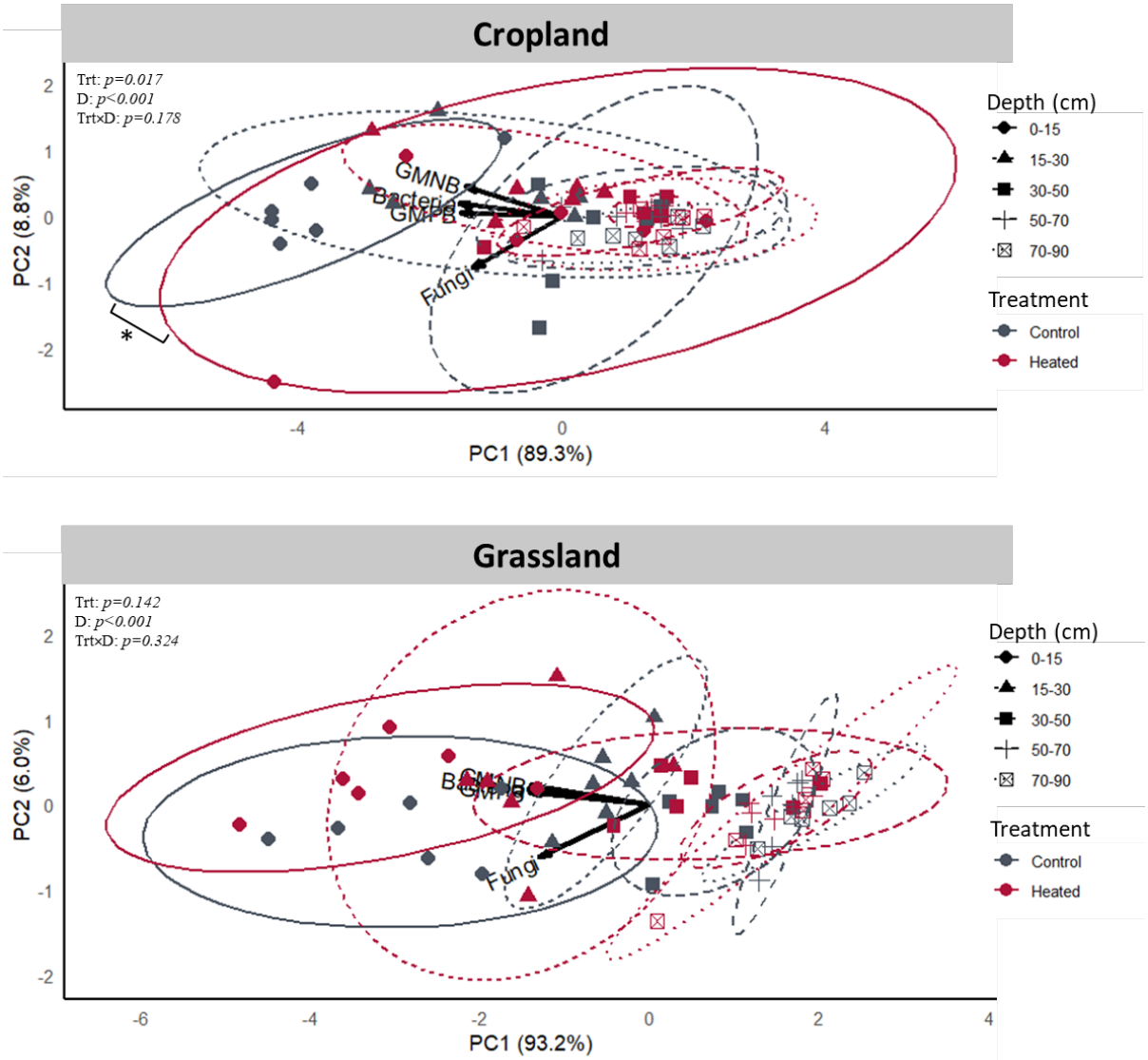


Fig. 20. Principal component analysis of the total soil microbial community composition in the control and heated plots (mean ± SE, *n*=6), sampled in May 2023 and 2024, two and three year after the onset of *in-situ* warming, throughout the 0-90 cm depth of soil profile under cropland and grassland. Here, microbial community composition included: (GMPB = gram (+) bacteria, GMNB = gram (-) bacteria, total bacteria and total fungi). In the figure, *p*-value represent the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D) as determined by PERMANOVA test. The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$).

4. Discussions

3.1 Warming effects on SOC and nitrogen under the two land management practices vary differently with depth

Our findings indicate that the effects of warming on SOC and N stocks are influenced by both land management practices and soil depths (Fig. 15). In surface soil (0-15 cm), warming led to decreases in both SOC and N stocks under cropland, consistent with our hypothesis (H1). In contrast, both SOC and N stocks increased in deeper soil layers (70-90 cm) under cropland. This gain contrasts with findings from similar whole-soil warming experiments in tropical and temperate forests, which indicated a loss of subsoil SOC after two years of warming (Hicks Pries et al., 2017; Nottingham et al., 2020). Moreover, these changes were not observed under grassland in our study, consistent with findings from other whole-soil warming experiments in high-altitude grassland, which reported no significant warming effects on SOC after three years in this ecosystem (Q. Zhang et al., 2023). Altogether, these results supported our hypothesis (H3) that agricultural land management can constrain the short-term warming effects on SOC.

The contrasting response to warming according to soil depths in different land management systems implies that factors such as vegetation type (annual vs. perennial) and/or differences in soil management practices could play a significant role in determining SOC and N dynamics in different soil layers (Rengel and Marschner, 2005; Romdhane et al., 2022). For instance, in cropland soils, the decrease in SOC and N stocks in surface soil layers (0-15 cm) under warming may indicate enhanced decomposition rates and/or reduced organic matter input at this depth. Indeed, our results indicate a slightly decreasing aboveground-biomass production under cropland (Table 8). In contrast, the increase in SOC and N stocks of deep soil layers (70-90 cm) suggests potential diffusive movement or redistribution of organic matter in response to warming, as supported by laboratory and field studies showing more diffusion of DOC and nutrients under warming (Moore and Dalva, 2001; Zhao et al., 2024; Zhou et al., 2023). These

shifts, coupled with changes in the C:N ratio (Fig. 15), might be explained by altered microbial processing of organic matter, driven by modifications in microbial activity and/or changes in microbial community composition (Mao et al., 2022; Roth et al., 2023; San Román et al., 2024; S. Wang et al., 2021). Conversely, under permanent vegetation in grassland, standing biomass and living roots may help retain SOC and limit C release under warming (Khan et al., 2023), potentially due to the stabilization of SOC through root-associated microbial activity (Pett-Ridge et al., 2021; Sanford, 2014). The distinct warming responses observed in differently managed ecosystems could be due to contrasting quantities, qualities and redistribution of C inputs and/or variations in microbial processing of organic matter (Morrison et al., 2019; Q. Zhang et al., 2023).

3.2 Warming effects on soil extracellular enzyme activity and microbial community composition under the two land management practices are contrasting

Our results showed that the extracellular enzyme response to warming was restricted to CN-acquisition enzymes (NAG and LAP), with variations observed depending on soil depths and land management practices (Fig. 16). The warming responses were contrasting and partially in agreement with findings from other studies (Conant et al., 2011; Dove et al., 2021; Fanin et al., 2022). Indeed, the warming-induced CN-acquisition enzymes response similarly to the observed changes of SOC and N stocks under warming, support our hypothesis (H1).

In grassland surface soil (0-15 cm), warming increased NAG potential to depolymerize microbial substrates containing N and C (e.g., peptidoglycan, chitin), while protein degradation potential by LAP decreased. This suggest that microbes changed their utilization of organic C and N source with warming, while overall N stocks and the combined activity of LAP and NAG remained stable. This stability is consistent with the stable N stocks observed in this study and findings from other studies (Dai et al., 2020; Risch et al., 2019; Séneca et al., 2021). Conversely,

in cropland surface soil (0-15 cm), only NAG activity decreased, reducing the overall potential of microbial community to degrade C and N-rich compounds. Meanwhile, the increased LAP activity in cropland deeper soil layer (70-90 cm) indicates an opposite response, where N-rich compounds were degraded in deep soil. These different warming responses of CN-acquisition enzymes led to specific eco-enzymatic stoichiometry responses in surface and deep soil layers under two land management practices (Fig. 17), suggesting that these changes could be explained by shifts in the nutritional demands of the microbial community, and supporting our hypothesis (H2). An alteration of N cycle and the associated stoichiometry of microbial resource is further supported by the warming-induced increase in TDN, despite stable DOC levels (Table 9).

Our findings further indicate that the effects of warming on microbial biomass were restricted to the surface soil (0-15 cm) and were negative only in cropland (Fig. 18). Moreover, the reduction aligned with a decrease of the surface soil SOC and N stocks (Fig. 15). This suggests a strong coupling between SOC storage and microbial biomass in cropland, potentially due to less labile organic inputs in the surface soil layers, as indicated by the reduced above-ground biomass production (Table 8). Additionally, the decrease of microbial biomass with warming in cropland surface soil (0-15 cm) may be explained by lower bacterial abundance, whereas fungal abundance remained stable (Fig. 18). The concurrent increase in the fungi-to-bacteria ratio in surface soil (0-15 cm) suggests that warming may selectively suppressing bacterial populations in cropland, possibly because of bacteria generally exhibit higher turnover rates under warming (Söllinger et al., 2022) and/or lower resistance to heat stress (De Vries and Shade, 2013). The depth-specific warming effects on GMP and GMN bacteria in cropland, with decreased abundances of both in surface soil (0-15 cm) but increased GMP bacteria in deeper soil layer (70-90 cm), suggest that warming selectively impacts bacterial groups at different soil depths. GMP bacteria are generally more resistance to warming (Naylor and Coleman-Derr,

2018; Piton et al., 2020) and better adapted to C limitation (Fanin et al., 2019), which may explain why they thrive better in deeper soil layers (70-90 cm) where resources are scarcer.

In contrast, the lack of warming effect on microbial biomass in grassland may reflect a system with more resistant microbial communities (Walker et al., 2018; Zhang et al., 2023), which is also supported by the PERMANOVA analysis (Fig. 20). These findings are consistent with studies reporting that microbial communities in cropland soils may be more sensitive to warming, while those in grassland soils may be more resistant to warming stress (Fierer et al., 2009; De Vries et al., 2012; De Vries and Shade, 2013; Yu et al., 2022), and also support our hypothesis (H3).

Indeed, PERMANOVA analysis showed no shifts in microbial community composition due to warming across soil depths for grassland (Fig. 20), which is consistent with other studies (Walker et al., 2018; Zhang et al., 2023). Conversely, PERMANOVA analysis for cropland revealed shifts in community composition due to warming. Therefore, the presence of clear warming effects on depth and management-specific potential enzyme activities or changes in microbial biomass and community composition was aligned with the warming-induced changes of surface and deep soil SOC and N stocks. Our findings thus suggest that the warming response of SOC and N dynamics may depend on its effect on microbial activity and enzyme synthesis, which can be influenced by agricultural land management practices and in particular the type of vegetation (Tecon and Or, 2017; Fanin et al., 2022).

4. Conclusions

In this study, we assessed how three years of whole-soil profile warming interacts with agricultural land management practices to influence SOC and N stocks, extracellular enzyme activity, microbial biomass and community composition and their controls across soil depths down to 1.0 m. We found that warming had no effect on SOC and N stocks along the soil profile

under grassland, whereas significant depth-dependent changes were observed under cropland. Microbial properties also appeared more sensitive to warming under cropland. Our findings suggest that soil biogeochemical response to warming can change across soil profiles depending on land management practices.

The distinct warming response of SOC and N stocks under different land management and depths were strongly associated to changes in CN-acquisition enzymes, eco-enzymatic stoichiometry, microbial biomass and bacterial community composition. These findings imply that the type of vegetation and/or agricultural management practices can play a significant role in determining warming effects on SOC and N dynamics in different soil layers, likely through their impact on the composition and activity of microbial communities, which might be direct or indirect through C inputs into the soil-system.

General discussion and implications

Understanding the effects of global warming on the soil's role in biogeochemical carbon cycling requires an integrated investigation of soil microbial communities and biogeochemical processes under diverse agricultural land management practices. Such insights are crucial for developing improved land management strategies for climate adaptation and mitigation, aiming to enhance soil C sequestration and agroecosystem resistance. My study investigated the interactions between soil warming and management in two contrasting systems, revealing critical mechanisms that drive SOC dynamics and microbial functioning across soil profiles.

In particular, I examined how warming interacts with management to affect seasonal soil CO_2 efflux and its origin within the soil profile (**Chapter 2**). Next, I studied soil microbial communities and their network complexity to assess the microbial resistance and soil functionality in differently managed soils under warming (**Chapter 3**). Finally, I explored how warming interacts with land management to affect microbial activity in relation to SOC dynamics and nutrient cycling (**Chapter 4**).

In **this section**, I will summarize the key findings including a graphical illustration (Fig. 21) and discuss their implications with the future prospects.

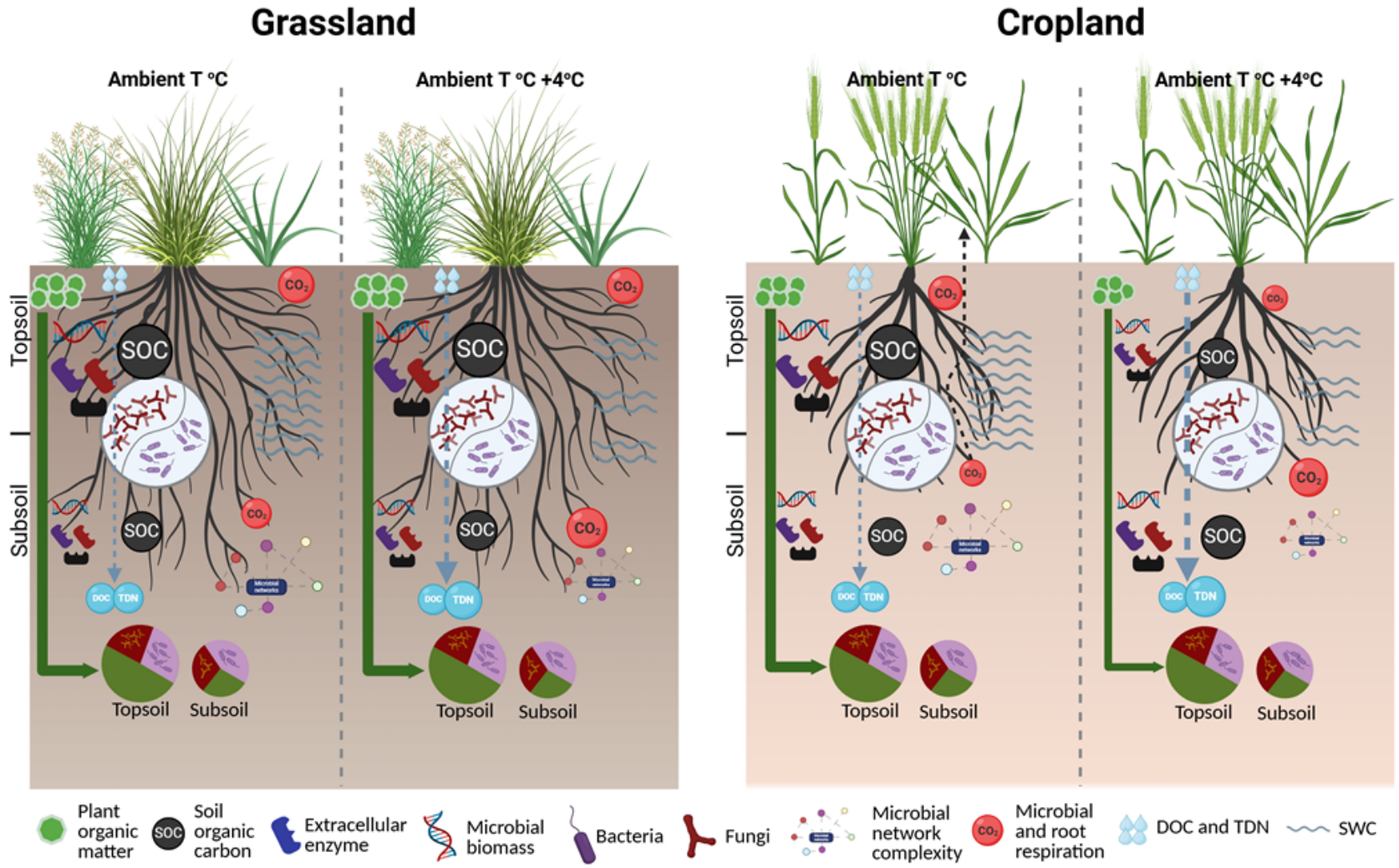


Fig. 21. Schematic illustration of whole-soil profile warming responses of C-cycling and biogeochemical processes under contrasting agricultural land management. In this diagram, the sizes of the elements denote the increase or decrease of key parameters under both land management practices in response to warming.

Our findings revealed that the warming response of CO_2 emission differed significantly between grassland and cropland. Specifically, we observed that subsoil CO_2 production was more sensitive to warming than topsoil CO_2 production in both land management systems (Fig. 21). However, warming did not negatively impact subsoil SOC stocks under both land management, instead, we found evidence of deep SOC gains under cropland (Fig. 21). These findings contrast with previous whole-soil warming observations in a temperate forest (Hicks Pries et al., 2017), which reported an increase in subsoil CO_2 production and an associated subsoil SOC loss (Soong et al., 2021). These differences highlight the critical role of vegetation type and land management in influencing soil C processes under warming conditions (Morrison et al., 2019). This is because warming could influence plant phenology, root dynamics, and their associated microbial activity, which are modulated by land management practices owing to distinct morphological and physiological traits of crop species (Berg and Smalla, 2009; Chao et al., 2014; Pugnaire et al., 2019). In our study, the warming-induced reductions in soil water content may emerge as a key driver, modulating plant phenology, root activity, and microbial processes, collectively influencing soil CO_2 efflux, depending on land management.

While grassland exhibited increased subsoil CO_2 production under warming, yet there was no significant warming effect on microbial biomass, richness, and community composition across soil profile, which aligned with observed stability of SOC and N stocks (Fig. 21). These findings are consistent with another whole-soil warming experiment in a high-altitude grassland, which also reported no significant changes in microbial biomass, diversity and SOC after three years of warming (Zhang et al., 2023). Therefore, our results suggest that warming may promote deeper root growth due to reduced soil moisture content (Joslin et al., 2000; Sotta et al., 2007), leading to increased subsoil root respiration, thereby contributing to the observed increase in subsoil CO_2 production. Despite the stability in microbial biomass, richness and community composition, warming caused minimal disruption to the complexity of microbial

networks in the whole-soil profile (Fig. 21), with notable effects limited to bacterial-bacterial interactions. These findings suggest that the initial higher complexity of microbial networks in grassland (Rillig et al., 2023; Yang et al., 2021) may have some ability to buffer environmental stresses, maintaining partial stability under warming.

Conversely, cropland showed a contrasting depth-dependent response, with reductions in topsoil CO_2 emissions (Fig. 21). This pattern aligns with the observations of surface SOC and N losses alongside deep soil SOC and N gains (Fig. 21), suggesting that warming altered the redistribution of OM and microbial processing within the soil profile (Wang et al., 2021). These shifts are further supported by a decrease in aboveground biomass production under warming (Fig. 21), highlighting the broader impact on plant-soil interactions. Enzyme activities also reflected these changes: surface soils showed reduced CN-acquisition enzyme potential for C and N degradation, while deep soils exhibited increased N degradation activity, likely driven by the redistribution of OM and supported by warming-induced changes in TDN (Fig. 21). Notably, microbial biomass in surface soils declined to warming, correlating with surface SOC and N losses (Fig. 21). Indeed, warming selectively suppressed bacterial communities, leading to increased fungi-to-bacteria ratio, possibly due to bacteria's higher turnover under warming (Söllinger et al., 2022). Similarly, microbial network complexity was severely disrupted to warming in the whole-soil profile (Fig. 21), including all microbial interactions. This suggests that the initial less complexity of microbial networks in cropland may experience greater disruption, likely due to the combined effects of warming and tillage-induced soil disturbance (Shi and Thakur, 2023; Siles et al., 2023).

1. Implications for land management and agroecosystem resistance

The contrasting depth-dependent responses of surface and deeper soil SOC stocks in cropland, compared to the uniformity observed in grassland, underscore the importance of adopting management and depth-specific strategies to address soil C dynamics under warming

conditions. Enhancing deep soil C sequestration in cropland while protecting the surface SOC pool may be a critical approach to mitigate C losses and improving agroecosystem resistance. Additionally, the observed decline in microbial network complexity, particularly in cropland, raises concerns about the long-term functional stability of these system under warming climate.

2. Future prospects

My findings emphasize the urgent need for long-term studies to unravel the microbial driven mechanisms underlying SOC dynamics and agroecosystem resistance across whole-soil profile, paving the way for the development of adaptive land management strategies that enhance soil C sequestration and mitigate the impacts of global climate change.

Key areas for further investigation include:

- **Distinguish heterotrophic and autotrophic respiration:** Future studies should separately assess heterotrophic and autotrophic contributions to soil CO_2 efflux to obtain a comprehensive understanding of soil respiration dynamic in response to warming.
- **Prioritizing root C quality and quantity:** The warming-induced responses of root C quality and quantity require focused investigation, as these factors may influence SOC dynamics and agroecosystem soil health.
- **Identifying warming susceptible SOM fractions:** Further research is needed to determine which SOM fractions (e.g., POM or MAOM), are more vulnerable to warming, as it may affect plant C input, microbial activity, and subsequent MAOM changes through priming effects, particularly in agricultural management systems.

Concluding remarks

In conclusion, this study reveals the complex interactions among soil carbon dynamics, microbial communities, and agricultural land management practices in response to warming. The warming led to reduced soil moisture content in both grassland and cropland, with surface soil CO_2 efflux varying by season and management practices. Subsoil CO_2 production was more sensitive to warming than those of topsoil, while microbial network complexity declined, with grassland demonstrating greater resistance compared to cropland.

This research highlights significant differences in how warming affects SOC and nutrient dynamics in agricultural ecosystems. In cropland, warming caused losses in surface SOC and N stocks, while deeper soil showed gains, driven by microbial shifts and enzyme activities. In contrast, grassland soils maintained stable SOC and N stocks, along with stable microbial communities across soil profile, reflecting their resistance under warming conditions.

Notably, the decline in microbial network complexity, especially in cropland, signals a potential risk to long-term microbial functionality and soil ecosystem stability. The results point to the need for further long-term studies to unravel microbial-driven mechanisms underlying SOC dynamics and to develop strategies for optimizing land management practices to improve soil C sequestration and agroecosystem resistance in the face of global climate change.

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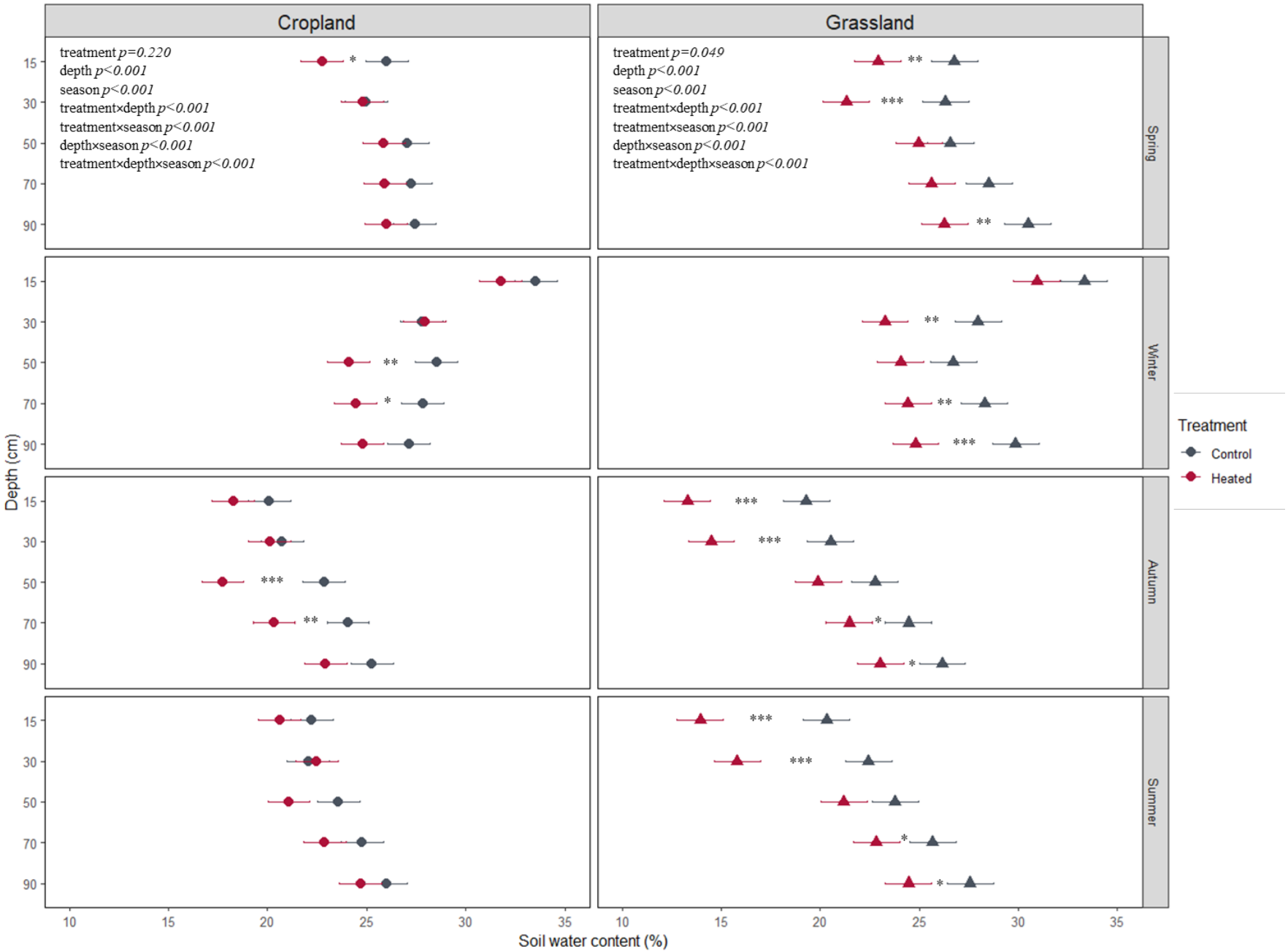
Appendices

Supplementary data for Chapter 2

Seasonal soil water content and warming effect

To evaluate the impacts of the warming treatment on seasonal soil water content, we ran mixed effects models in RStudio (R Core Team, 2022) using the nlme package (Pinheiro et al., 2017) and restricted maximum likelihood with blocks (n=3) as a random effect. Using Q-Q plots and residual histograms, we visually examined all models for homoscedasticity and normality of distribution. To test the fixed effects of treatment, depth, season, and their interaction on soil water content from within the soil profile, we used plots crossed within the sampling date as random effects (to account for violations of independence), separately for cropland and grassland.

Our result imply that depth-wise soil water content was depended on the season (significant, $p<0.001$) for both land management practices (Supp. Fig. S1). During the winter season, topsoil (0-15 cm) showed higher water content, while other seasons showed the opposite trend for both cropland and grassland. Despite the seasonal variations, *in-situ* warming reduced soil water content throughout the season for both land management (Supp. Fig. S1).



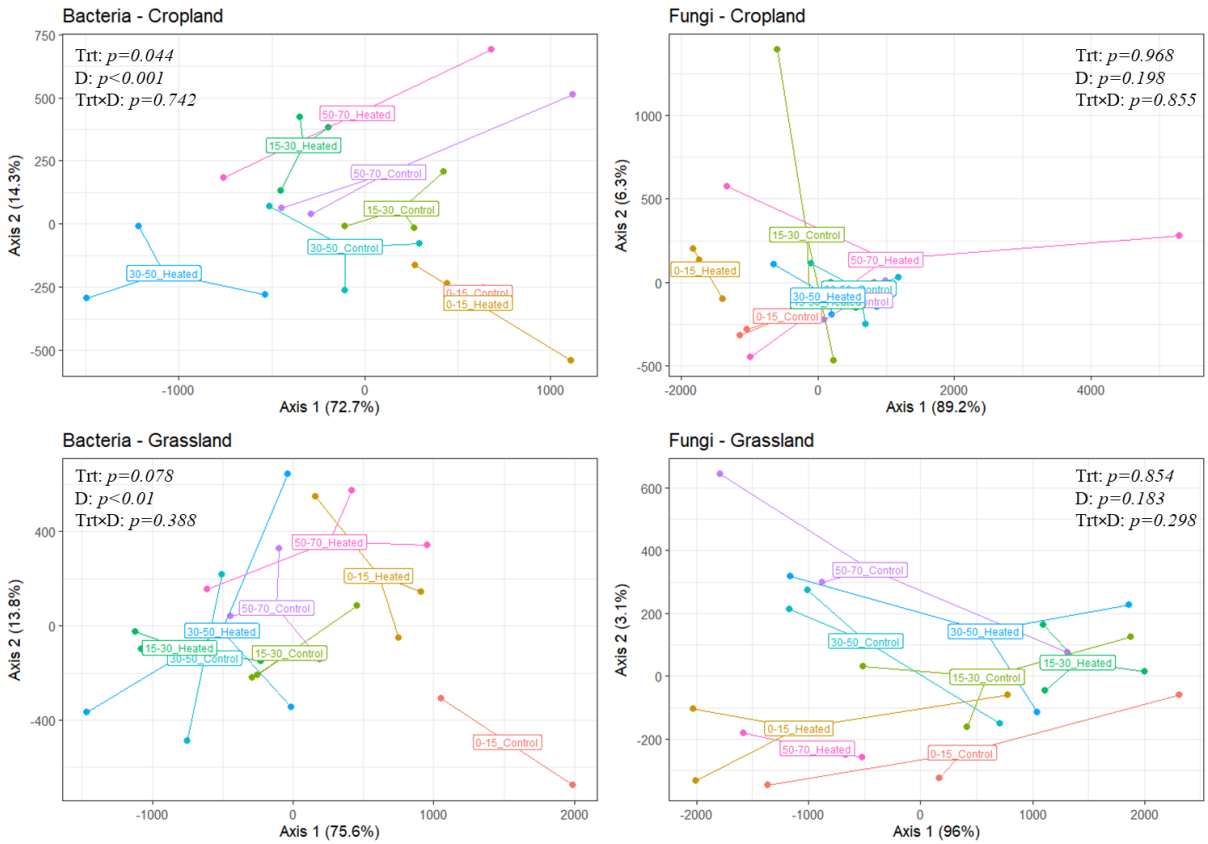
Supplementary Fig. S1. Mean seasonal variation of soil water content in the control and heated plots (mean ± SE, *n*=3) throughout the 1.0 m depth of soil profile over the study period (July 2021 to June 2022). In the figure, *p* values represented the statistically significant effects of treatment, depth, season, and their interaction (treatment×depth×season). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

Supplementary data for Chapter 3

Warming effects on microbial community structure across the soil profile

The principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA) was performed to assess the effects of warming treatment, depth, and their interaction on microbial community structure, separately for cropland and grassland. The PCA and PERMANOVA tests were performed using the *factoextra* and *vegan* R packages (Oksanen et al., 2012).

Our results indicate that the bacterial community structure differed significantly with depth under both land management practices ($p \leq 0.01$; Supp. Fig. S2). In contrast, the fungal community structure did not show significant differences with depth regardless of land management practices (Supp. Fig. S2). Warming shifted the whole-soil bacterial community structure, with significant changes observed in cropland ($p=0.044$) and marginally significant changes in grassland ($p=0.078$), but did not affect the fungal community structure under both management practices (Supp. Fig. S2). The interaction between warming treatment and depth was non-significant for both land management practices (Supp. Fig. S2).



Supplementary Fig. S2. Principal component analysis of the composition of bacterial and fungal structural communities in the control and heated plots (*n*=3), sampled in May 2022, one year after the onset of *in-situ* warming, throughout the 0-70 cm depth of soil profile under cropland and grassland. In the figure, *p*-values denote the statistically significant effects of treatment (Trt), depth (D), and their interaction (Trt×D*) as determined by PERMANOVA test.*

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Management impacts on whole soil warming responses of CO_2 production and efflux in temperate climate

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ABSTRACT

Although global warming has the potential to increase soil CO_2 efflux, the magnitude of these changes are uncertain, as CO_2 production rates in deep soil are poorly constrained. In particular, management effects on the warming responses at depth are unknown. Here, we conducted an *in-situ* soil warming experiment down to 2.0-m depth in an agricultural Cambisol to study the warming responses of (1) CO_2 production across different depths and (2) CO_2 efflux from topsoil in different seasons under two management practices. To this end, we measured whole-soil profile water content, CO_2 production and CO_2 efflux under continuous grassland and cropland in response to elevated temperature (+4°C).

Warming decreased soil water content for both management practices. We found contrasting warming effects on surface CO_2 efflux, depending on season and land management practices. Subsoil CO_2 production was more sensitive to warming than topsoil CO_2 production with grassland subsoil showing a greater warming response than cropland subsoil. Topsoil CO_2 production decreased in response to warming in the cropland but not the grassland. We concluded that warming responses of CO_2 production and efflux are affected by soil management practices. Their effect on biological processes (roots and microbial activity) and factors affecting gas diffusivity, such as soil water availability and soil physical organization need to be assessed to model warming effects on carbon exchange between soil and the atmosphere in agricultural systems.

1. Introduction

According to the Representative Concentration Pathways (RCPs) 8.5 scenario, the global soil temperature is expected to rise to +4.5 ± 1.1°C above pre-industrial levels down to 1.0 m depth by the end of the 21st century (worst IPCC scenario) (Soong et al., 2020). These increasing temperatures will affect carbon (C) cycling in terrestrial ecosystems and possibly cause positive or negative feedbacks in terms of CO_2 exchange between the soil and atmospheric reservoirs (Carey et al., 2016; Davidson and Janssens, 2006). One of the most important mechanisms driving feedbacks to climate change is the increase in microbial activity in response to warming, which can increase the decomposition of soil organic carbon (SOC) and the soil CO_2 efflux to the atmosphere (Crowther et al., 2016). SOC is the largest biologically active C pool in terrestrial ecosystems, storing twice as much C as the earth's atmosphere and vegetation combined (up to 2500 Pg C) (Friedlingstein et al., 2020; IPCC, 2013). But it is still uncertain how this reservoir will respond to warming because the mechanisms controlling SOC turnover and microbial access to SOC are not fully understood (Bradford et al., 2016; Schmidt et al., 2011; Gestel et al., 2018).

In view of the international efforts to increase SOC stocks in agricultural systems through the development of sustainable agricultural practices (Rumpel et al., 2020; Smith et al., 2015), warming effects on C fluxes in managed agricultural systems are of crucial importance. Warming may affect the CO_2 soil efflux through its impact on the production and transport of CO_2 within and between individual soil depths. This was evidenced in recent *in-situ* whole soil warming (+4°C) experiments, in temperate and tropical forests, which found that warming

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caused substantial increases in soil CO_2 efflux due to the stimulation of soil profile CO_2 production (Hicks Pries et al., 2017; Nottingham et al., 2020). This increase was induced by the heterotrophic decomposition of SOC pools rather than autotrophic sources (Nottingham et al., 2020; Soong et al., 2021).

Rising temperatures are expected to stimulate CO_2 efflux by their effect on the plant-soil system (Bond-Lamberty and Thomson, 2010; Jochheim et al., 2022; Nottingham et al., 2020) and the magnitude of these responses are likely controlled by water availability (Védère et al., 2022). Water regulates plant and microbial activity, substrate availability, and soil gas diffusivity (Chen et al., 2016; McGrath et al., 2022; Wood et al., 2013). Also, seasonal climate variations affect soil temperature, soil water content, and plant phenology in temperate ecosystems (Vincent et al., 2006), leading to variable soil CO_2 efflux across time (Vanhala, 2002; Vincent et al., 2006). The controlling factors of CO_2 production and efflux, such as soil water content, microbial activity, root abundance and activity, which control substrate supply, and soil tortuosity are all influenced by soil management practices (Kuzyakov, 2006; Oertel et al., 2016; Pagenkemper et al., 2014; Vargas and Allen, 2008). Insight into how soil warming interacts with management to affect seasonal soil CO_2 efflux and its origin within the soil profile will enhance our ability to predict terrestrial C fluxes and C sink potential under future projected climate change.

The majority of publications addressing the effects of agricultural management on SOC turnover have focused on topsoil, ignoring the amount and climate vulnerability of SOC in deep soil layers (Voort et al., 2019; Yost & Hartemink, 2020; Moreland et al., 2021). It has been assumed that SOC stored in deep soil is relatively stable and unresponsive to agricultural practices. This assumption was mainly based on radiocarbon dating studies showing that subsurface C could be hundreds to thousands of years old (Hobley et al., 2017; Wang et al., 1999). Although decomposition rates in deeper soils are slower than in surface soils, recent studies have shown that part of subsoil C could turn over much faster due to preferential input of labile organic matter which could induce positive priming effects in subsoil (Chabbi et al., 2009; Min et al., 2020; Rumpel and Kögel-Knabner, 2011) and also be more responsive to changing abiotic conditions (Hicks Pries et al., 2017; Nottingham et al., 2020; Ofiti et al., 2021; Zhang et al., 2016) and agricultural management practices (Harrison et al., 2011; Osanai et al., 2020; Samson et al., 2021; Shahbaz et al., 2017) than previously assumed. Moreover, the microbial communities in subsoils are also quite different from those in surface soils, owing to lower SOC availability and more favorable conditions for oligotrophs due to nutrient-poor conditions (Ekelund et al., 2001; Fierer et al., 2003; Oertel et al., 2016; Scheffers et al., 2016; Stone et al., 2014; Zifcakova, 2020). Because of the soil depth profile with gradients of many abiotic and biotic factors affecting the mechanisms that control SOC input, loss, transformation, and stabilization, deep SOC is likely to respond differently to soil warming than surface SOC (Hicks Pries et al., 2023; Von Lützow and Kögel-Knabner, 2009). However, up to now it is unknown how deep soil C under different agricultural practices will respond to warming.

In this study, we thus aimed to provide *in-situ* experimental evidence on the SOC response to warming in managed agricultural soils down to 1.0 m depth. We used a whole-soil warming experiment established on an agricultural Cambisol to investigate how soil water content, CO_2 production, and CO_2 efflux respond to +4°C of warming under continuous grassland and temporary grassland in its cropland phase. In particular, we had the following study questions: (1) How does warming affect soil water content under contrasting land management?; (2) How does whole-soil warming affect the CO_2 efflux under contrasting management practices and seasons?; (3) How does the CO_2 production among differ depths respond to warming under contrasting land management? We hypothesized that in response to *in-situ* warming (H1) water content will decrease across all soil depths, because warming induces higher evapotranspiration, (H2) CO_2 efflux will increase year round in soils under continuous grassland but only seasonally in cropland soils, and (H3) CO_2 production will increase across the whole soil profile in grassland but not cropland soils, because grasslands show more root activity and root-derived C inputs throughout the year.

2. Materials and methods

2.1. Study area description

The experimental field site is part of the long-term Observatory for Environmental Research: Agro-ecosystems, Biogeochemical Cycles and Biodiversity (SOERE ACBB, http://www.soere-acbb.com/index.php/fr/), hosted by the INRAE (French National Institute of Agricultural Research and Environment), situated in Lusignan, Nouvelle-Aquitaine (46°25′12.91″ N; 0°07′29.35″ E), western France. The climate of the experimental site is oceanic with mean annual precipitation of around 760 mm, with most of it occurring from November to March. The mean annual temperature is about 12°C, with a monthly maximum of 27.9°C in August and a monthly minimum of −3.1°C in January (data from Météo France, 2007 to 2021). The site is flat with a slight slope gradient (0.5 percent). The soils are complex because of their particular pedogenetic evolution (Ducloux and Chesseron, 1988). The soil is classified as a Cambisol (FAO soil group) with loamy texture in the upper horizons, and clayey texture at depth with a high content of oxides and kaolinite, classified as a Paleo-Ferralsol. Pedoclimatic characteristics are shown in Table 1.

2.2. Land management history

The long-term experiment (SOERE ACBB) started in 2005 on an area previously covered by oak forest and then devoted to conventional agro-ecosystem management (grassland, grain cropping, or temporary (ley) grassland) for at least the next 100 years. The aim of the experiment is to elucidate the impact of temporary grassland introduction into the cropping cycle (maize (*Zea mays* L.), winter wheat (*Triticum* spp.), and winter barley (*Hordeum vulgare* L.)) on biogeochemical cycling. The experiment is conducted with several treatments and two controls (continuous grassland and continuous cropland). Temporary (ley) grassland treatments differ in duration (3 or 6 years of ley), fertilization strategy (with or without N fertilization) and harvesting regime (cutting or pasture). Croplands are ploughed to a depth of 30 cm (using a mouldboard plough), and crop residues (straw and yield) are returned to the soil after harvesting. For grassland treatments, a mixture of perennial ryegrass (*Lolium perenne* cv. Milca), cocksfoot (*Dactylis glomerata* cv. Ludac), and tall fescue (*Festuca arundinacea* cv. Soni) is used. Grasslands are hayed three to four times per year depending on productivity or grazed by cattle. Plant root biomass is greater in the continuous grassland than in the ley grassland and cropland down to 90 cm depth (Table 2).

2.3. In-situ experimental soil warming

In 2019, we established an *in-situ* soil warming (+4°C) experiment in the continuous grassland and in the ley grassland that began its cropland phase in 2020 after 6 years of temporary grassland (Table 3 and Fig. 1). The treatments are called 'grassland' and 'cropland' hereafter.

The *in-situ* warming experiment heated the soil to +4°C above ambient temperature (°C) across 10 m^2 circular plots to a depth of 1.0 m with 2.0 m long resistance heating cables while retaining the natural temperature gradient within the soil profile based on Hicks Pries et al. (2017) and Hanson et al. (2011). The warming experiment consists of three ($n = 3$) replicated plot pairs: control (ambient temperature) and heated (ambient temperature +4°C). There was approximately a 10 m distance between the control and heated pair plots (Fig. 2b). Briefly, warming was maintained throughout the 3.5 m diameter circular plot and down to 1.0 m depth with twenty-two 2.0-m-long steel pipes, which were installed vertically around 0.50 m apart and 0.10 m beyond the

Table 1
Soil properties by horizon of the experimental site (Chabbi et al., 2009; Moni et al., 2010). Mean and coefficient of variation expressed as a percentage of the mean. Both grassland and cropland treatments are on the same soil type.

Horizons	Soil depth /cm	Bulk density /g cm^{-3}	$pH_{(H2O)}$	Particle size distribution			CEC	Base saturation	Oxides-Hydroxides			
				Sand	Silt	Clay			Al_o	Fe_o	Fe_d	Al_d
				———/g kg^{-1} soil———			/cmol$_c$ kg^{-1}	/%	———/g kg^{-1} soil———			
Lp (n = 6)	0–30	1.43 ± 5%	6.3 ± 3%	105 ± 14%	727 ± 3%	168 ± 87%	7.08 ± 11%	0.98 ± 7%	1.20 ± 19%	3.22 ± 22%	20.97 ± 25%	3.08 ± 25%
S (n = 6)	30–59	1.45 ± 4%	6.6 ± 4%	95 ± 9%	594 ± 7%	311 ± 11%	8.6 ± 22%	0.94 ± 6%	1.65 ± 14%	1.53 ± 17%	27.19 ± 27%	5.11 ± 18%
S2 (n = 5)	59–84	1.60 ± 7%	6.9 ± 5%	103 ± 11%	520 ± 9%	377 ± 21%	10.8 ± 27%	0.94 ± 5%	2.01 ± 9%	1.82 ± 15%	45.27 ± 22%	7.20 ± 21%
C1 (n = 5)	84–143	1.59 ± 8%	6.6 ± 4%	98 ± 8%	330 ± 11%	572 ± 9%	11.6 ± 16%	0.93 ± 9%	1.95 ± 19%	2.03 ± 14%	62.79 ± 36%	7.42 ± 22%
C2 (n = 6)	>143	1.61 ± 6%	5.2 ± 3%	110 ± 10%	242 ± 13%	642 ± 12%	9.9 ± 17%	0.82 ± 10%	1.87 ± 15%	2.27 ± 11%	67.02 ± 32%	6.16 ± 27%

Here, CEC stands for cation exchange capacity; Fe_o and Al_o standas for amorphous forms of Fe and Al; Fe_d and Al_d stands for non-crystalline forms of Fe and Al.

Table 2
Plant root mass from whole soil profile (mean ± SE, $n = 5$) of the experimental site in the control plots with two different land management practice.

Field	Year	Root mass (kg ha^{-1}) in different soil depth		
		0–30 cm	30–60 cm	60–90 cm
Cropland	2019*	7160 ± 340	918 ± 26	222 ± 9
	2020	4250 ± 150	643 ± 8	314 ± 6
	2021	3560 ± 190	726 ± 7	258 ± 9
Grassland	2019	9850 ± 260	1200 ± 45	484 ± 8
	2020	11600 ± 1100	1250 ± 65	496 ± 10
	2021	12800 ± 1600	2000 ± 13	563 ± 9

*Data collected from temporary grassland in 2019, after that was in its cropland phase after 6 years of ley grassland (see Table 3).

edge of the plots (Fig. 2a). In warmed plots, self-regulating heating cables (TECHNITRACE (CAMT EA 35 W/m 230 V), France) were placed inside the pipes, which were then filled with sand, while control plots did not have that type of heating cable. We installed additional heating cables 5 cm below the soil surface in a horizontal zigzagging pattern across the circular plot, around 0.30 m apart, to compensate for surface heat loss (Fig. 2a). Similar unpowered heated cables were not installed in the control plots, modified from Hicks Pries et al. (2017). The heaters were powered by an electric power supply, which was controlled by a silicon-controlled rectifier (SCRs, Watlow, Missouri, USA) via a current-to-voltage converter with a unipolar multiplexer (1KOhms resistance). The amount of electricity (source of heating) supplied to the deep heaters was based on the temperature difference between control-heated paired thermistors at 50, 75, and 100 cm depths at 0.75 m radial distance from the center of the plot. Similarly, the temperature difference of thermistors at 15 and 30 cm depth at radial distances of 0 and 0.75 m from the center of the plot was used to calculate the power supplied to the surface heaters of each paired plot. We installed this *in-situ* soil warming experiment in two phases: (1) initial installation in 2019 and 2020, during which the warming system was turned on, and (2) conditioning in 2020 and 2021 before field data collection started in June 2021 (Fig. 2c). The conditioning phase was due to low soil warming with respect to our target of +4°C, and we reached our target after 1.5 years of turning on the warming system.

2.4. Automated temperature and moisture data measurements

Portable dataloggers (CR1000, Campbell Scientific, Utah, USA) recorded continuous soil profile temperature and moisture data at 10-second intervals. In each plot, a PVC tube containing six temperature probes (OMEGA 44,007 thermistor) was placed at different depths (5, 15, 30, 50, 75, and 100 cm) with five replicates at 0.75 m radial distance from each other to monitor temperature, modified from Hicks Pries et al. (2017). The sensors at 15 and 75 cm depth were also used to regulate the surface and depth temperatures in order to ensure that the +4°C relative to the control is maintained throughout the soil profile. Another tube including five capacitance sensors equipped with enviroSCAN probes (Sentek, Australia) measured soil moisture at different depths (15, 30, 50, 70, and 90 cm) at a radial distance of 0.75 m from the center of each plot. The soil moisture readings were calibrated by comparing the sensor values at each depth to the volumetric water content recorded in the nearest (within 0.30 m) soil samples. We repeated this soil sampling two times over one year, in both dry and wet periods, to minimize the calibration errors.

2.5. Evaluation of in-situ soil warming (+4°C) performance

Our *in-situ* soil warming experiment shows a homogeneous temperature gradient (+4 ± 0.2°C for cropland and +4 ± 0.3°C for grassland) down to 1.0 m depth of the soil profile, except 0–15 cm depth, throughout the whole study period (Figs. 3a and 3b). The 0–15 cm depth showed a higher variability (+4 ± 0.5°C for cropland and +4 ± 0.4°C for grassland) concerning our target (+4°C) because there is no surface heating to compensate for the heat loss due to close contact of ambient air temperature and radiation compared to deep soils. However, the relative warming effect was consistent over the entire study period (Fig. 3a) and did not change with weather (seasonal fluctuations) or soil moisture conditions.

2.6. Leaf area index

During the growing season (March to June 2022), we measured leaf area (m^2) by scanning the leaf blades in a leaf area scanner (LI-2000, Li-

Table 3
Long-term land-use management history under an experimental site since 2005 in the Nouvelle-Aquitaine (Lusignan) region of western France.

Year:	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Cropland:	Ley Grassland (harvested 3 to 4 times/year)						Maize	Wheat	Barley	Ley Grassland (harvested 3 to 4 times/year)						Maize	Wheat	Barley
Grassland:	Continuous Grassland (harvested 3 to 4 times/year) for annual forage production																	

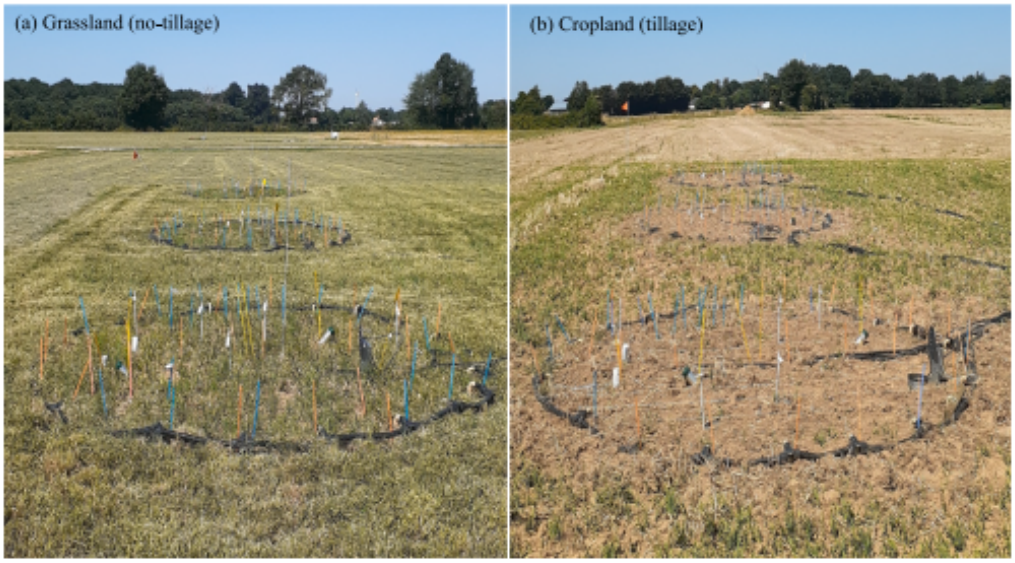


Fig. 1. *In-situ* whole-soil warming experiment under contrasting grassland management: (a) continuous grassland; and (b) cropland (cropland phase after 6 years of ley grassland) in a temperate region of western France.

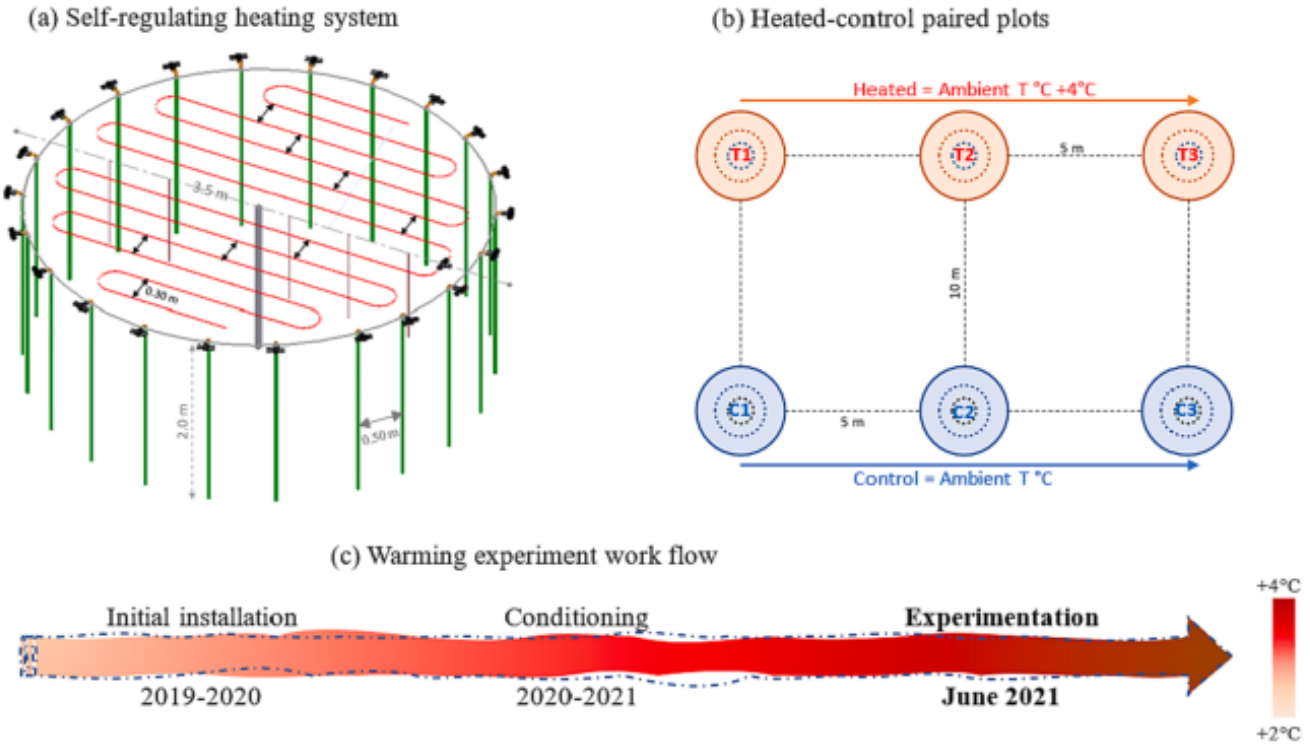


Fig. 2. *In-situ* soil warming experimental design under contrasting grassland management: (a) whole-soil warming methodology based on self-regulating heating cable down to 2.0 m soil profile (the red zigzag line represents the buried surface heating location at 5 cm depth while the green and gray bar represents the location of the deep heating system down to 2.0 m depth); (b) heated-control paired (replicates, $n = 3$) plots; and (c) warming experiment work-flow (after 1.5 years, we have +4°C, which has continued) in a temperate region of western France.

Cor, Lincoln, Nebraska, USA) to estimate the leaf area index (LAI, m^2 m^{-2}) based on ground cover (m^2).

2.7. Soil organic carbon content

In late May 2022, we collected soil samples from control-heated paired plots (n = 3) at different soil depths (0–15 cm, 15–30 cm, 30–50 cm, 50–70 cm, and 70–90 cm) before harvesting the vegetation cover, including cropland and grassland. To elaborate, soils were randomly sampled using a mechanical soil auger (8 cm in diameter) with five replicates (n = 5). Subsequently, all replicates were combined into a single composite sample for each plot and depth. These samples were then air-dried, sieved to 2 mm, and ground into a fine powder. Following this preparation, the concentration of soil organic carbon (SOC) was

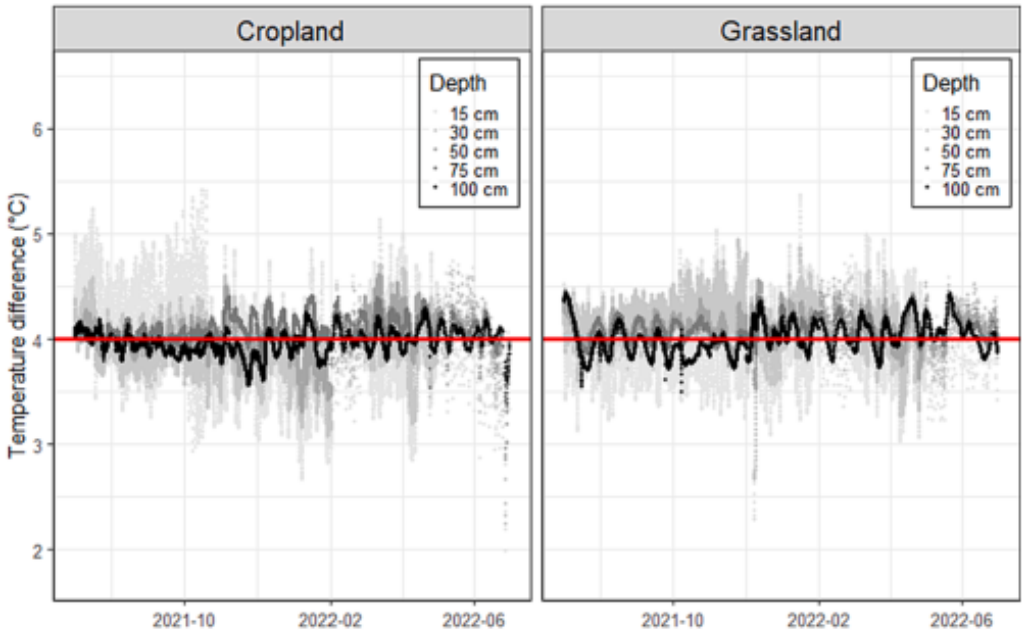


Fig. 3a. The average temperature difference between the control-heated pair plots ($n = 3$) at five depths over the study period (July 2021 to July 2022). The red line is the +4°C target. Discontinuities in warming (drops followed by sharp increases) were due to electrical outages.

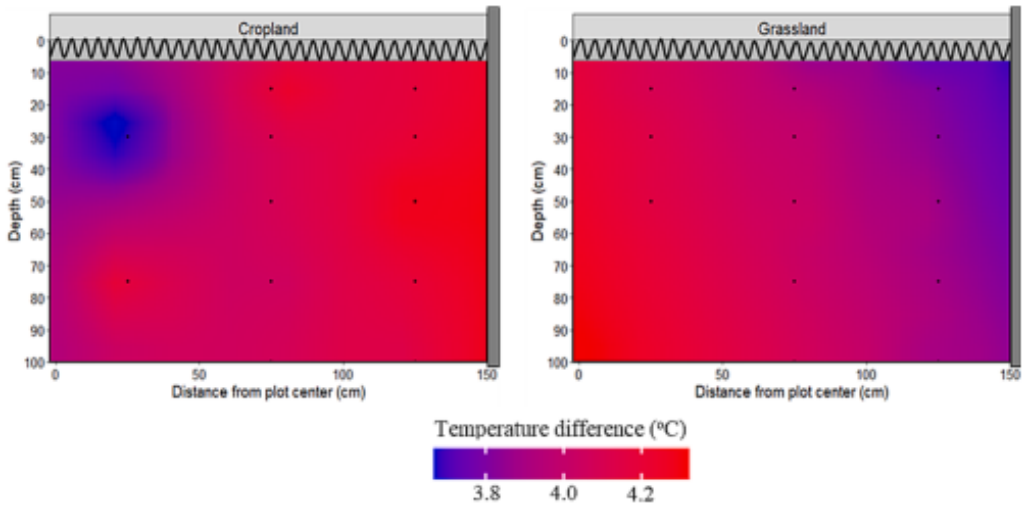


Fig. 3b. A radial cross-section of the study plot shows the average temperature difference ($n = 3$ plot pairs) from August to October 2021. The black dots represent the location of the temperature sensors. The horizontal zigzag line (black) is the location of the surface heaters and the vertical grey bar represents the 2.0 m long deep heaters, which are located 25 cm from the edge of the plot at 175 cm from the plot center. The data were interpolated using the automap package in R.

determined using a CHN auto-analyzer (Flash EA, Thermo Electron Corporation, Bremen, Germany).

2.8. Soil CO_2 efflux

Measurements of surface soil respiration (CO_2 efflux) were performed from July 2021 to June 2022 using soil respiration auto-chambers (8100A, LI-COR, Lincoln, Nebraska), which continuously measured the soil respiration fluxes every 90 min in each plot. During each flux measurement, the air temperature was also recorded using a temperature probe (OMEGA 44,007 thermistor), which was connected to the auto-chamber lid above the soil surface. The soil CO_2 efflux was calculated as follows:

$$f = \frac{dc}{dt} \times \frac{hMP}{RT} \quad (1)$$

Where f denotes the surface CO_2 efflux rates ($\mu g\ m^{-2}\ s^{-1}$), dc/dt is the rate of increase of the CO_2 concentration inside the chamber with time and is determined by the slope of linear regression; h is the height of the chamber; M is the molar mass of CO_2; P is the atmospheric pressure; R is the ideal gas constant; and T is the chamber air temperature in Kelvin. After that, the surface CO_2 efflux was converted to g C m^{-2} d^{-1} units.

To prevent CO_2 efflux from aboveground plant parts (leaves and stems), living plants inside the auto-chambers were manually eradicated once a week if they grew, and the removed plant material was left inside the chambers to die. Therefore, the CO_2 efflux measured in the soil did not include aboveground respiration from living plants (e.g., leaves and

stems) but included belowground root respiration.

2.9. Soil profile CO_2 production

Gas measurements were carried out using a set of gas wells at 15, 30, 50, 70, and 90 cm depths of the soil profile, which had been installed at each plot at a distance of 0.20 m from the center. At 70 and 90 cm, the set up included two additional replicates to better account for deep soil heterogeneity. The gas wells were 6.35 mm (diameter) stainless steel tubes inserted at a 45° angle into the soil to the desired depth and topped with straight swage pipe-fittings with septa at the distal end (TECHNITRACE, France). We collected soil gas samples every fifteen days during six months (January 2022 to June 2022). Samples were collected from the gas wells with a 60 ml syringe, after clearing the headspace in each well. For gas analysis, 3, 20 ml samples were immediately injected into a portable infrared gas analyzer (EGM-5, PP Systems, USA) in the field and stored the gas concentration (ppm) reading with a nonvolatile semiconductor memory (TECHNITRACE, France). In addition, the actual CO_2 concentration from the EGM-5 data was calculated using a four-point standard calibration curve ranging from 0 to 20,000 ppm.

We modeled depth-resolved CO_2 production (μmol m^{-3} s^{-1}) from soil CO_2 concentrations (ppm) using Fick's first Law as a flux gradient approach (Davidson et al., 2006; Hicks Pries et al., 2017; Vargas et al., 2010):

$$F = -D\frac{dC}{dz} \tag{2}$$

Where F denotes the flux density of CO_2 across a horizontal surface on each depth of soil (μmol m^{-2} s^{-1}), D indicates the diffusion coefficient (mm^2 s^{-1}) of air into soil pores, and the change in CO_2 mol fraction at each depth is represented by dC/dz, which was calculated using the first derivative of a curve fit to the depth profile of CO_2 mol fraction. For each depth and time interval, the diffusion coefficient (D) was calculated as follows:

$$D = D_o{}^*\xi \tag{3}$$

$$D_o = D_{ao}\left(\frac{T}{293.15}\right)^{1.75}\left(\frac{101.3}{P}\right) \tag{4}$$

Where D_o is the diffusion coefficient of CO_2 in the air at the soil temperature (T) and local air pressure (P), D_{ao} is a reference value for CO_2 in the air (15.7 mm^2 s^{-1}), and ξ is the dimensionless tortuosity factor. Tortuosity was estimated using six different equations (Jassal et al., 2005; Millington, 1959; Moldrup et al., 2001; Moyes and Bowling, 2013), and we picked the equation that resulted in the best match between the measured surface flux and the estimated surface flux from soil profile data. The most effective equation was determined by an empirical relationship (Hicks Pries et al., 2017; Moyes and Bowling, 2013):

$$\xi = 0.95\varepsilon^{1.93} \tag{5}$$

Where ε is the calculated air-filled porosity (m^3 m^{-3}) from the total soil porosity (based on dry soil bulk density and solid particle density) and volumetric water content at each soil depth and measurement time. Finally, CO_2 production (μmol m^{-3} s^{-1}) was calculated from a given depth interval by subtracting the difference in flux densities (μmol m^{-2} s^{-1}) across that interval and dividing by the depth (m) increment. After that, the CO_2 production was converted to g C m^{-3} d^{-1} units.

2.10. Statistical analysis

To evaluate the impacts of the warming treatment on various measured responses, we ran mixed effects models in RStudio (R Core Team, 2022) using the *nlme* package (Pinheiro et al., 2017) and restricted maximum likelihood with blocks ($n = 3$) as a random effect. Using Q-Q plots and residual histograms, we visually examined all models for homoscedasticity and normality of distribution, and we used a log transformation to meet model assumptions when necessary. Afterward, we used slightly different statistical model structures owing to the varied data structures of SOC concentration, soil water content, CO_2 production and CO_2 efflux. Plots were utilized as random effects to examine the fixed effects of treatment, depth, and their interaction on SOC content within the soil profile. This analysis was conducted separately for cropland and grassland.

To test the fixed effects of treatment, depth, and their interaction on soil water content, we used plots crossed with the sampling date as random effects (to account for violations of independence), separately for cropland and grassland. For surface soil CO_2 efflux, we used a different model that included treatment, seasons, and their interaction as fixed effects, and plots crossed with sampling date as random effects, to test the treatment and seasonal effects on surface soil CO_2 efflux, separately for cropland and grassland. To test the fixed effects of treatment, depth, and their interaction on soil CO_2 production from within the soil profile, we used plots crossed with the sampling date as random effects, separately for cropland and grassland. Besides that, we used treatment, field, and their interaction as fixed effects, and plots crossed with sampling date as random effects, to test the treatment and management effects on leaf area index. Also, a pairwise mean comparison test was performed using the *emmeans* package in RStudio (Searle et al., 2023) when significant differences were observed for any response variables from mixed effect models.

We also calculated the warming response for each block at each sampling date, to test whether warming affected whole-soil profile CO_2 production and surface CO_2 efflux. The warming response was expressed as a percentage difference between warmed and control conditions, which was normalized by the control values of soil CO_2 production and surface CO_2 efflux. A similar warming response was also tested for SOC concentrations.

$$Warmingresponse(\%) = \frac{Warm - Control}{Control} \times 100 \tag{6}$$

3. Results

3.1. Warming effects on soil profile water content

In control plots, annual average soil water contents across the different soil depths ranged from 23.7 to 26.4% in cropland and 24.3 to 28.5% in grassland (Fig. 4). The soil water content significantly ($p \leq 0.05$) decreased in most depths with *in-situ* warming for grassland, and a decreasing trend was noted for cropland, which was only statistically significant at 50 cm ($p \leq 0.05$). In addition, we observed a significant interaction between warming treatment and depth ($p < 0.001$) for both cropland and grassland. Indeed, grassland showed a stronger warming response than cropland, as warming reduced the water content under grassland by 9.7 to 22.9% and by 0.35 to 13.2% under cropland compared to control plots. The greatest differences between the two management practices were found at 30 cm depth with warming, where soil water content was strongly decreased for grassland and slightly decreased for cropland (Fig. 4).

The depth-wise soil water content also depended on the season (significant, $p < 0.001$) for both land management practices (supplementary Fig. S1). During the winter season, topsoil (0–15 cm) showed higher water content, while during other seasons deeper soils had a higher water content for both cropland and grassland. Despite the seasonal variations, *in-situ* warming reduced soil water content throughout the year for both land management practices.

3.2. Warming effects on seasonal CO_2 efflux

The mean surface CO_2 efflux (g C m^{-2} d^{-1}) in the four seasons is presented in Fig. 5. In control plots, CO_2 efflux ranged from 0.52 to 1.35

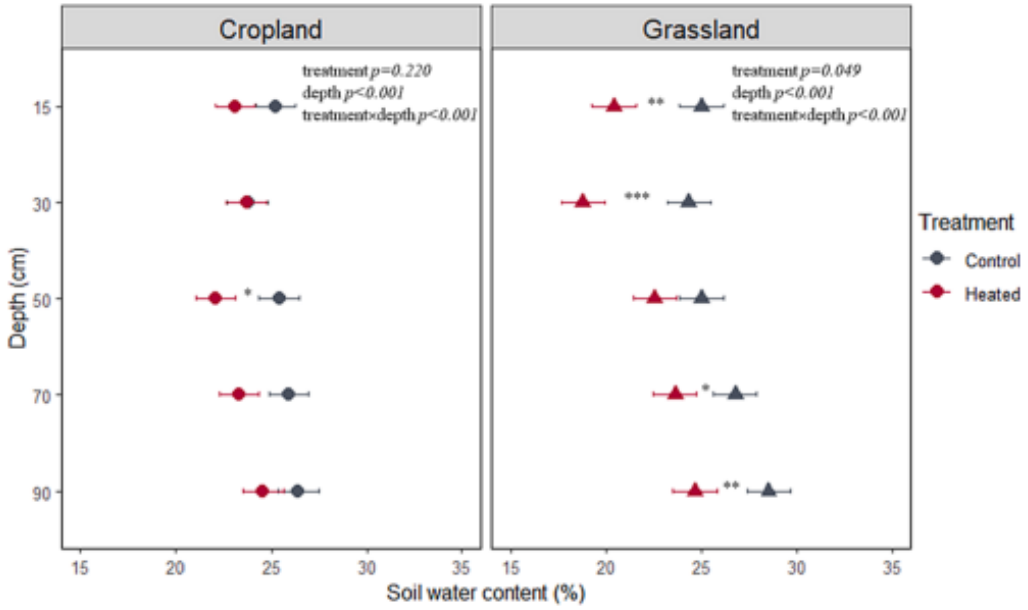


Fig. 4. Mean soil water content (%) in the control and heated plots (mean ± SE, $n = 3$) throughout the 1.0 m depth of soil profile over the study period (July 2021 to June 2022). In the figure, the *p*-values denote the statistically significant effects of treatment, depth, and their interaction (treatment × depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

g C m^{-2} d^{-1} under cropland and 0.50 to 2.47 g C m^{-2} d^{-1} under grassland (Fig. 5). The response of CO_2 efflux to warming depended on the season (Fig. 5). Comparing the different seasons for the grassland, our results show that *in-situ* warming only increased (non-significantly) surface CO_2 efflux in winter, while warming significantly ($p \leq 0.05$) reduced CO_2 efflux in other seasons (Fig. 5). In contrast, *in-situ* warming had no effect on surface CO_2 efflux in cropland during the summer and winter, while in autumn and spring warming reduced CO_2 efflux (non-significantly) (Fig. 5). Overall, the whole-soil warming response of annual surface CO_2 efflux was reduced by 21.8% under cropland (from 399 g C m^{-2} y^{-1} in control plots to 312 g C m^{-2} y^{-1} in heated plots) and by 20.6% under grassland (from 528 g C m^{-2} y^{-1} in control plots to 419 g C m^{-2} y^{-1} in heated plots).

3.3. Warming effects on soil profile CO_2 production

The CO_2 production in the different soil depths, which was measured during six months, is presented in Fig. 6. In control plots, CO_2 production ranged from 0.54 to 4.68 g C m^{-3} d^{-1} under cropland and 0.84 to 5.21 g C m^{-3} d^{-1} under grassland (Fig. 6). In grassland, the CO_2 production significantly ($p \leq 0.05$) increased with *in-situ* warming in subsoil (below 30 cm), while statistically non-significant differences were observed between the two treatments in topsoil (0–30 cm) (Fig. 6). In contrast, in cropland, the CO_2 production significantly ($p \leq 0.05$)

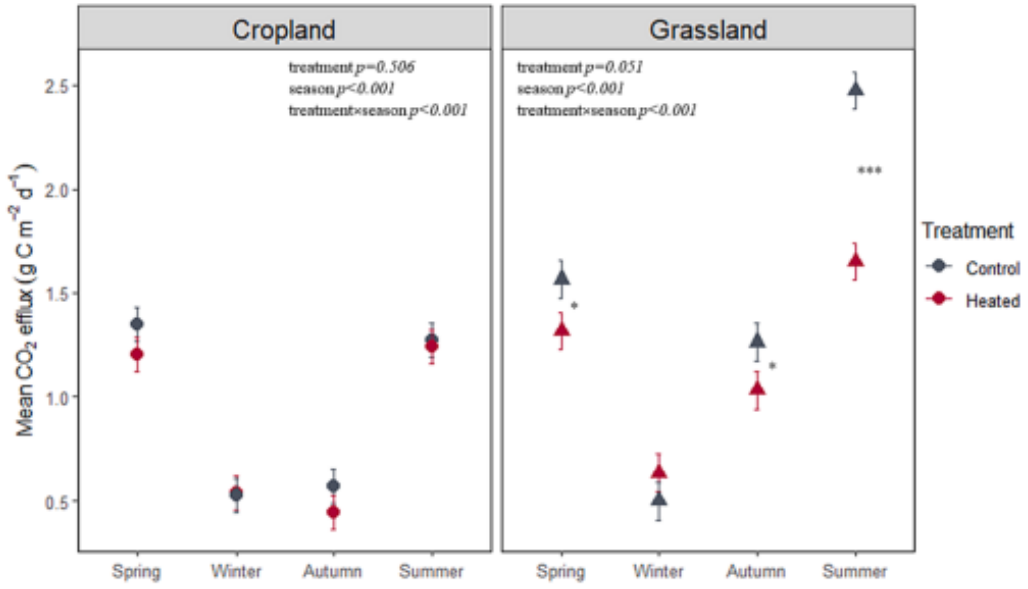


Fig. 5. Mean seasonal variations of soil CO_2 efflux (g C m^{-2} d^{-1}) in the control and heated plots (mean ± SE, $n = 3$) from the whole-soil profile over the study period (July 2021 to June 2022). In the figure, the *p*-values denote the statistically significant effects of treatment, season, and their interaction (treatment × season). The asterisk indicates any significant treatment effect within season as determined by post hoc analysis (*$p \leq 0.05$; ***$p \leq 0.001$).

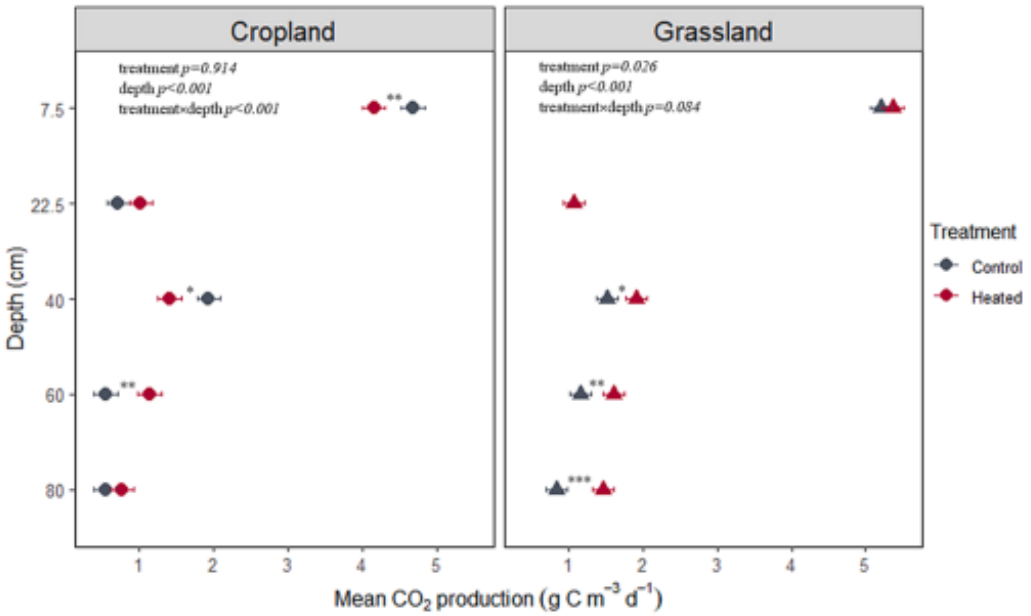


Fig. 6. Mean CO_2 production (g C m^{-3} d^{-1}) in the control and heated plots (mean ± SE, $n = 3$) with depth increment (0–15, 15–30, 30–50, 50–70, and 70–90 cm) over the study period (January 2022 to June 2022). In the figure, the *p*-values denote the statistically significant effects of treatment, depth, and their interaction (treatment × depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

decreased with *in-situ* warming at the 0–15 cm and 30–50 cm depths, but an increasing trend was also noted below 50 cm (Fig. 6). The interaction between treatment and depth was significant ($p < 0.001$) for cropland but not for grassland ($p = 0.084$).

Overall, we found that warming-induced CO_2 production in subsoil was higher for both grassland and cropland as compared to their controls, with a larger increase in grassland (42%) than in cropland (10%). In contrast, the warming-induced CO_2 production rate in topsoil (0–30 cm) differed between grassland and cropland: warming increased CO_2 production by 2.4% in grassland but decreased CO_2 production by 4.2% in cropland. However, it should be noted that the depthwise CO_2 production measurements occurred during the winter and spring seasons, while we do not have data for the summer and autumn seasons. The warming response on cumulative CO_2 production from the whole-soil profile during six months was increased by 1.2% under cropland (from 84 g C m^{-3} half.y^{-1} in control plots to 85 g C m^{-3} half.y^{-1} in heated plots) and by 16.3% under grassland (from 98 g C m^{-3} half.y^{-1} in control plots to 114 g C m^{-3} half.y^{-1} in heated plots).

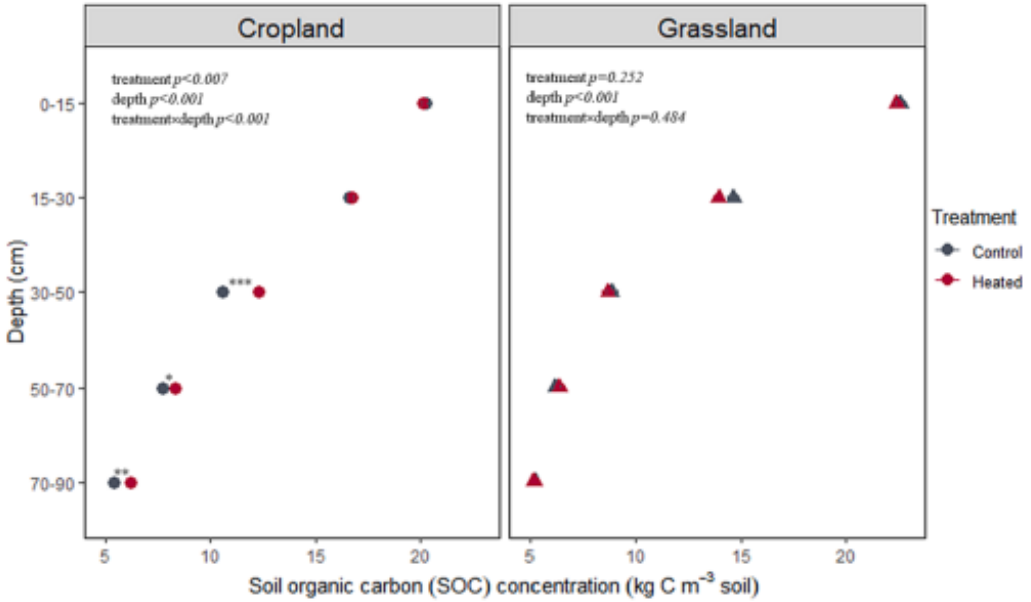


Fig. 7. Soil organic carbon (SOC) concentration (kg C m^{-3} soil) in the control and heated plots (mean ± SE, $n = 3$) with depth increment (0–15, 15–30, 30–50, 50–70, and 70–90 cm), sampled in May 2022, one year after the onset of *in-situ* warming. In the figure, the *p*-values denote the statistically significant effects of treatment, depth, and their interaction (treatment × depth). The asterisk indicates any significant treatment effect within depth as determined by post hoc analysis (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

3.4. Warming effects on soil profile carbon content

In the control plots, SOC concentrations varied across different soil depths, ranging from 20.24 to 5.42 kg C m^{-3} soil in cropland and from 22.59 to 5.20 kg C m^{-3} soil in grassland (Fig. 7). *In-situ* warming did not affect the topsoil SOC content under both land management practices. However, a divergent response to warming was observed in the subsoil (>30 cm): it significantly increased under cropland but not under grassland (Fig. 7). The interaction between treatment and depth was found to be significant ($p < 0.001$) for cropland but not for grassland ($p = 0.484$).

Overall, we observed that the warming-induced SOC content in subsoil was higher in cropland (12.7%) but lower in grassland (0.09%). In contrast, the warming-induced SOC content in topsoil decreased for both cropland (0.05%) and grassland (2.42%) compared to their respective controls. Over the course of one year, the warming response on SOC content across the entire soil profile increased by 4.9% in cropland (from 60.6 kg C m^{-3} in control plots to 63.6 kg C m^{-3} in heated plots) and decreased by 1.6% in grassland (from 57.4 kg C m^{-3} in control plots to 56.5 kg C m^{-3} in heated plots).

3.5. Warming effects on leaf area index

In control plots, the leaf area index was 3.2 under cropland and 3.4 under grassland (Table 4). It significantly increased with *in-situ* warming for grassland ($p < 0.01$) and also showed an increasing, but non-significant trend for cropland (Table 4).

4. Discussion

4.1. Negative warming effects on whole-soil profile water content under the two land management practices

Our findings revealed that *in-situ* warming had a negative effect on soil water content in the whole-soil profile for both land management practices (Fig. 4). These results are in agreement with a recent *meta*-analysis by Xu et al. (2013), which showed that warming generally leads to soil drying in agricultural ecosystems. The two main processes of water loss from the soil reservoir include evaporation from the soil surface and transpiration by plants (Chapin et al., 2002). Both may be affected by land management practices (Liancourt et al., 2012). In this study, we showed that grassland soil was more vulnerable to soil water loss under *in-situ* warming compared to cropland. Despite the lack of air warming, warming-induced reduction in soil water content could be attributed to increased plant growth and transpiration processes with a higher leaf area index (Table 4), as also found by other authors (Chen et al., 2021a; Wan et al., 2002; Xia et al., 2010). However, the plants' effects on soil water might be species- or management-and/or site-specific (Wang et al., 2012). In particular, in grassland with a complex root system exploring deeper soil layers it might be stronger than croplands. Indeed in grasslands, perennial plants could respire throughout the year involving (i) transpiration by plant leaves and (ii) water consumption by plant roots for their physiological and metabolic activities (Du et al., 2011). In contrast, cropland soil is characterized by periods of bare soil, plant regrowth and harvest, involving less root production (Table 2) (Hu & Chabbi, 2021) and consequently probably much less water consumption by plant roots and transpiration. As soil water becomes depleted under *in-situ* warming, this could alter the plants' C allocation strategies depending on land management practices, thereby affecting the C inputs and turnover in the soil profile (Li et al., 2019; Mokany et al., 2006; Védère et al., 2022).

Table 4
Warming response of leaf area index (mean ± SE, $n = 3$) under the two different land management practices during the plant growing season (March to June 2022) with different treatments (control: heated).

	Leaf area index (m^2 m^{-2})	
Field	Control	Heated
Cropland	3.2 ± 0.2^{b}	3.6 ± 0.2^{ab}
Grassland	3.4 ± 0.2^{b}	4.6 ± 0.2^{a}

Here, different letters indicate the statistically significant effects of treatment and field interaction, as determined by post hoc analysis ($p < 0.01$).

4.2. Contrasting warming response of seasonal CO_2 efflux under the two land management practices

Our data indicated that *in-situ* warming suppressed surface CO_2 efflux for both land management practices in the spring and autumn, whereas the other seasons (winter and summer) showed a contrasting warming response of surface CO_2 efflux (Fig. 5). *In-situ* warming could be a constraint for the spring and autumn seasons by slowing the processes that lead to both autotrophic and heterotrophic respirations that are controlled by precipitation and soil moisture determining plant growth (Miao et al., 2020; Quansah et al., 2015). In contrast, the different warming responses of soil CO_2 efflux in the winter and summer for both land management practices might be related to the continuous rhizodeposition supplied by the perennial root systems in grassland while cropland soil is bare during these periods (Hu & Chabbi, 2022; McGowan et al., 2019). In grassland, the warming response was negative in summer but positive in winter, which could be due to the warming-induced lower soil water content and seasonal fluctuation of plant growth and their root activity (Du et al., 2019; Hu et al., 2017). For example, the combination of high soil temperature and low soil water content might constrain root and microbial activity under summer warming, while in winter adequate moisture fuels greater microbial activity (supplementary Fig. S1) (Davidson et al., 1998; Vincent et al., 2006). In cropland, *in-situ* warming did not affect surface CO_2 efflux during summer or winter, which could be because the plants are only actively growing in the spring, providing evidence for plant activity as a control (Chen et al., 2021b).

The contrasting warming response of seasonal CO_2 efflux leads to an overall reduction in whole-soil warming annual surface CO_2 efflux, depending on land management practices. Our findings differ from those found in various terrestrial ecosystems where warming stimulated soil CO_2 efflux (Hicks Pries et al., 2017; Lin et al., 2011; Luo et al., 2010; Ofiti et al., 2021; Schindlbacher et al., 2009; Yan et al., 2022). Nevertheless, our data are consistent with studies reporting reduced soil and microbial respiration under warming in an old-field grasslands (Wan et al., 2007), in some seasons in tallgrass prairie (Verburg et al., 2005), and in an alpine meadow (Saleska et al., 1999). However, one recent *meta*-analysis demonstrated that on average, global soil will be a source of C to the atmosphere under a future warmer climate (Wang et al., 2022), supporting the expectation of positive SOC loss as a warming feedback (García-Palacios et al., 2021). In our study, warming-induced reductions in soil water content may be strongly influencing surface CO_2 efflux, through its influence on microbial communities and their functionalities, which are temperature- and moisture-dependent (Curiel Yuste et al., 2007; Davidson and Janssens, 2006). Water availability is a critical factor that can affect plant and microbial respiration. When water is limited, plants slow down photosynthesis to conserve water (Wang et al., 2018), leading to a decrease in plant contributions to soil respiration (Ribas-Carbo et al., 2005). Similarly, water limitation can reduce microbial activity and their contribution to respiration as insufficient water can impede substrate diffusion to microbial cells (Lehmann et al., 2020; Moyano et al., 2013; Védère et al., 2022). Moreover, *in-situ* warming may reduce the contribution of seasonal fine root respiration as a source of surface CO_2 efflux. In warmer climates, grassland ecosystems may increase their total root system length but lose a proportion of fine roots growing in the superficial layer of soil (Pilon et al., 2013).

4.3. Warming alters soil CO_2 production along soil depths under the two land management practices

Our results showed greater positive effects of warming on subsoil CO_2 production than in topsoil for both agricultural practices (Fig. 6). This is consistent with previous whole-soil warming observations in a temperate forest (Hicks Pries et al., 2017), but not in a tropical forest (McGrath et al., 2022). The stronger warming effect on CO_2 production in subsoil than in topsoil could be due to a contrasting response of microbial metabolisms and/or root respiration in both parts of the soil profile. In subsoil, microbial activity is generally much lower than in topsoil (Rumpel and Kögel-Knabner, 2011), which was explained by the absence of fresh organic matter input (Fontaine et al., 2007). Under warming, energy input into subsoil through root growth and dissolved organic matter could be increased and stimulate copiotrophic populations at greater depths, leading to CO_2 production through decomposition of fresh root litter (Müller et al., 2016; Sanaullah et al., 2016; Soong et al., 2021). Root growth deeper in the soil profile could be stimulated by lower soil water content in warmed soil, which forces plants to explore deeper soil horizons (Joslin et al., 2000; Sotta et al., 2007).

Warming effects on CO_2 production were contrasting in the two management systems. Subsoil CO_2 production was more pronounced under warming of grassland soil compared to cropland. This might be explained by greater root production and activity under grassland than cropland, as indicated by the greater standing root biomass in this agroecosystem (Table 2). Conversly, we observed that warming-induced subsoil SOC content was significantly higher in cropland but slightly lower in grassland. These findings suggest that the turnover or accumulation of subsoil SOC, as well as the subsequent mechanisms for CO_2 production under warming conditions, may vary based on different management practices. For instance, if warming leads to an increase in above-ground biomass, soil disturbance caused by tillage might be advantageous. This disturbance helps in transferring crop residues, dead roots, and topsoil C into subsoil layers where C accumulates, as shown in studies by Yang et al. (2022). Consequently, this process results in higher subsoil SOC content in cropland. Additionally, the organic materials added through this process could serve as a food source for microorganisms, potentially leading to increased CO_2 production in the short term.

In contrast, warming might cause a greater allocation of belowground C in grassland compared to cropland. This allocation is primarily derived from rhizodeposition provided by the perennial root system, as indicated in Table 2 (Hu & Chabbi, 2022; McGowan et al., 2019). Consequently, continuous interactions between living root systems and subsoil microbes could have contributed to higher CO_2 production in grassland. This is supported by the negligible SOC loss observed under warming conditions.

In topsoil, warming-induced CO_2 production was differently affected by management. Under grassland, increased CO_2 production in topsoil could be attributed to a greater contribution of plant roots and rhizosphere microbes or saprotrophic microorganisms under warming conditions, which are the primary contributors to SOC decomposition (Schindlbacher et al., 2009; Zhou et al., 2007), as evidenced by lower SOC content under warming conditions. While decreasing CO_2 production in response to warming in cropland may be attributed to a double effect of both warming and tillage-induced soil disturbance (ploughing horizon at 30 cm depth), which can lead to shifts in soil microbial community and function due to lower plant root biomass (Table 2), resulting in lower CO_2 production and less sensitivity to warming-induced SOC decomposition, as evidenced by negligible SOC loss.

4.4. Future prospects

Although an increase in CO_2 production in subsoil is well documented in this study (Fig. 6) and supported by another whole-soil warming study in a temperate forest (Hicks Pries et al., 2017), the biotic fixation and movement of CO_2 in the context of projected global warming under different agricultural management practices are still unknown. Despite a few recent studies confirming that soil microbes can fix CO_2 in temperate soils (Nel and Cramer, 2019; Spohn et al., 2020). Moreover, warming might change the microbial community composition and in particular increase fungal contribution, leading to the formation of more stable pore structures (Miller and Jastrow, 2000). These effects may depending on soil management, as there is lower fungal contribution in cropland than in grassland due to ploughing events (Schnoor et al., 2011). In order to get a complete picture of soil responses, heterotropic respiration should in future studies be distinguished from autotropic respiration.

Additionally, CO_2 flux within the soil profile may also be influenced by its microstructural properties. Indeed, a recent study found that grassland soil has a larger macropore diameter due to the presence of large biopores that are periodically destroyed in cropland (Schlüter et al., 2022). The processes affecting soil structure may have an impact on soil gas diffusivity, which determines whether CO_2 produced at depth is lost to the atmosphere or fixed in situ (Liao et al., 2023). Thus, we propose that for understanding and modeling the effects of soil warming on CO_2 efflux under different agricultural management practices, the parameters influencing CO_2 diffusivity, such as soil architecture need to be taken into account in addition to factors affecting CO_2 production such as root activity and microbial community composition.

5. Conclusion

In this study we investigated seasonality of whole soil warming responses of CO_2 efflux. Moreover, we compared CO_2 production across soil depths down to 1 m. We conclude that *in-situ* soil warming led to soil drying, the extent of which depended on soil management. Soil drying was more pronounced in grassland compared to cropland, supporting our initial hypothesis (H1). We also found contrasting seasonal dynamics of surface CO_2 efflux. While our findings indicate that *in-situ* warming suppressed surface CO_2 efflux for both land management practices in the spring and autumn, winter and summer showed greater increases and decreases in response to warming, respectively, under grassland compared to cropland. These findings reject our second hypothesis (H2), which states that CO_2 efflux would increase year round in response to warming under continuous grassland but only seasonally in cropland soils.

Subsoil (>30 cm) CO_2 production was more vulnerable to warming than in the topsoil, and this increase due to warming was more pronounced in grassland compared to cropland, confirming our last hypothesis (H3). In topsoil (0–30 cm), warming decreased CO_2 production in cropland but not in the grassland. Overall, land management had a significant impact on CO_2 production throughout the soil profile, which may driven by both autotrophic and heterotrophic sources. Thus, we suggest that management influences the warming responses of CO_2 production and efflux by its effects on soil physical organization in addition to plant and microbial activity with root carbon input.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116725.

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