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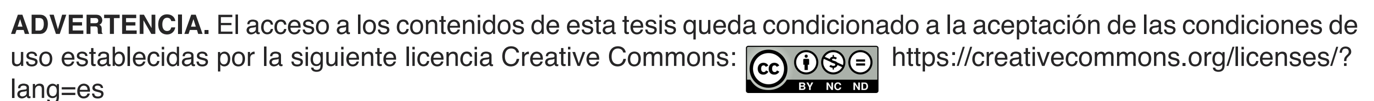
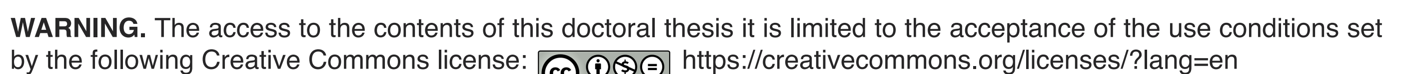
ADVERTENCIA. El acceso a los contenidos de esta tesis queda condicionado a la aceptación de las condiciones de uso establecidas por la siguiente licencia Creative Commons:  https://creativecommons.org/licenses/?lang=es

WARNING. The access to the contents of this doctoral thesis it is limited to the acceptance of the use conditions set by the following Creative Commons license:  https://creativecommons.org/licenses/?lang=en

Climate change effects on soil respiration and belowground biogenic volatile organic compounds in subalpine and Mediterranean forests

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Climate change effects on soil respiration and belowground biogenic volatile organic compounds in subalpine and Mediterranean forests

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PhD Thesis

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Abstract

Soil systems are vital for the global carbon cycle, but there is uncertainty about how they respond to environmental changes. Understanding soil responses to environmental changes is therefore crucial for comprehending the carbon cycle. Climate change is causing global modifications, affecting local climates, ecosystems, and soil environments differently. The Tibetan Plateau, a climate-sensitive region and a significant carbon reservoir, experiences higher warming rates than other regions, leading to changes in snow cover. The Mediterranean region is also sensitive to global changes, with reduced rainfall and increased drought stress altering below-ground biochemical cycling processes. In this thesis, I investigated how these two distinct ecosystems (the Tibetan Plateau and the Mediterranean) respond to climate change, namely warming and drought, respectively.

In the Tibetan Plateau, the response of soil properties and biological processes (including soil respiration, aggregate structure, microorganisms, enzymes, and root biomass) to snow removal was investigated using a methodology designed to simulate warming-induced snow reduction. I found the reduction in snow cover decreased the concentration of phospholipid fatty acids and enzyme activity, but did not alter their stoichiometric ratios. Additionally, the microbial changes were most pronounced in small microaggregates, indicating that microbial responses to snow removal differed between soil aggregate classes (Chapter 1). The subsequent reduction in snow cover significantly increased freeze-thaw intensity and frequency, as well as nitrogen availability, but reduced root biomass, leading to a reduction of soil CO_2 efflux by an average of 17% in winter. However, the effect of snow cover reduction had no carry-over effect on subsequent growth season soil respiration and microbial characteristics,

suggesting strong microbial resilience to environmental change (Chapter 2).

Another way that climate change can affect soils is through changes in emissions of biogenic volatile organic compounds (BVOCs). All living organisms release non-methane BVOCs, which are reactive gases that have both negative and positive climate forcing impacts. Soil BVOC emission has previously been considered insignificant and the soil sink capacity of BVOCs has been underestimated. Therefore, I reviewed and summarised recent studies about sources of BVOCs in soil and how global change is affecting the exchange of BVOCs between the soil and the atmosphere in different ecosystems across the globe. Forest soils mainly emit isoprenoids while tundra and cropland mainly emit non-isoprenoids, and 17−36 % of annual VOCs emissions may be taken up by the ground from the atmosphere. I also showed that climate change impacts on belowground VOCs vary depending on the ecosystem, and that the warming response of VOCs from soil may not be in accordance with patterns shown by aboveground sources (Chapter 3).

Mediterranean forests face severe challenges from persistent drought and nitrogen (N) deposition. I carried out a drought and N deposition manipulation experiment in a Mediterranean holm oak forest and measured seasonal soil BVOC and CO_2 fluxes over two years. Prolonged drought led to reduced soil water content, affecting the balance of volatile organic compounds between the soil and the atmosphere, meanwhile, N deposition increased the release of monoterpenes by litter. Although seasonal differences played a crucial role in regulating the VOC sources and sinks of soil, their influence may diminish as the soil dries out due to drought (Chapter 4).

In conclusion, my results highlight snow reduction and drought have significant impacts on soil biochemical cycling processes, potentially altering the role of soil in

carbon cycling. My findings demonstrate the importance of the soil processes and differences between ecosystems in terms of climate change responses, and emphasize the need for improved modelling of soil C cycling (particularly BVOCs) to improve our understanding of the impacts of soil characteristics on ecosystem functioning in a changing world.

General introduction

Climate change

Anthropogenic activities and pollution are changing the climate and causing major environmental perturbations for the world with drastic consequences for its inhabitants (IPCC, 2022). The frequency and intensity of extreme weather events caused by climate change, such as heatwaves, precipitation, and drought, have been consistently increasing (Fig. 1) and have been reported all over the world (https://ourworldindata.org). In particular, climate change has significant impacts on forest ecosystems. Warmer and drier conditions facilitate disturbances such as fire, drought and insect outbreaks (Morán-Ordóñez et al., 2020; Samaniego et al., 2018), while warmer and wetter conditions increase disturbances from wind and pathogens (Seidl et al., 2017). These disturbances can lead to changes in forest structure, composition, and functioning. Climate change also affects the dominance of tree mycorrhizal associations, with potential consequences for forest productivity and carbon sequestration (Jo et al., 2019). Forest ecosystems are expected to experience conditions outside their natural range of variability, increasing the probability of widespread mortality and large-scale disturbance events (Peñuelas et al., 2018; Strahan et al., 2016). Changes in climate can directly impact plant physiology and productivity, as well as indirectly affect ecosystems through changes in species composition and

diversity (Morin et al., 2018).

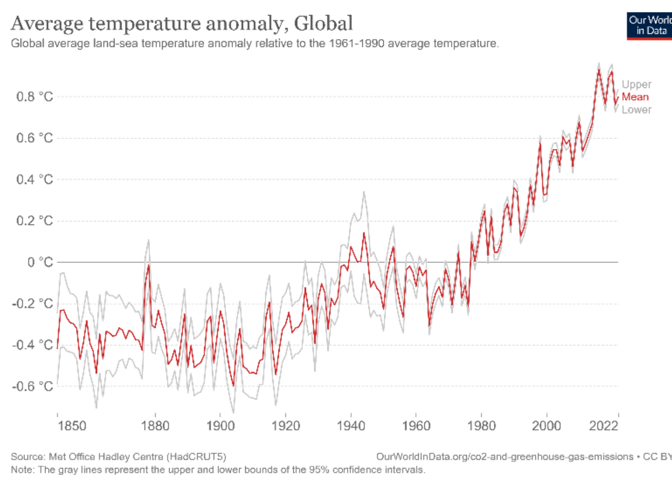

Figure 1. Global average land-sea temperature anomaly relative to the 1961-1990 average temperature (source: https://ourworldindata.org).

Soil ecosystem under climate change

Soil is the foundation base for the global cycles of carbon and nutrients, and the physical and biochemical properties of soil play important roles in carbon sequestration and ecosystem stability. The direction of ongoing climate change is towards warmer and drier weather in most regions in the coming years. Typically, cold biome regions, such as the Tibetan Plateau and the Arctic region, are particularly sensitive to the increase of temperature, resulting in higher emissions of carbon-contained gases (such as, carbon dioxide, volatile organic compounds and greenhouse gases) (Kramshøj et al., 2016). However, in the Mediterranean region, the longer and more frequent drought is dramatically affecting soil microbial communities, greenhouse emission and ecosystem VOCs exchange (Appiagyei et al., 2022; Asensio et al., 2021; Aurelle et al., 2022; Lidón et al., 2021; Peguero et al., 2021; Yáñez-Serrano et al., 2021).

Changes in temperature and precipitation patterns directly affect soil properties and processes. The turnover time of carbon in terrestrial ecosystems, which is influenced

by climate, soil, and vegetation type, plays a crucial role in the feedback between the terrestrial carbon cycle and climate (Carvalhais et al., 2014). Climate change can alter the abundance and composition of soil microbial communities, with warming and changes in precipitation affecting fungal and bacterial abundance and community structure (Castro et al., 2010; Pugnaire et al., 2019). Soil ecosystem functioning is influenced by climate change through changes in plant community composition, which indirectly affect soil communities and their inputs (Kardol et al., 2010; Williams and Vries, 2020; Xiong et al., 2020). Climate change can also impact soil respiration, net nitrogen mineralization, and litter decomposition, with warming and changes in precipitation influencing these processes (Emmett et al., 2004; Zhang et al., 2021). Changes in soil microbial diversity and biomass are driven by soil carbon content across global biomes, with soil carbon content associated with the microbial diversity-biomass relationship (Bastida et al., 2021).

Soil microbial communities and biodiversity can be impacted by climate change, with reductions in microbial diversity observed under warming and reduced soil moisture conditions (Wu et al., 2022). Additionally, climate change can indirectly affect soil biodiversity through extreme weather events such as heavy rainfall and drought (Tibbett et al., 2020). Overall, climate change has wide-ranging effects on soil ecosystems, including changes in carbon turnover, microbial communities, ecosystem functioning, biodiversity, and soil properties. Understanding these impacts is crucial for managing and conserving soil ecosystems in the face of climate change.

Climate change in the Tibetan Plateau forest

Climate change has significant impacts on the Tibetan Plateau forest ecosystem. The

region has experienced rapid climate warming, with temperatures increasing at a rate of 0.4°C per decade over the past 50 years (Ma et al., 2017). This warming, coupled with changes in precipitation patterns, has influenced net primary production, biogeochemical cycles, litter decomposition, and rangeland quality in the region. The Tibetan Plateau is highly vulnerable to climate change, with significant environmental changes such as glacier retreat, lake expansion, and permafrost degradation (Yang et al., 2013). These changes have brought environmental risks and disasters to the surrounding regions.

Changes in temperature and precipitation patterns directly impact soil properties and processes (Yin et al., 2012). Soil moisture plays a fundamental role in controlling land surface energy partitioning, surface runoff, soil drainage, canopy transpiration, and carbon cycling (Yang et al., 2013). Given that the Tibetan Plateau is particular sensitive to climate warming, and has suffered a great reduction in snow cover over the past decades, climate change in that region could dramatically alter soil structure and functions, particularly in response to increases in temperature and snow melt. The Tibetan Plateau soils contain massive amounts of carbon, that when thawed could potentially release large amounts of CO_2.

Overall, climate change has significant implications for the Tibetan Plateau forest ecosystem. Understanding these impacts is crucial for effective conservation and management strategies in the face of ongoing climate change.

Climate change in Mediterranean forests

Climate change has significant impacts on Mediterranean ecosystems, where the warming weather is inducing more frequent and severe droughts (Peñuelas et al., 2018;

Peñuelas and Llusià, 2003). There has been an increase in the frequency and intensity of drought and nitrogen (N) deposition in the Mediterranean region (Asensio et al., 2021; Mu et al., 2018, p. 20; Preece et al., 2020). Drought is linked to decreases in plant productivity, intensive plant mortality and alterations in soil biogeochemistry (Ogaya and Peñuelas, 2007; Vallicrosa et al., 2021). Additionally, changes in forest structure and composition influence carbon storage, tree productivity, and water use (Mechergui et al., 2021; Ruiz-Benito et al., 2013).

The soil ecosystem in Mediterranean forests is significantly affected by climate change, with the main effect being a significant decrease in soil moisture availability and water balance (Asensio et al., 2007). Reduced soil moisture in that region can lead to change in microbial activity, nutrient availability and litter decomposers (Peguero et al., 2021; Preece et al., 2020). The composition and activity of soil microbial communities, which play crucial roles in nutrient cycling and organic matter decomposition, are also significantly affected by drought (De Vries et al., 2018; Williams and Vries, 2020; Yuste et al., 2011). Overall, climate change poses significant challenges to Mediterranean forests and soils, highlighting the need for adaptive management strategies to mitigate its impacts and enhance ecosystem resilience.

Soil respiration

Soil respiration refers to the process by which carbon dioxide (CO_2) is released from the soil into the atmosphere through the metabolic activities of soil organisms, such as plant roots, microbes and soil fauna (Baggs., 2006). It is estimated that global soil respiration releases 77 Pg C yr^{-1} from soil components (Raich and Potter 1995), of which 50 Pg C yr^{-1} from decompositions of litter and soil organic matter (SOM), and 18 Pg C yr^{-1} from live roots (Raich and Schlesinger 1992). The soil pool from which

soil respiration releases carbon is about four times the atmospheric pool. It is a major component of the global carbon cycle and plays a crucial role in the exchange of carbon between the terrestrial ecosystem and the atmosphere (Schlesinger and Andrews 2000; Xu and Shang 2016). Under climate change, soil respiration becomes particularly important as it can have significant implications for the carbon balance of ecosystems and the global climate system (Raich and Schlesinger 1992).

The response of soil respiration to climate change is a complex process influenced by various factors (Conant et al. 2000; Meyer et al., 2018; Zhou et al. 2016). The rising temperatures associated with climate change can stimulate soil respiration rates. The temperature sensitivity of soil respiration, often quantified by the $Q10$ value, indicates that as temperatures increase, the metabolic activity of soil organisms accelerates, leading to higher rates of soil respiration (Hursh et al., 2017; Meyer et al. 2018). However, the relationship between temperature and soil respiration is not linear, and there may a threshold temperature beyond which respiration rates decrease (Carey et al., 2016). Otherwise, changes in precipitation patterns due to climate change can alter soil moisture levels, which in turn can affect the activity and abundance of soil organisms and impact soil respiration rates (Preece et al., 2019, 2020). Furthermore, the availability of organic matter in the soil serves as a substrate for soil organisms, and changes in climate can influence the decomposition rates of organic matter and consequently impact soil respiration rates (Gray et al., 2012; Yuste et al., 2011).

Overall, while it is expected that rising temperatures associated with climate change will stimulate soil respiration rates, the response of soil respiration to climate change is influenced by multiple factors, including temperature, moisture, organic matter availability, and the balance between autotrophic and heterotrophic processes (Asensio et al., 2007; Baggs 2006; Conant et al., 2000; Monson et al., 2006). More

research is needed to improve our understanding of these complex interactions and their implications for the carbon balance of ecosystems and the global climate system.

Volatile organic compounds

Volatile organic compounds (VOCs) are characterized by having a low molecular mass and high vapour pressure, which are properties that allow them to have a high chemical activity and affect the atmosphere environment (Kulmala et al., 2004; Paasonen et al., 2013). For example, VOCs emission can induce the formation of particles in the otherwise unpolluted Arctic air, leading to increased cloud cover (Paasonen et al., 2013). On the other hand, VOCs also have the potential to prolong the lifetime of methane in the atmosphere through the depletion of hydroxyl radicals via oxidation reactions, thus strengthening the global warming potential of methane (Hellén et al., 2018; Peñuelas and Staudt, 2010).

All organisms emit a unique and common mixture of BVOCs to the surroundings, where they build a close connection among organisms above and/or below ground, acting as a language for communication among different organisms (Bilas et al., 2021; Insam and Seewald, 2010; Schenkel et al., 2015; Stotzky et al., 1976). Globally, the source of VOCs derived from anthropogenic and biogenic sources, which ranges from 139-163 TgC yr^{-1} and 424-591 TgC yr^{-1}, respectively (Duan et al., 2023). The vast majority of BVOCs are terpenoids, including isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$), and sesquiterpenes ($C_{15}H_{24}$), which contribute 50%-60%, 15% and 3% of the total BVOC emissions, respectively (Duan et al., 2023; Lun et al., 2020; Sindelarova et al., 2022). Moreover, the majority of biogenic VOCs originate from vegetation (Guenther et al., 2012; Yáñez-Serrano et al., 2020), and regions with high BVOC emissions are located in the tropics (Sindelarova et al., 2022; Yáñez-Serrano et al., 2020).

BVOCs have been extensively studied in many different organisms over the last decades, with their functions and interactions being described (Insam and Seewald., 2010; Peñuelas et al., 2014; Stotzky et al., 1976). In recent years, more unique BVOCs have been identified, and examples of interactions between organisms mediated by BVOCs are numerous (Lemfack et al., 2017).

In soil ecosystem, the most important source of BVOCs are plant roots, microorganisms, and soil organic matter decomposition processes (Tang et al., 2019; Tsuruta et al., 2018; Viros et al., 2020). Like in aboveground ecosystems, BVOCs in the soil interact in complex multispecies networks, affecting behaviour and population and community structure of responding organisms, with both promoting and suppressing effects (Bennett and Klironomos, 2019; Bilas et al., 2021; De Long et al., 2018; Schenkel et al., 2015; Yuan et al., 2017). Therefore, BVOCs play a vital role in the soil ecosystem.

Generally, soils account for 1-10% of total ecosystem BVOC emissions to the atmosphere (Peñuelas et al., 2014), but this number varies considerably between various types of ecosystems and seasons (Aaltonen et al., 2013; Llusià et al., 2022; Mu et al., 2022; Romero-Olivares et al., 2022). More and more field studies have demonstrated that the fluxes of BVOC are dominated by soil properties and climate conditions (Bachy et al., 2018; Bourtsoukidis et al., 2018; Mäki et al., 2019; Mielnik et al., 2018; Mu et al., 2022). Terpenoids and oxygenated BVOCs are among those most emitted in soil systems, and they are widely surveyed in boreal and Mediterranean forests (Aaltonen et al., 2013, 2011; Asensio et al., 2007a, 2007b; Barreira et al., 2017; Hellén et al., 2018; Janson, 1993). Litter and roots can both be significant sources of terpenes, but they appear to have distinct emission profiles. Litter tends to release more monoterpenes (Isidorov and Zaitsev, 2022; Viros et al., 2021, 2020), while roots tend

to emit more sesquiterpenes (Lin et al., 2007; Tsuruta et al., 2018). Similarly, soil bacteria and fungi exhibit divergent emission profiles (Peñuelas et al., 2014). Bacterial VOCs are dominated by (in descending order) alkenes, alcohols, ketones, terpenes, benzenoids, pyrazines, acids and esters. Whereas, fungal VOCs are dominated by alcohols, benzenoids, aldehydes, alkenes, acids, esters and ketones.

Soil BVOC emissions are bidirectional, although the BVOC uptake capacity of soils is not well understood, and whether soils primarily are net sinks or net sources is uncertain (Asensio et al., 2007b; Insam and Seewald, 2010; Peñuelas et al., 2014). Based on available field studies, soils in Mediterranean, subtropical, and tropical forests demonstrate a significant soil sink function (Asensio et al., 2007b; Carrión et al., 2020; Huang et al., 2021; Llusià et al., 2022). However, this feature is rarely observed in tundra, boreal, and temperate forests (Faubert et al., 2010; Isidorov et al., 2022; Mäki et al., 2019; Romero-Olivares et al., 2022; Zhang-Turpeinen et al., 2021). Laboratory studies have shown that microorganisms in soil utilize BVOCs as a carbon source, and microbial consumption is likely to be a significant mechanism by which soils can act as sinks for BVOCs (Albers et al., 2018; Gray et al., 2015; Hohener et al., 2003; Ramirez et al., 2010). Therefore, studying the source-sink function of BVOCs in forest soils is a critical process for comprehending the effects of climate change on terrestrial ecology.

Objectives of the thesis

In this thesis I investigate how various factors associated with climate change affect soil function, the fluxes of CO_2, and BVOCs in Tibetan Plateau forest and Mediterranean forest. More specifically, In the Tibetan Plateau forest, I investigate the

effect of short-term experimental warming on the structure and activity of soil microbial communities and overall soil CO_2 efflux. In the Mediterranean I investigate the balance of soil BVOCs occurring in response to long-term drought and short-term nitrogen fertilization. The general aim of my research was to give a broader understanding of how climate change affects soil structure, the microbial community and soil BVOC fluxes. The objectives of the specific chapters in the dissertation are as follows:

Chapter 1

In chapter I the aim was to study the effect of short-term experimental warming on the structure and activity of soil microbial communities in this cold ecosystem during the period of 2016-2017. We used a snow-exclusion experiment to examine the influence of increased soil frost on microbial biomass and activity in aggregate fractions in a Tibetan alpine spruce forest. I hypothesized that snow-exclusion would significantly increase soil frost in winter season and that such an increase of frequency would change soil aggregate fractions and therefore affect the concentrations of phospholipid fatty acids (PLFAs) and the activities of extracellular enzymes.

Chapter 2

The release of carbon dioxide from warming Tibetan alpine spruce forest soils has been little studied, therefore the aim of this study was to assess the impact of snow-exclusion on the soil CO_2 efflux and biological activity in a subalpine spruce forest on the eastern Tibetan Plateau. In addition, I tested if higher frost due to snow-exclusion had immediate and carry-over effects (in the coming seasons) on soil respiration and microbial activity. I hypothesized that more intense frost in the soil, as a result of the exclusion of snow, would decrease microbial activity and soil respiration in winter, and frost-reduced biological processes would carry over into the subsequent snow-free

growing season.

Chapter 3

The soil system constitutes a significant locus for the generation, emission, and sequestration of BVOCs; however, the precise mechanisms underpinning these processes remain insufficiently elucidated. In pursuit of enhanced understanding, I gathered data on soil BVOCs across diverse ecosystems, concomitantly recording relevant environmental variables. Subsequently, I conducted a comprehensive review of existing insights into soil BVOC fluxes and the ensuing fate of BVOCs within the soil environment. This synthesis culminated in a comprehensive review of VOC exchange dynamics between soils and the atmosphere.

Chapter 4

The main aim of this chapter was to investigate the response of soil VOCs to global change, focusing on the Mediterranean forest ecosystem. During the period spanning 2018 to 2020, I examined the exchange rates of soil VOCs under conditions of experimental drought and nitrogen addition. The overarching hypotheses posited were twofold: firstly, that diminished soil moisture stemming from prolonged drought or seasonal climatic variations would stimulate the absorption of soil BVOCs; and secondly, that transient nitrogen fertilization would amplify soil BVOC emissions by intensifying soil microbial activity.

References

Aaltonen, H., Aalto, J., Kolari, P., Pihlatie, M., Pumpanen, J., Kulmala, M., Nikinmaa, E., Vesala, T., Bäck, J., 2013. Continuous VOC flux measurements on boreal forest floor. Plant Soil 369, 241–256.

Aaltonen, H., Pumpanen, J., Pihlatie, M., Hakola, H., Hellén, H., Kulmala, L., Vesala, T., Bäck, J., 2011. Boreal pine forest floor biogenic volatile organic compound emissions peak in early summer and autumn. Agric. For. Meteorol. 151, 682–691.

Albers, C.N., Kramshøj, M., Rinnan, R., 2018. Rapid mineralization of biogenic volatile organic compounds in temperate and Arctic soils. Biogeosciences 15, 3591–3601.

Appiagyei, B.D., Belhoucine-Guezouguli, L., Bessah, E., Morsli, B., Fernandes, P.A.M., 2022. A Review on Climate Change Impacts on Forest Ecosystem Services in the Mediterranean Basin. J. Landsc. Ecol. 15, 1–26.

Asensio, D., Peñuelas, J., Llusià, J., Ogaya, R., Filella, I., 2007a. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. Soil Biol. Biochem., 39, 2471–2484.

Asensio, D., Penuelas, J., Ogaya, R., Llusia, J., 2007b. Seasonal soil VOC exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmos. Environ. 41, 2456–2466.

Asensio, D., Zuccarini, P., Ogaya, R., Marañón-Jiménez, S., Sardans, J., Peñuelas, J., 2021. Simulated climate change and seasonal drought increase carbon and phosphorus demand in Mediterranean forest soils. Soil Biol. Biochem. 163, 108424.

Aurelle, D., Thomas, S., Albert, C.H., Bally, M., Bondeau, A., Boudouresque, C.-F., Cahill, A.E., Carlotti, F., Chenuil, A., Cramer, W., Davi, H., Jode, A.D., Ereskovsky, A.V., Farnet, A., Fernandez, C., Gauquelin, T., Mirleau, P., Monnet, A., Prévosto, B., Rossi, V., Sartoretto, S., Wambeke, F.V., Fady, B., 2022. Biodiversity, Climate Change, and Adaptation in the Mediterranean. Ecosphere 13(4): e3915.

Bachy, A., Aubinet, M., Amelynck, C., Schoon, N., Bodson, B., Moureaux, C., Delaplace, P., De Ligne, A., Heinesch, B., 2018. Methanol exchange dynamics between a temperate cropland soil and the atmosphere. Atmos. Environ. 176, 229–239.

Baggs, E. M. 2006. Partitioning the components of soil respiration: a research challenge. Plant and Soil 284, 1–5.

Barreira, L.M.F., Duporte, G., Parshintsev, J., Hartonen, K., Jussila, M., Aalto, J., Back, J., Kulmala, M., Riekkola, M.L., 2017. Emissions of biogenic volatile organic compounds from the boreal forest floor and understory: a study by solid-phase microextraction and portable gas chromatography-mass spectrometry. Boreal Environ. Res. 22, 393–413.

Bastida, F., Eldridge, D.J., García, C., Kenny Png, G., Bardgett, R.D., Delgado-Baquerizo, M., 2021. Soil microbial diversity-biomass relationships are driven by soil carbon content across global biomes. ISME J. 15, 2081–2091.

Bennett, J.A., Klironomos, J., 2019. Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. New Phytol. 222, 91–96.

Bilas, R.D., Bretman, A., Bennett, T., 2021. Friends, neighbors and enemies: an overview of the communal and social biology of plants. Plant Cell Environ. 44, 997–1013.

Bourtsoukidis, E., Behrendt, T., Yañez-Serrano, A.M., Hellén, H., Diamantopoulos, E., Catão, E., Ashworth, K., Pozzer, A., Quesada, C.A., Martins, D.L., Sá, M., Araujo, A., Brito, J., Artaxo, P., Kesselmeier, J., Lelieveld, J., Williams, J., 2018. Strong sesquiterpene emissions from Amazonian soils. Nat. Commun. 9, 2226.

Carey, J. C., J. Tang, P. H. Templer, K. D. Kroeger, T. W. Crowther, A. J. Burton, J. S. Dukes, B. Emmett, S. D. Frey, M. A. Heskel, L. Jiang, M. B. Machmuller, J. Mohan,

A. M. Panetta, P. B. Reich, S. Reinsch, X. Wang, S. D. Allison, C. Bamminger, S. Bridgham, S. L. Collins, G. De Dato, W. C. Eddy, B. J. Enquist, M. Estiarte, J. Harte, A. Henderson, B. R. Johnson, K. S. Larsen, Y. Luo, S. Marhan, J. M. Melillo, J. Peñuelas, L. Pfeifer-Meister, C. Poll, E. Rastetter, A. B. Reinmann, L. L. Reynolds, I. K. Schmidt, G. R. Shaver, A. L. Strong, V. Suseela, and A. Tietema. 2016. Temperature response of soil respiration largely unaltered with experimental warming. PNAS 113, 13797–13802.

Carrión, O., Gibson, L., Elias, D.M.O., McNamara, N.P., van Alen, T.A., Op den Camp, H.J.M., Supramaniam, C.V., McGenity, T.J., Murrell, J.C., 2020. Diversity of isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation. Microbiome A 8, 81.

Carvalhais, N., Forkel, M., Khomik, M., Bellarby, J., Jung, M., Migliavacca, M., Mu, M., Saatchi, S., Santoro, M., Thurner, M., Weber, U., Ahrens, B., Beer, C., Cescatti, A., Randerson, J.T., Reichstein, M., 2014. Global covariation of carbon turnover times with climate in terrestrial ecosystems. Nature. 514(7521), 213–217.

Castro, H.F., Classen, A.T., Austin, E., Norby, R.J., Schadt, C.W., 2010. Soil Microbial community responses to multiple experimental climate change drivers. Appl. Environ. Microbiol. 76(4), 999–1007.

Conant, R.T., Klopatek, J.M., Klopatek, C.C. 2000. Environmental Factors Controlling Soil Respiration in Three Semiarid Ecosystems. Soil Science Society of America Journal 64, 383–390.

De Long, J.R., Fry, E.L., Veen, G.F., Kardol, P., Bennett, A., 2018. Why are plant-soil feedbacks so unpredictable, and what to do about it? Funct. Ecol. 33, 118–128.

De Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin,

S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K.E., Oliver, A., Ostle, N., Prosser, J.I., Thion, C., Thomson, B., Bardgett, R.D., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nat. Commun. 9, 3033.

Duan, C., Liao, H., Wang, K., Ren, Y., 2023. The research hotspots and trends of volatile organic compound emissions from anthropogenic and natural sources: A systematic quantitative review. Environ. Res. 216, 114386.

Emmett, B.A., Beier, C., Estiarte, M., Tietema, A., Kristensen, H.L., Peñuelas, J., Schmidt, I.K., Sowerby, A., 2004. The Response of Soil Processes to Climate Change: Results From Manipulation Studies of Shrublands Across an Environmental Gradient. Ecosystems 7, 625–637.

Faubert, P., Tiiva, P., Rinnan, Å., Michelsen, A., Holopainen, J.K., Rinnan, R., 2010. Doubled volatile organic compound emissions from subarctic tundra under simulated climate warming. New Phytol. 187, 199–208.

Gray, C.M., Fierer, N., 2012. Impacts of nitrogen fertilization on volatile organic compound emissions from decomposing plant litter. Glob. Chang. Biol. 18(2), 739–748.

Gray, C.M., Helmig, D., Fierer, N., 2015. Bacteria and fungi associated with isoprene consumption in soil. Elem. Sci. Anthr. 3, 000053.

Guenther, A.B., Jiang, X., Heald, C.L., Sakulyanontvittaya, T., Duhl, T., Emmons, L.K., Wang, X., 2012. The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. Geosci. Model Dev. 5, 1471–1492.

Hellén, H., Praplan, A.P., Tykkä, T., Ylivinkka, I., Vakkari, V., Bäck, J., Petäjä, T., Kulmala, M., Hakola, H., 2018. Long-term measurements of volatile organic

compounds highlight the importance of sesquiterpenes for the atmospheric chemistry of a boreal forest. Atmospheric Chem. Phys. 18, 13839–13863.

Hohener, P., Duwig, C., Pasteris, G., Kaufmann, K., Dakhel, N., Harms, H., 2003. Biodegradation of petroleum hydrocarbon vapors: laboratory studies on rates and kinetics in unsaturated alluvial sand. J. Contam. Hydrol. 66, 93–115.

Huang, X., Zheng, L., Guo, P., Yi, Z., 2021. Nitrogen addition inhibits total monoterpene emissions in subtropical forest floor of South China. Soil Ecol. Lett. 3, 63–72.

Hursh, A., Ballantyne, A., Cooper, L., Maneta, M., Kimball, J., Watts, J., 2017. The sensitivity of soil respiration to soil temperature, moisture, and carbon supply at the global scale. Glob. Chang. Biol. 23, 2090–2103.

Insam, H., Seewald, M.S.A., 2010. Volatile organic compounds (VOCs) in soils. Biol. Fertil. Soils 46, 199–213.

IPCC, 2022. IPCC (2022). AR6 Climate Change 2022: Mitigation of Climate Change–IPCC.

Isidorov, V.A., Pirożnikow, E., Spirina, V.L., Vasyanin, A.N., Kulakova, S.A., Abdulmanova, I.F., Zaitsev, A.A., 2022. Emission of volatile organic compounds by plants on the floor of boreal and mid-latitude forests. J. Atmospheric Chem. 79, 153–166.

Isidorov, V.A., Zaitsev, A.A., 2022. Reviews and syntheses: VOC emissions from soil cover in boreal and temperate natural ecosystems of the Northern Hemisphere. Biogeosciences 19, 4715–4746.

Janson, R.W., 1993. Monoterpene emissions from Scots pine and Norwegian spruce. J. Geophys. Res: Atmo. 98, 2839–2850.

Jo, I., Fei, S., Oswalt, C.M., Domke, G.M., Phillips, R.P., 2019. Shifts in Dominant

Tree Mycorrhizal Associations in Response to Anthropogenic Impacts. Sci. Adv. 5(4): eaav6358.

Kardol, P., Cregger, M.A., Campany, C.E., Classen, A.T., 2010. Soil Ecosystem Functioning Under Climate Change: Plant Species and Community Effects. Ecology 91(3):767–781

Kramshøj, M., Vedel-Petersen, I., Schollert, M., Rinnan, Å., Nymand, J., Ro-Poulsen, H., Rinnan, R., 2016. Large increases in Arctic biogenic volatile emissions are a direct effect of warming. Nat. Geosci. 9, 349–352.

Kulmala, M., Suni, T., Lehtinen, K.E.J., Dal Maso, M., Boy, M., Reissell, A., Rannik, U., Aalto, P., Keronen, P., Hakola, H., Back, J.B., Hoffmann, T., Vesala, T., Hari, P., 2004. A new feedback mechanism linking forests, aerosols, and climate. Atmospheric Chem. Phys. 4, 557–562.

Lemfack, M.C., Gohlke, B.O., Toguem, S.M.T., Preissner, S., Piechulla, B., Preissner, R., 2017. mVOC 2.0: a database of microbial volatiles. Nucleic Acids Res. 46, 1261–1265.

Lidón, A., Lull, C., Bussi, G., Bussi, G., Molina, A., 2021. Thinning Decreased Soil Respiration Differently in Two Dryland Mediterranean Forests With Contrasted Soil Temperature and Humidity Regimes. Eur. J. For. Res. 140, 1469–1485.

Lin, C., Owen, S., Penuelas, J., 2007. Volatile organic compounds in the roots and rhizosphere of Pinus spp. Soil Biol. Biochem. 39, 951–960.

Llusià, J., Asensio, D., Sardans, J., Filella, I., Peguero, G., Grau, O., Ogaya, R., Gargallo-Garriga, A., Verryckt, L.T., Van Langenhove, L., Brechet, L.M., Courtois, E., Stahl, C., Janssens, I.A., Peñuelas, J., 2022. Contrasting nitrogen and phosphorus fertilization effects on soil terpene exchanges in a tropical forest. Sci. Total Environ. 802, 149769.

Lun, X., Lin, Y., Chai, F., Fan, C., Li, H., Liu, J., 2020. Reviews of emission of biogenic volatile organic compounds (BVOCs) in Asia. J. Environ. Sci. 95, 266–277.

Ma, Z., Liu, H., Mi, Z., Zhang, Z., Wang, Y., Xu, W., Jiang, L., He, J.S., 2017. Climate Warming Reduces the Temporal Stability of Plant Community Biomass Production. Nat. Commun. 8:15378.

Mäki, M., Aaltonen, H., Heinonsalo, J., Hellén, H., Pumpanen, J., Bäck, J., 2019. Boreal forest soil is a significant and diverse source of volatile organic compounds. Plant Soil 441, 89–110.

Mechergui, K., Altamimi, A.S., Jaouadi, W., Naghmouchi, S., 2021. Climate Change Impacts on Spatial Distribution, Tree-Ring Growth, and Water Use of Stone Pine (*Pinus Pinea* L.) Forests in the Mediterranean Region and Silvicultural Practices to Limit Those Impacts. Iforest 14: 104–112.

Meyer, N., Welp, G., Amelung, W., 2018. The Temperature Sensitivity ($Q10$) of Soil Respiration: Controlling Factors and Spatial Prediction at Regional Scale Based on Environmental Soil Classes. Global Biogeochemical Cycles 32, 306–323.

Mielnik, A., Link, M., Mattila, J., Fulgham, S.R., Farmer, D.K., 2018. Emission of formic and acetic acids from two Colorado soils. Env. Sci. Process Impacts 20, 1537–1545.

Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., Schmidt, S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. Nature 439, 711–714.

Morán-Ordóñez, A., Duane, A., Gil-Tena, A., Cáceres, M.D., Aquilué, N., Geijzendorffer, I.R., Fortin, M.J., Brotons, L., 2020. Future Impact of Climate Extremes in the Mediterranean: Soil Erosion Projections When Fire and Extreme Rainfall Meet. Land Degrad. Dev. 31: 3040–3054.

Mu, Z., Asensio, D., Llusià, J., Filella, I., Ogaya, R., Yi, Z., Peñuelas, J., 2022. Annual and seasonal variations in soil volatile organic compound concentrations in a Mediterranean shrubland and holm oak forest. Geoderma 405, 115401.

Mu, Z., Llusià, J., Liu, D., Ogaya, R., Asensio, D., Zhang, C., Peñuelas, J., 2018. Seasonal and diurnal variations of plant isoprenoid emissions from two dominant species in Mediterranean shrubland and forest submitted to experimental drought. Atmos. Environ. 191, 105–115.

Ogaya, R., Peñuelas, J., 2007. Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. Plant Ecol. 189, 291–299.

Paasonen, P., Asmi, A., Petaja, T., Kajos, M.K., Aijala, M., Junninen, H., Holst, T., Abbatt, J.P.D., Arneth, A., Birmili, W., van der Gon, H.D., Hamed, A., Hoffer, A., Laakso, L., Laaksonen, A., Leaitch, W.R., Plass-Dulmer, C., Pryor, S.C., Raisanen, P., Swietlicki, E., Wiedensohler, A., Worsnop, D.R., Kerminen, V.M., Kulmala, M., 2013. Warming-induced increase in aerosol number concentration likely to moderate climate change. Nat. Geosci. 6, 438–442.

Peguero, G., Folch, E., Liu, L., Ogaya, R., Peñuelas, J., 2021. Divergent effects of drought and nitrogen deposition on microbial and arthropod soil communities in a Mediterranean forest. Eur. J. Soil Biol. 103. 103275.

Peñuelas, J., Asensio, D., Tholl, D., Wenke, K., Rosenkranz, M., Piechulla, B., Schnitzler, J.P., 2014. Biogenic volatile emissions from the soil. Plant Cell Environ. 37, 1866–1891.

Peñuelas, J., Llusià, J., 2003. BVOCs: plant defense against climate warming? Trends Plant Sci. 8, 105–109.

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., Carnicer, J.,

Bartrons, M., Rivas-Ubach, A., Grau, O., Peguero, G., Margalef, O., Pla-Rabés, S., Stefanescu, C., Asensio, D., Preece, C., Liu, L., Verger, A., Rico, L., Barbeta, A., Achotegui-Castells, A., Gargallo-Garriga, A., Sperlich, D., Farré-Armengol, G., Fernández-Martínez, M., Liu, D., Zhang, C., Urbina, I., Camino, M., Vives, M., Nadal-Sala, D., Sabaté, S., Gracia, C., Terradas, J., 2018. Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia. Environ. Exp. Bot. 152, 49–59.

Peñuelas, J., Staudt, M., 2010. BVOCs and global change. Trends Plant Sci. 15, 133–44.

Preece, C., Farre-Armengol, G., Peñuelas, J., 2020. Drought is a stronger driver of soil respiration and microbial communities than nitrogen or phosphorus addition in two Mediterranean tree species. Sci. Total Environ. 735, 139554.

Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol. Biochem. 131, 28–39.

Raich, J.W., Potter, C.S., 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochemical Cycles 9, 23–36.

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44, 81–99.

Ramirez, K.S., Lauber, C.L., Fierer, N., 2010. Microbial consumption and production of volatile organic compounds at the soil-litter interface. Biogeochemistry 99, 97–107.

Romero-Olivares, A.L., Davie-Martin, C.L., Kramshøj, M., Rinnan, R., Frey, S.D., 2022. Soil volatile organic compound emissions in response to soil warming and

nitrogen deposition. Elem. Sci. Anthr. 10, 00065.

Ruiz-Benito, P., Gómez-Aparicio, L., Paquette, A., Messier, C., Kattge, J., de Zavala, M., 2013. Diversity Increases Carbon Storage and Tree Productivity in Spanish Forests. Glob. Ecol. Biogeogr. 23, 311–322.

Samaniego, L., Thober, S., Kumar, R., Wanders, N., Rakovec, O., Pan, M.X., Zink, M.C., Sheffield, J., Wood, E.F., Marx, A., 2018. Anthropogenic Warming Exacerbates European Soil Moisture Droughts. Nat. Clim. Change 8, 421–426.

Schenkel, D., Lemfack, M.C., Piechulla, B., Splivallo, R., 2015. A meta-analysis approach for assessing the diversity and specificity of belowground root and microbial volatiles. Front. Plant Sci. 6, 707.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G., Wild, J., Mencuccini, M., Petr, M., Honkaniemi, J., Lexer, M.J., Trotsiuk, V., Mairota, P., Svoboda, M., Sedmák, R., Nagel, T., Reyer, C.P.O., 2017. Forest Disturbances Under Climate Change. Nat. Clim. Change 7, 395–402.

Sindelarova, K., Markova, J., Simpson, D., Huszar, P., Karlicky, J., Darras, S., Granier, C., 2022. High-resolution biogenic global emission inventory for the time period 2000–2019 for air quality modelling. Earth Syst. Sci. Data 14, 251–270.

Stotzky, G., Schenck, S., Papavizas, G.C., 1976. Volatile Organic Compounds and Microorganisms. CRC Crit. Rev. Microbiol. 4, 333–382.

Strahan, R.T., Meador, A.J.S., Huffman, D., Laughlin, D.C., 2016. Shifts in Community-Level Traits and Functional Diversity in a Mixed Conifer Forest: A Legacy of Land-Use Change. J. Appl. Ecol. 53, 1755–1765.

Tang, J., Schurgers, G., Rinnan, R., 2019. Process Understanding of Soil BVOC Fluxes

in Natural Ecosystems: A Review. Rev. Geophys. 57, 966–986.

Tibbett, M., Fraser, T.D., Duddigan, S., 2020. Identifying Potential Threats to Soil Biodiversity. Peerj 8, e9271.

Tsuruta, J., Okumura, M., Makita, N., Kosugi, Y., Miyama, T., Kume, T., Tohno, S., 2018. A comparison of the biogenic volatile organic compound emissions from the fine roots of 15 tree species in Japan and Taiwan. J. For. Res. 23, 242–251.

Vallicrosa, H., Sardans, J., Ogaya, R., Fernández, P.R., Peñuelas, J., 2021. Short-Term N-Fertilization Differently Affects the Leaf and Leaf Litter Chemistry of the Dominant Species in a Mediterranean Forest under Drought Conditions. Forests 12, 605.

Viros, J., Fernandez, C., Wortham, H., Gavinet, J., Lecareux, C., Ormeño, E., 2020. Litter of Mediterranean species as a source of volatile organic compounds. Atmos. Environ., 242, 117815.

Viros, J., Santonja, M., Temime-Roussel, B., Wortham, H., Fernandez, C., Ormeño, E., 2021. Volatilome of Aleppo Pine litter over decomposition process. Ecol. Evol. 6862–6880.

Williams, A., Vries, F.T., 2020. Plant root exudation under drought: implications for ecosystem functioning. New Phytol. 225, 1899–1905.

Wu, Linwei, Zhang, Y., Guo, X., Ning, D., Zhou, X., Feng, J., Yuan, M., Liu, S., Guo, J., Gao, Z., Ma, J., Kuang, J., Jian, S., Han, S., Yang, Z., Ouyang, Y., Fu, Y., Xiao, N., Liu, X., Wu, Liyou, Zhou, A., Yang, Y., Tiedje, J.M., Zhou, J., 2022. Reduction of Microbial Diversity in Grassland Soil Is Driven by Long-Term Climate Warming. Nat. Microbiol. 7(7), 1054–1062.

Xu, M., Shang, H., 2016. Contribution of soil respiration to the global carbon equation. Journal of Plant Physiology 203, 16–28.

Yáñez-Serrano, A.M., Bach, A., Bartolomé-Català, D., Matthaios, V., Seco, R., Llusia, J., Filella, I., Peñuelas, J., 2021. Dynamics of volatile organic compounds in a western Mediterranean oak forest. Atmos. Environ. 257.,118447.

Yáñez-Serrano, A.M., Bourtsoukidis, E., Alves, E.G., Bauwens, M., Stavrakou, T., Llusià, J., Filella, I., Guenther, A., Williams, J., Artaxo, P., Sindelarova, K., Doubalova, J., Kesselmeier, J., Peñuelas, J., 2020. Amazonian biogenic volatile organic compounds under global change. Glob. Chang. Biol. 26, 4722–4751.

Yang, K., Qin, J., Zhao, L., Chen, Y., Tang, W., Han, M., Lazhu, Chen, Z., Lv, N., Ding, B., Wu, H., Lin, C., 2013. A Multiscale Soil Moisture and Freeze–Thaw Monitoring Network on the Third Pole. Bull. Amer. Meteoro. Soc. 94, 1907–1916.

Yin, H., Chen, Z., Liu, Q., 2012. Effects of Experimental Warming on Soil N Transformations of Two Coniferous Species, Eastern Tibetan Plateau, China. Soil Biol. Biochem. 50, 77–84.

Yuan, J., Zhao, M., Li, R., Huang, Q., Raza, W., Rensing, C., Shen, Q., 2017. Microbial volatile compounds alter the soil microbial community. Env. Sci. Pollut. Res. Int. 24, 22485–22493.

Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486.

Zhang, L., Ren, Y., Yang, K., Li, Z., Tan, B., Liu, Y., Li, H., You, C., Liu, S., Wang, L., Yin, R., Zhang, J., Xu, Z., 2021. Immediate and legacy effects of snow exclusion on soil fungal diversity and community composition. For. Ecosyst. 8, 22.

Zhang-Turpeinen, H., Kivimäenpää, M., Berninger, F., Köster, K., Zhao, P., Peng Zhao, Peng Zhao, Zhou, X., Zhou, X., Pumpanen, J., 2021. Age-related response of forest floor biogenic volatile organic compound fluxes to boreal forest succession after

wildfires. Agric. For. Meteorol. 308, 108584.

Zhou, L., Zhou, X., Shao, J., Nie, Y., He, Y., Jiang, L., Wu, Z., Hosseini, B.S., 2016. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. Glob. Chang. Biol. 22, 3157–3169.

CHAPTER 1. Divergent effects of snow exclusion on microbial variables across aggregate size classes

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Abstract

Projected changes in winter climate can have large implications for the functioning of terrestrial ecosystems. In particular, increased soil frost associated with reduced insulating snow cover can affect the structure and activity of soil microbial communities in cold ecosystems, but little is known about the variability of these effects among the fractions of soil aggregates. We used a snow-exclusion experiment to examine the influence of increased soil frost on microbial biomass and activity in aggregate fractions in a Tibetan alpine spruce forest. We measured the concentrations of phospholipid fatty acids (PLFAs) and the activities of extracellular enzymes involved in carbon and nutrient cycling in large macroaggregates (> 2 mm), small macroaggregates (0.25-2 mm) and microaggregates (< 0.25 mm) during early thawing in 2016 and 2017. Increased frost severity due to snow exclusion tended to reduce the concentrations of total, bacterial and fungal PLFAs and the activities of β-glucosidase, β-N-acetyl-glucosaminidase and acid phosphatase, especially in the small macroaggregates, but did not affect the microbial community or enzymatic stoichiometry. Most of the microbial variables, however, varied across the aggregate fractions, and aggregate size generally had much stronger effects than did snow removal. Our results indicated that the microbial communities and enzymatic activities were more sensitive to a change in snow cover in the small macroaggregates than the other two fractions. These findings highlight the ecological importance of microbial processes in aggregates in Tibetan forests experiencing large decreases in snowfall.

Keywords: winter climate change; snow exclusion; soil enzyme; soil frost; microbial communities; soil aggregate.

1.1. Introduction

The Tibetan Plateau has experienced dramatic climate change in recent decades, particularly in winter (Chen et al., 2013). Winter snowfall has been decreasing in this region, and this trend is accelerating under strong winter warming (Wang et al., 2016). Seasonal snow cover is one of the most important determinants that regulate the physical and biochemical properties of soil in cold biomes (Aanderud et al., 2013, Groffman et al., 2001, Yu et al., 2016). The biological forces that modulate soil aggregates are vulnerable to physical disruptions associated with winter soil environments (Tian et al., 2021). Therefore, the lack of an insulating snow cover due to climate change may consequently alter winter soil conditions (e.g., temperature, frost and freeze–thaw cycles), which could in turn affect the structure and biological activity of aggregates in snowy ecosystems, such as Tibetan forests.

Soil aggregates play an essential role in the fluxes of water, carbon (C) and nutrients to microbial communities (Wilpiszeski, et al., 2019). Aggregates form by the combined action of cohesion and fragmentation and are microhabitats for microorganisms; they directly affect microbes that live in aggregates and in return are mediated by microbes (Jiang et al., 2013; Kristiansen et al., 2006). Current laboratory studies have demonstrated that the freeze-thaw cycle inconsistently affects biological activities in aggregates, with positive, negative and neutral impacts having been reported, depending on the frequency and strength of the freezing and thawing (Oztas et al., 2003; Tian et al., 2021; Walker et al., 2006). Freezing can disrupt the balance of microbes in aggregates by directly killing microorganisms and indirectly destroying the structure of the aggregates (Oztas et al., 2003). An *in situ* manipulation experiment found that increased winter soil freezing due to snow removal altered the distribution

of soil aggregates and aggregate-associated nutrient cycling in a northern hardwood forest (Steinweg et al., 2008). Others have also found that removing the snow cover inhibited microbial biomass and activity in bulk soils (Aanderud et al., 2013; Tan et al., 2014). We do not know, however, if excluding snow cover would decrease microbial biomass and activity in soil aggregates.

Soil generally consists of sand, clay and silt particles physically bonded into aggregates. The cycling of C and nutrients in bulk soil is primarily controlled by microbial activity, and such processes may differ among aggregate size classes (Mendes et al., 1999; Wilpiszeski et al., 2019). Soil microbial biomass and enzymatic activities have been reported to be higher in micro- than macroaggregates (Allison and Jastrow., 2006; Feng et al., 2016), but opposite results have also been reported (Dorodnikov et al., 2009; Fansler et al., 2005). Drivers of global change, including temperature, CO_2 level and land-use change, can affect biological activities in aggregates (Dorodnikov et al., 2009; Fang et al., 2016), and these climate change may differentially affect biological activity in different sizes of aggregates had demonstrated in recent studies (Fang et al., 2016; Nie et al., 2014). We therefore tested if changes in snow cover would induce divergent effects on microbial biomass and activity across aggregate size classes.

The subalpine forest ecosystems on the eastern Tibetan Plateau are susceptible to climate change, with important consequences for the global C balance (Li et al., 2017; Tan et al., 2014). Winter snowpack in this zone has unique properties, such as shorter duration and thinner cover than at high latitudes (Wang et al., 2017). Soil temperature in winter is near the physical melting point (Li et al., 2017), so a change in snow cover has great potential to alter winter soil temperature and further affect physical and biological processes in Tibetan forest soils. Our previous studies have reported that

snow exclusion inhibits soil respiration but increases nitrogen (N) availability in winter (Li et al., 2017; Yang et al., 2019). We also found that snow exclusion reduced biological activities in the bulk soil (Yang et al., 2019). As mentioned above, the lack of snow cover may potentially affect biological processes in aggregates, so exploring aggregate-based microbial biomass and activity may help us to understand the responses of C and nutrient cycling in this system. We conducted a snow-manipulation experiment in a Tibetan spruce forest to examine the effects of snow exclusion on the biomass and composition of microbial communities and on enzymatic activities in fractions of soil aggregates. We hypothesized that snow exclusion would inhibit microbial biomass and activity and that its effect would differ among aggregate size classes.

1.2. Materials and methods

1.2.1. Site description and experimental design

This study was conducted in a stand of dragon spruce (*Picea asperata* Mast.) at the Long-term Research Station of Alpine Forest Ecosystems of Sichuan Agricultural University (31°15′N, 102°53′E; 3021 m a.s.l.) in the Bipeng Valley of the Miyaluo Nature Reserve, Li County, southwestern China. The mean annual precipitation and temperature are 850 mm and 3.0 °C, respectively. Snow generally begins to accumulate in late November and melts in late March the following year. More details of the environmental conditions of the study site have been described elsewhere (Li et al., 2017). We excluded winter snowfall using shelters to intensify soil frost. Six wooden roofs (3 m × 3 m ground area) were installed in the forest in November 2015 to prevent the accumulation of snow on the ground. Each snow-exclusion plot was paired with

one control plot where snow cover was not manipulated.

Soil temperatures 5 cm below the surface were recorded in each plot. Snow depth was measured using a metal ruler about every two weeks. Soil-moisture content was measured using a Theta probe (Delta-T Devices, Cambridge, UK). Freeze-thaw cycles were counted as the number of times the temperature crossed the 0 °C isotherm and then returned to above-zero temperatures for > 3 h (Li et al., 2017). The seasonal dynamics of soil temperature and moisture content have been reported in our prior study (Yang et al., 2019). Winter snow depth varied among years (Table 1). Snow exclusion reduced winter soil temperature but did not affect moisture content. Snow exclusion, however, increased the number of freeze-thaw cycles and the duration of frozen soil.

Table 1. Climatic and soil properties of the study site during the winters of 2016 and 2017.

	2016		2017	
Variable	Control	Snow exclusion	Control	Snow exclusion
Maximum snow cover (cm)	40	-	23	-
Mean soil temperature at 5 cm (°C)	−0.3	−0.6	0	−0.2
Minimum daily soil temperature (°C)	−2.2	−0.5	−2.4	−1.3
Mean soil-moisture content at 5 cm (%)	19	21	18	22
Number of freeze-thaw cycles	13	25	17	35
Duration of frozen soil (d)	83	99	103	108

1.2.2. Soil sampling and aggregate size fractionation

Soil samples were collected in the early thawing periods (early April) of 2016 and 2017. Two cores were collected from each plot using an auger (15 cm deep and 10 cm in diameter) and were mixed to form one composite sample. Aggregates were isolated as

described by Kristiansen et al., (2006). Large macroaggregates (> 2 mm), small macroaggregates (0.25-2 mm) and microaggregates (< 0.25 mm) were separated using methods described by Yang et al. (2019). Our previous study found that the snow-exclusion treatment did not affect the distribution of the aggregate size classes in the thawing periods of 2016 and 2017 (Yang et al., 2019).

1.2.3. Assays of soil phospholipid fatty acids and enzymatic activities

Phospholipid fatty acids (PLFAs) were extracted and quantified using the methods described by White et al (1996). Further details of the methods have been described in our previous studies (He et al., 2017; Yang et al., 2019). Briefly, the PLFAs 15:0, i15:0, a15:0, 16:0, i16:0, 17:0, i17:0, a17:0, 16:1w7c, 16:1w5t, 16:1w9c, 18:1w7c, 18:0, cy17:0, cy19:0 and 20:5 were used as bacterial markers (Frostegård et al., 1993). The polyunsaturated PLFAs 18:3, 18:1w9c, 18:2w6, 9c and 20:1w9c were used as fungal markers (Tornberg et al., 2003). The PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 were used as markers of gram-positive (G^+) bacteria, and the PLFAs 16:1w7c, 16:1w9c, 18:1w7c, cy17:0 and cy19:0 were used as markers of gram-negative (G^-) bacteria (Zelles L., 1999). The total PLFAs were the sum of all identified GC peaks.

We assessed the activities of four enzymes involved in the cycling of soil C, N and phosphorus (P), β-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (AP), using the methods described by Allison and Jastrow (2006). Substrate solutions were 5 mM pNP-β-glucopyranoside for BG, 2 mM pNP-β-N-acetylglucosaminide for NAG, 5 mM leucine p-nitroanilide for LAP and 5 mM pNP-phosphate for AP. Soil enzymatic stoichiometries were calculated as (Sinsabaugh et al., 2008): C:N = Ln(BG)/Ln(LAP + NAG), C:P = Ln(BG)/Ln(AP)

and N:P = Ln(LAP + NAG)/Ln(AP).

1.2.4. Data analysis

Repeated-measures ANOVAs were conducted to test the effects of snow exclusion, aggregate size and sampling date and their interactions on enzymatic activities and variables of the soil microbial communities. A Bonferroni post hoc test was used to examine the effect of the snow-exclusion treatment on the variables for each aggregate size class when the interaction between treatment and sampling date was significant ($P < 0.05$), and a paired *t*-test was used when the interaction was not significant. All data were tested for the assumptions of an ANOVA before analysis. All statistical tests were performed using SPSS version 17.0 (IBM SPSS Statistics Inc, Chicago, USA).

1.3. Results

1.3.1. Soil microbial communities

The snow-exclusion treatment significantly negatively affected total ($F = 9.62$, $P < 0.01$; Table 1, Fig. 1a) and bacterial ($F = 8.974$, $P < 0.01$; Table 2, Fig. 1b) PLFA concentrations and marginally significantly affected fungal PLFA concentration ($F = 3.847$, $P < 0.1$; Table 1, Fig. 1c). Snow exclusion decreased total, bacterial and fungal PLFA concentrations in the small macroaggregates and microaggregates in 2016 (all $P < 0.05$; Table 2, Fig. 1a-c) and tended to decrease G^+ and G^- bacterial PLFA concentrations in the small macroaggregates in 2016, but not significantly (Table 2, Fig. 1c, f). Snow exclusion, however, did not affect any of the microbial variables in 2017 (all $P > 0.05$; Fig. 1a-f). The concentrations of the microbial biomarkers (total, bacterial and fungal PLFAs) were often higher in the second year, regardless of frost severity

(Table 2, Fig. 1). PLFA concentration in the aggregates was generally higher in the small macroaggregates, followed by microaggregates and large macroaggregates, regardless of snow amount and sampling date. Snow exclusion, aggregate size, sampling date and their interactions did not affect the bacteria:fungi (B:F) or G^+:G^- ratios (Table 2, Fig. 1d, g).

Table 2. *F* values from the repeated-measures ANOVA of the responses of total, bacterial, fungal, bacteria:fungi (B:F), G^+ bacterial (G^+), G^- bacterial (G^-) and G^+:G^- PLFA concentrations to snow exclusion (SE), aggregate size (AS) and sampling date (SD). n=6.

Factor	Total	Bacteria	Fungi	B:F	G^+	G^-	G^+:G^-
SE	9.620**	8.974**	3.847†	0.373	0.138	0.027	0.008
AS	29.061***	26.606***	26.606***	0.385	9.825***	5.022*	0.135
SD	137.809**	124.369**	92.313**	0.029	1.008	0.427	0.192
SE×AS	0.479	0.437	0.412	0.074	0.215	0.363	0.056
SE×SD	0.010	0.012	0.012	0.059	0.962	0.237	0.092
AS×SD	5.286*	4.798*	4.017*	0.459	0.327	0.451	0.143
SE×AS×SD	0.240	0.247	0.010	0.140	0.844	0.132	0.097

†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

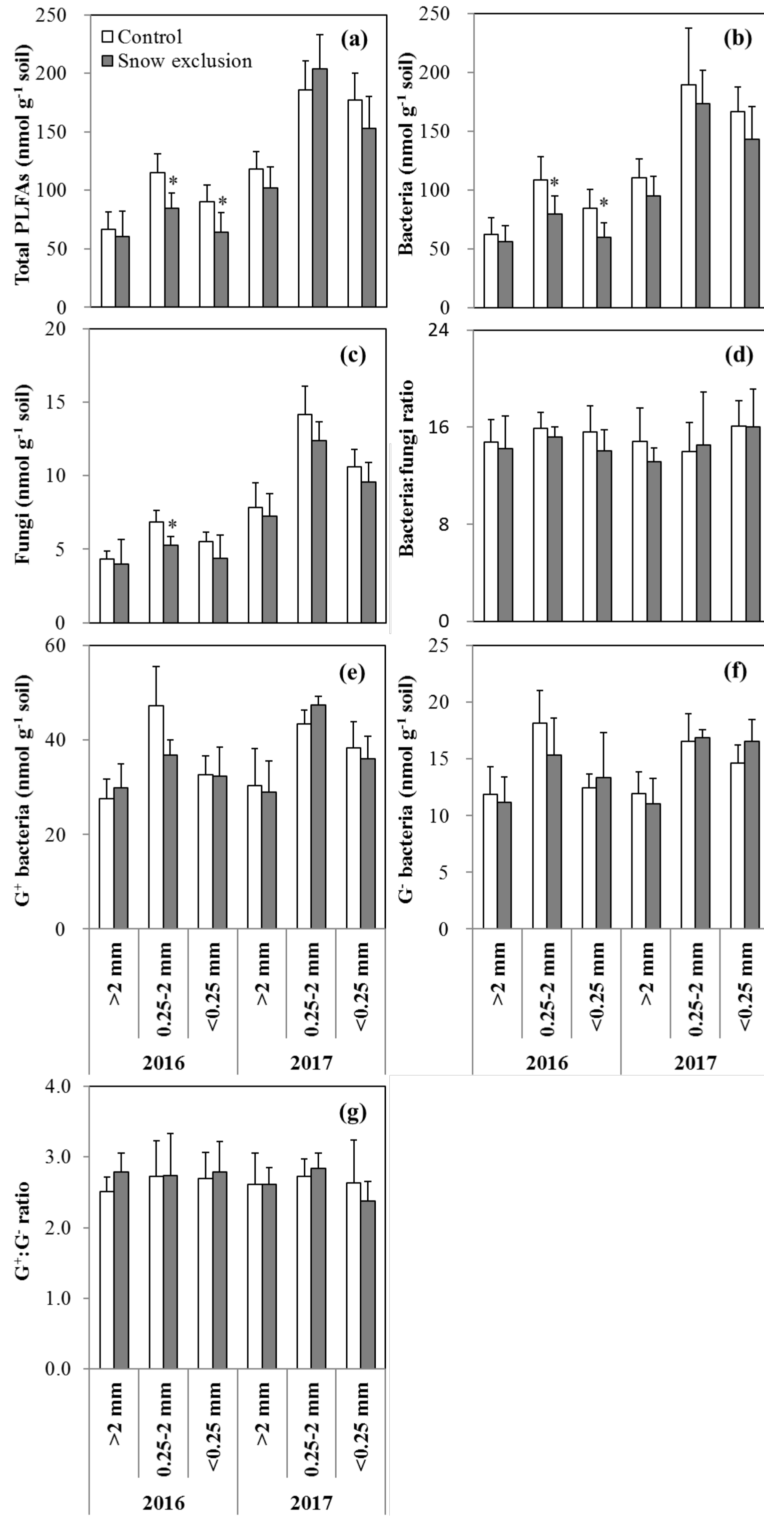

Figure 1. Concentrations of phospholipid fatty acids (PLFAs) and concentration ratios in the three aggregate fractions in the snow-exclusion treatment and control plots (mean ± SE, n=6). Significant differences between the control and treatment for the fractions are indicated by asterisks ($P < 0.05$).

1.3.2. Enzymatic activities and stoichiometries

The intensified frost marginally affected the activity of the BG C-degrading enzyme ($F = 3.125$, $P < 0.1$; Table 3, Fig. 2a) and significantly affected the activity of the AP P-degrading enzyme ($F = 5.389$, $P < 0.05$; Table 3, Fig. 2d). The activities of BG, NAG and AP in the small macroaggregates in 2016 were lower in the snow-exclusion treatment than the control plots but did not differ significantly between snow amounts for the other two size classes (all $P > 0.05$, Fig. 2a). Snow exclusion did not significantly affect the activities of the four enzymes in 2017 (Fig. 1a-d). The activities tended to be higher in 2016 than 2017, regardless of snow amount (Table 3, Fig. 2). The ANOVA found that the activities of the enzymes varied significantly among the size classes (all $P < 0.01$, Fig. 2a-d). The activities tended to be higher in the small macroaggregates than the large macroaggregates and microaggregates, regardless of snow amount and sampling date.

The snow-exclusion treatment did not significantly affect the total C:N, C:P and N:P enzymatic-activity ratios (all $P > 0.05$; Table 3, Fig. 2e, f). Aggregate size, however, significantly affected the total C:N and N:P activity ratios ($P < 0.05$, Table 3). The C:N activity ratio tended to increase as aggregate size decreased across the snow manipulations (Fig. 2e). The N:P activity ratio was larger for the small macroaggregates than the other two size classes, regardless of snow amount and sampling date (Fig. 2g). Snow exclusion, aggregate size, sampling date and their interactions did not affect the C:P activity ratio (Table 3, Fig. 2f).

Table 3. *F* values from the repeated-measures ANOVA of the responses of the activities of β-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (AP) and enzymatic stoichiometries (C:N, C:P and N:P) to snow exclusion (SE), aggregate size (AS) and sampling date (SD). n=6.

Factor	BG	NAG	LAP	AP	C:N	C:P	N:P
SE	3.125†	0.952	0.324	5.389*	0.271	1.012	0.087
AS	9.575**	6.776**	2.452	5.723**	4.459*	1.380	3.876*
SD	9.653	1.270	7.016*	5.735*	0.006	2.764	0.435
SE×AS	0.206	0.350	0.677	0.528	0.201	0.045	0.238
SE×SD	1.345	1.237	0.241	1.319	0.320	0.014	0.473
AS×SD	2.864†	1.100	1.128	1.314	0.236	3.501*	0.873
SE×AS×SD	0.216	0.465	0.427	0.054	0.489	0.081	0.746

†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$

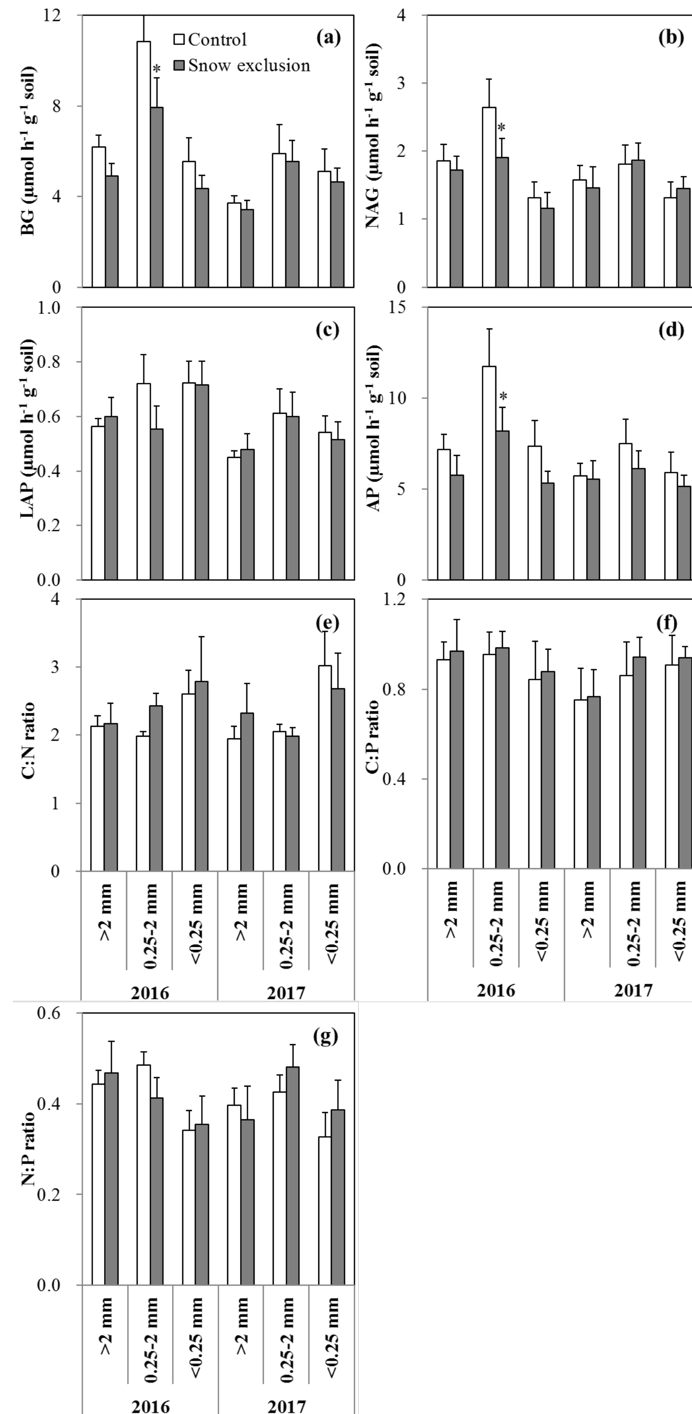

Figure 2. Activities of β-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (AP) and C:N:P enzymatic stoichiometry in the three aggregate fractions in the snow-exclusion treatment and control plots (mean ± SE, n=6). Significant differences between the control and treatment for the fractions are indicated by asterisks ($P < 0.05$).

1.4. Discussion

1.4.1. Effects of snow exclusion on the microbial variables in the aggregates

Soil microbes can adjust their composition and activity in response to environmental changes (Fang et al., 2016). Our previous study reported that snow exclusion reduced total PLFAs in the bulk soil early in the winters of 2016 and 2017 (Yang et al., 2019). Similar to the bulk soil, snow exclusion in our current study also decreased total, bacterial and fungal PLFA biomarkers, particularly in the small macroaggregates. The decrease in microbial biomarkers could thus be mainly attributed to a decline in the amount of small macroaggregates and/or microaggregates. This response has important implications for nutrient cycling in Tibetan forest soil, because small macroaggregates account for more than half of the weight of bulk soil (Yang et al., 2019). The response of microbial biomass in soil aggregates to *in situ* changes in snow cover has very rarely been studied, so we could not directly compare our results to those of other studies. Many microcosmic experiments, however, have suggested that freeze-thaw cycles and soil freezing could kill microbes by the rupture of cell membranes by ice crystals (Gavazov et al., 2017; Jusselme et al., 2016; Walker et al., 2006), Snow exclusion would then increase frost depth and the number of freeze-thaw cycles (Li et al., 2017; Table 1). Such changes in winter soil conditions associated with snow exclusion may partly account for the decline in microbial biomass.

The activities of soil enzymes, as important microbial indicators, are often assayed in studies of global change (Burns et al., 2013). Previous studies have demonstrated that snow removal lowered enzymatic activities in bulk soils in mixed-hardwood forests and subalpine forests (Tan et al., 2014; Sorensen et al., 2016). Similarly, the snow-exclusion treatment in our study significantly decreased the activities of BG, NAG and AP in the small macroaggregates. A decrease in soil temperature due to snow exclusion

may partly inhibit the activities of enzymes, because they are often temperature-dependent (Tabatabai.,1982). Microorganisms are also a major source of enzyme synthesis (Waring et al., 2014). Snow exclusion in our study also reduced the concentrations of the microbial biomarkers in the small macroaggregates. Such responses of microbial biomass could to some extent decrease the potential for a microbial community to secrete enzymes in small macroaggregates.

As mentioned above, soil microbial biomass and activity in the small macroaggregates may be more vulnerable to changes in snow cover than those in the other two aggregate fractions. Studies have also reported that the activities of soil enzymes respond disproportionally to drivers of global change across aggregate classes (Dorodnikov et al., 2009; Fang et al., 2016; Nie et al., 2014). Small macroaggregates at our site account for >50% of the weight of bulk soil (Yang et al. 2019). Soil microbes in small macroaggregates could thus theoretically be more easily affected by changes induced by snow exclusion under winter soil conditions than microbes in the other two aggregate fractions. The underlying mechanisms have not been clearly elucidated. Additional detailed studies integrating potential factors are needed to better understand biological processes in aggregates.

Microbial biomass varied between years, with total PLFAs higher in 2017 than 2016, irrespective of size class and snow exclusion. Our earlier data also identified a higher winter respiration rate in 2017 than 2016, also indicating microbial differences between the two years (Yang et al, 2019). This interannual variation was mainly attributed to the differences in winter temperature between the two years. The mean winter air temperatures were –2.1 and –0.9 °C in 2016 and 2017, respectively (Yang et al. 2019). Relatively mild winters may favor the growth of soil microbes. Snow exclusion also lowered microbial biomass and enzymatic activities in the early thawing

of 2016 but did not affect them in the early thawing of 2017. Maximum snow depths were 40 cm in 2016 but only 23 cm 2017. The changes in winter soil conditions induced by snow exclusion were larger in 2016 than 2017, which may account for our results to some extent. Our experiment was carried out in two contrasting winters (cold and thick snowpack in 2016 and mild and thin snowpack in 2017), so it offered a good opportunity for exploring the influence of a change in snow cover under different winter conditions on microbial biomass and activity in soil aggregates. Our findings also indirectly indicated that changes in snow cover have important implications for belowground biological processes in Tibetan forest soils.

1.4.2. Microbial variables across aggregate size classes

A recent review suggested that aggregate-based approaches could advance our understanding of how geochemical and community interactions govern nutrient cycling in soil (Wilpiszeski et al., 2019). Microbial biomass has been found to be lower in microaggregates than macroaggregates in agroecosystems (Guggenberger et al., 1999; Jiang et al., 2013), but studies in a semi-arid grassland and a subtropical forest observed that microbial biomass decreased as aggregate size increased (Fang et al., 2016; Wang et al., 2015). We used PLFA analysis to determine the biomass and structure of the microbial communities. The biomarker concentrations of the microbial groups tended to be higher in the small macroaggregates than the microaggregates and large macroaggregates. Such inconsistent results may be mainly attributed to the separation methods and substrates used. Dry- and wet-sieving are two main methods used, both with advantages and disadvantages (Blaud et al., 2017). For example, soil microorganisms and associated activities are sensitive to wetting, so dry-sieving is better than wet-sieving for measuring temporal microbial activity (Blaud et al., 2017).

One study focused on substrates from three soils with different vegetation using the same dry-sieving and found that the distribution of soil microorganisms in aggregates depended on soil type (Murugan et al., 2019). Most studies indicate that microbial communities generally vary greatly across aggregate classes, regardless of the methods used, suggesting that aggregates are key functional units in soils.

Aggregate size also significantly affected the activities of enzymes involved in the cycling of soil C, N and P. The activities of BG, NAG and AP were higher in the small macroaggregates than the microaggregates and/or large macroaggregates. Dorodnikov et al (2009) observed a similar pattern for chitinase and phosphatase in crop soil. Other studies, however, have reported that the activities of enzymes increased as aggregate size decreased (Grandy et al., 2008; Nie et al., 2014). The activities of some enzymes, though, may also not differ across aggregate fractions (Fang et al., 2016). Aggregates provide a spatially heterogeneous habitat for the production of enzymes due to differences in microorganisms among the size classes (Jiang et al., 2013). The pattern of activities among the aggregate sizes in our study may be partly attributed to the distribution of microbes across the aggregates. Nie et al (2014) found that BG activity increased as aggregate size decreased but that NAG and LAP activities did not vary among the aggregate fractions. Our study found that aggregate size affected BG, NAG and AP activities but not LAP activity. The synthesis of an enzyme can be mainly mediated by a specific microbial group or factor (e.g., pH or substrate availability), so the patterns of activities across aggregate fractions may depend on the enzyme and the soil texture.

1.4.3 Limitations and implications

A recent study reported that changes in snow cover may induce legacy effects on soil biological processes in the subsequent growing season (Zhao et al., 2017). Our study only focused on microbial biomass and activities of extracellular enzymes in soil aggregates in the early thawing periods of 2016 and 2017. We were thus unable to test whether legacy effects exist at aggregate scales. Divergent responses were detected in two contrasting winters with different snow conditions, suggesting that seasonal snow cover is a crucial indicator that mediates winter soil biological processes in this region on the eastern Tibetan Plateau. In addition to interannual differences in snowfall, extreme climatic events could be more likely under future climate change, implying complex and uncertain winter climate change in snowy Tibetan forests.

1.5. Conclusions

This study explored the potential effects of increased severity of soil frost on microbial biomass and enzymatic activities in soil aggregates in a subalpine spruce forest on the Tibetan Plateau of China. The increase in frost severity decreased total, bacterial and fungal PLFA biomarker concentrations and the activities of enzymes involved in C, N and P cycling, especially in the small macroaggregates, suggesting that the functions and activities of the small macroaggregates were more vulnerable to winter climate change. Microbial biomass and enzymatic activity also often differed significantly among the aggregate fractions, further indicating that aggregates are key functional units in soil. Aggregate size had a stronger effect than change in snow cover on the parameters measured. The lower microbial activities and the higher proportion of the weight of bulk soil in the small macroaggregates implied that the lack of snow may

decrease microbially driven processes in winter. These findings may further account for the lower winter soil respiration in snow-exclusion plots reported by Yang et al (2019). Our study advances our understanding of belowground responses to winter climate change in cold regions.

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References

Aanderud, Z.T., Jones, S.E., Schoolmaster, Jr.D.R., Fierer, N., Lennon, J.T., 2013. Sensitivity of soil respiration and microbial communities to altered snowfall. Soil. Biol. Biochem. 57, 217–227.

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil. Biol. Biochem. 38, 3245–3256.

Blaud, A., Menon, M., van der Zaan, B., Lair, G.J., Banwart, S.A., 2017. Effects of Dry and Wet Sieving of Soil on Identification and Interpretation of Microbial Community Composition. In: Advances in Agronomy (eds. Banwart, SA & Sparks,

DL). Academic Press, pp. 119–142.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N, Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil. Biol. Biochem. 58, 216–234.

Chen, H., Zhu, Q.A., Peng, C.H., Wu, N., Wang, Y.F., Fang, X.Q., Gao, Y.H., Zhu, D., Yang, G., Tian, J.Q., Kang, X., Piao, S.L., Ouyang, H., Xiang, W.H., Luo, Z.B., Jiang, H., Song, X.Z., Zhang, Y., Yu, G.R., Zhao, X.Q., Gong, P., Yao, T.D., Wu, J,H., 2013. The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. Glob. Chang. Biol. 19, 2940–2955.

Chen, X., Li, Z., Liu, M., Jiang, C., Che, Y., 2015. Microbial community and functional diversity associated with different aggregate fractions of a paddy soil fertilized with organic manure and/or NPK fertilizer for 20 years. J. Soils. Sediments. 15, 292–301.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Glob. Chang. Biol. 15, 1603–1614.

Fang, X., Zhou, G., Li, Y., Liu, S., Chu, G., Xu, Z., Liu, J., 2016. Warming effects on biomass and composition of microbial communities and enzyme activities within soil aggregates in subtropical forest. Biol. Fertil. Soils. 52, 353–365.

Fansler, S.J., Smith, J.L., Bolton, H., Bailey, V.L., 2005. Distribution of two C cycle enzymes in soil aggregates of a prairie chronosequence. Biol. Fertil. Soils. 42, 7–23.

Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L., 2001. Colder soils in a warmer world: a snow manipulation study in a northern

hardwood forest ecosystem. Biogeochemistry 56, 135–150.

Gavazov, K., Ingrisch, J., Hasibeder, R., Mills, R.T.E., Buttler, A., Gleixner, G., Pumpanen, J., Bahn, M., 2017. Winter ecology of a subalpine grassland: effects of snow removal on soil respiration, microbial structure and function. Sci. Total. Environ. 590, 316–324.

Grandy, A.S., Sinsabaugh, R.L., Neff, J.C., Stursova, M., Zak, D.R., 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91, 37–49.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliot, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil. Sci. Soc. Am. J. 63, 1188–1198.

He, R., Yang, K., Li, Z., Schädler, M., Yang, W., Wu, F., Tan, B., Zhang, L., Xu, Z., 2017. Effects of forest conversion onsoil microbial communities depend on soil layer onthe eastern Tibetan Plateau of China. PLoS ONE 12(10): e0186053.

Jiang, Y., Sun, B., Jin, C., Wang, F., 2013. Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil. Soil. Biol. Biochem. 60, 1–9.

Jusselme, M.D., Saccone, P., Zinger, L., Faure, M., Le Roux, X., Guillaumaud, N., Bernard, L., Clement, J.C., Poly, F., 2016. Variations in snow depth modify N-related soil microbial abundances and functioning during winter in subalpine grassland. Soil. Biol. Biochem. 92, 27–37.

Kreyling, J.: The ecological importance of winter in temperate, boreal, and arctic ecosystems in times of climate change, in: Progress in Botany Vol. 81, edited by: Cánovas, F. M., Lüttge, U., Leuschner, C., Risueño, M.C., Springer International Publishing, Cham, 377–399, 2020.

Kristiansen, S.M., Schjnning, P., Thomsen, I.K., Olesen, J.E., Kristensen, K., Christensen, B.T., 2006. Similarity of differently sized macro-aggregates in arable soils of different texture. Geoderma 137, 147–154.

Li, Z.J., Yang, W.Q., Yue, K., Justine, M.F., He, R.Y., Yang, K.J., Zhuang, L.Y., Wu, F.Z., Tan, B., Zhang, L., Xu, Z.F., 2017. Effects of snow absence on winter soil nitrogen dynamics in a subalpine spruce forest of southwestern China. Geoderma 307, 107–113.

Mendes, I.C., Bandick, A.K., Dick, R.P., Bottomley, P.J., 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops. Soil. Sci. Soc. Am. J. 63, 873–881.

Murugan, R., Djukic, I., Keiblinger, K., Zehetner, F., Bierbaumer, M., Zechmeister-Bolternstern, S., Joergernsen R.T., 2019. Spatial distribution of microbial biomass and residues across soil aggregate fractions at different elevations in the Central Austrian Alps. Geoderma. 339, 1–8.

Nie, M., Pendall, E., Bell, C., Wallenstein, M.D., 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil. Biol. Biochem., 68, 357–365.

Oztas, T., Fayetorbay, F., 2003. Effect of freezing and thawing processes on soil aggregate stability. Catena, 52, 1–8.

Ranjard, L., Poly, F., Combrisson, J., Richaume, A., Gourbiere, F., Thioulouse, J., Nazaret, S., 2000. Heterogeneous cell density and genetic structure of bacterial pools associated with various soil microenvironments as determined by enumeration and DNA fingerprinting approach (RISA). Microbial. Eco. 39, 263–272.

Repo, T., Sirki, S., Heinonen, J., Lavigne, A., Roitto, M., Koljonen, E., Koljonen, E., Sutinen, S., Finer, L., 2014. Effects of frozen soil on growth and longevity of fine

roots of Norway spruce. Forest. Ecol. Manage. 313, 112–122.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. Soil. Biol. Biochem. 43, 1417–1425.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.

Sorensen, P.O., Templer, P.H., Finzi, A.C., 2016. Contrasting effects of winter snowpack and soil frost on growing season microbial biomass and enzyme activity in two mixed-hardwood forests. Biogeochemistry 128, 141–154.

Stark, S., Mannisto, M.K., Eskelinen, A., 2014. Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. Plant. Soil. 383, 373–385.

Steinweg, J.M., Fisk, M.C., McAlexander, B., Groffman, P.M., Hardy, J.P., 2008. Experimental snowpack reduction alters organic matter and net N mineralization potential of soil macroaggregates in a northern hardwood forest. Biol. Fertil. Soils. 45, 1–10.

Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, second ed. American Society of Agronomy—Soil Science Society of America, Madison, W, 903–947.

Tan, B., Wu, F.Z., Yang, W.Q., He, X.H., 2014. Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest. Appl. Soil. Ecol. 76, 34–

41.

Tian, J., Zong, N., Hartley, I.P., He, N., Zhang, J., Powlson, D., Zhou, J., Kuzyakov, Y., Zhang, F., Yu, G., Dungait, J.A.J., 2021. Microbial metabolic response to winter warming stabilizes soil carbon. Glob Change Biol.

Tornberg, K., Bååth, E., Olsson, S., 2003. Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. Biol Fertil Soils. 37, 190–197.

Walker, V. K., Palmer, G. R., Voordouw, G., 2006. Freeze-thaw tolerance and clues to the winter survival of a soil community. Appl. Envir. Microl. 72, 1784–1792.

Wang, J., Zhang, M.J., Wang, S.J., Ren, Z.G., Che, Y.J., Qiang, F., Qu, D.Y., 2016. Decrease in snowfall/rainfall ratio in the Tibetan Plateau from 1961 to 2013. J. Geogr. Sci. 26, 1277–1288.

Wang, R.Z., Dorodnikov, M., Yang, S., Zhang, Y.Y., Filley, T.R., Turco, R.F., Zhang, Y.G., Xu, Z.W., Li, H., Jiang, Y., 2015. Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. Soil. Biol. Biochem. 81, 159–167.

Waring, B., Weintraub, S.R., Sinsabaugh, R.L., 2014. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117, 101–113.

White, D.C., Stair, J.O., Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. J. Ind. Microbiol. 17, 185–196.

Wilpiszeski, R.L., Aufrecht, J.A., Retterer, S.T., Sullivan, M.B., Graham, D.E., Pierce, E.M., Zablocki, O.D., Palumbo, A.V., Elias, D.A., 2019. Soil aggregate microbial communities: towards understanding microbiome interactions at biologically relevant scales. Appl. Environ. Microbiol. 85, e0032419.

Yang, K.J., Peng, C.H., Peñuelas, J., Kardol, P., Li, Z.J., Zhang. L., Ni, X.Y., Yue, K., Tan, B., Yin, R., Xu, Z.F., 2019. Immediate and carry-over effects of increased soil frost on soil respiration and microbial activity in a spruce forest. Soil. Biol. Biochem. 135, 51–59.

Yu, Z., Wang, J., Liu, S., Piao, S., Ciais, P., Running, S.W. Poulter, B., Rentch, J. S., Sun P. S., 2016. Decrease in winter respiration explains 25% of the annual northern forest carbon sink enhancement over the last 30 years. Glob. Chang. Biol. 25, 586–595.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fertil. Soils. 29, 111–129.

Zhao, J., Peichl, M., Nilsson, M. B., 2017. Long-term enhanced winter soil frost alters growing season CO_2 fluxes through its impact on vegetation development in a boreal peatland. Glob. Chang. Biol. 23, 3139–3153.

CHAPTER 2. Immediate and carry-over effects of increased soil frost on soil respiration and microbial activity in a spruce forest

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Abstract

Increased soil frost associated with winter climate change could have immediate and carry-over effects on biological processes in high-altitude forest soils, but the nature of these processes remain poorly understood. We conducted a snow-exclusion experiment to investigate the immediate and cross-seasonal effects of increased soil frost on soil CO_2 efflux and biological activity in a subalpine spruce forest on the eastern Tibetan Plateau, China. The increased frost reduced soil CO_2 efflux by ~15 and ~19% in the winters of 2015/2016 and 2016/2017, respectively. Increased frost also tended to decrease soil basal respiration, the amount of microbial phospholipid fatty acids and the activities of enzymes involved in soil carbon cycling during the winters. Winter soil nitrogen availabilities were higher in the snow-exclusion treatment than in the control plots. However, these effects did not carry over to the following growing season. Our results suggest that increased frost reduces winter soil respiration by direct environmental effects (e.g., soil temperature) and indirect biological processes (e.g., microbial biomass and activity), whereas increased frost did not induce any cross-seasonal effects. These findings underscore the ecological importance of seasonal snowpack and microbe-associated carbon processes in subalpine forests where winter snowfall is decreasing substantially.

2.1. Introduction

Seasonal snow cover is a major control of biogeochemical cycling in cold environments (Jusselme et al., 2016). Many snowy areas at high latitudes and altitudes have experienced substantial climate change in recent decades, and this trend is predicted to continue in this century (IPCC, 2013). Climate-induced changes are particularly rapid in high latitude and alpine ecosystems, where rising temperatures have profound effects on winter conditions, such as snowfall, soil frost and extreme climatic events (Liu et al., 2012; IPCC, 2013). Winter precipitation in these regions is more likely to occur in the form of rain rather than snow due to winter warming (Wang et al., 2016). The lack of insulating snow cover could consequently increase soil frost (Groffman et al., 2001a; Bokhorst et al., 2013), which could in turn have complex and large impacts on soil microbiological and biochemical processes in cold forests.

Winter biological processes and their controls are not as well understood as growing-season processes, despite the importance of winter warming and biological activity in cold systems (Sanders-DeMott and Templer, 2017). Recent studies have found that soil biological processes are sensitive to warming-induced changes in winter conditions, especially snow cover and soil frost (Haei and Laudon, 2015; Li et al., 2016). Snow removal has negative or neutral influences on winter soil respiration in boreal and temperate forests (Groffman et al., 2006; Aanderud et al., 2013; Bokhorst et al., 2013), but changes in snow cover can also alter biological processes in snow-free periods (Muhr et al., 2009; Wubs et al., 2018). Snow exclusion can suppress soil respiration in the snow-free season in high-latitude ecosystems (Öquist and Laudon, 2008; Zhao et al. 2017). To our knowledge, however, soil biological responses to changing soil frost have rarely been investigated in both snow-covered and snow-free periods in the same experiment. A better understanding of the impacts of intensified

soil frost on the biotic and abiotic controls over the dynamics of soil C in both snow-covered and snow-free seasons is thus essential for accurately modeling and predicting potential C feedbacks in a warmer world.

Altered soil frost may directly and indirectly affect soil C cycling, such as by affecting soil temperature and moisture (Aanderud et al., 2013; Song et al., 2017), soil microbial biomass and activity (Monson et al., 2006; Sorensen et al., 2016) and substrate quality and quantity (Brooks et al., 2004; Steinweg et al., 2008; Comerford et al., 2013). The direction and magnitude of biological responses to increased frost may be determined by the combined effect of these processes. Diverse techniques have provided insight in recent years into the impacts of winter climate change on soil C cycling as the importance of winter processes has increased (Li et al., 2016). Most field-manipulation studies have focused mainly on high-latitude systems, including peatlands and boreal forests (Sanders-DeMott and Templer, 2017). Soil biological responses from low-latitude cold systems with unique winter conditions, such as Tibetan subalpine forests, however, remain unknown.

The Tibetan Plateau, the Earth's 'Third Pole', has warmed substantially, especially in winter (Chen et al., 2013). Winter snowfall has decreased at a rate of 0.6 mm y^{-1} in recent decades (Wang et al., 2016; Xu et al., 2017). Seasonal snowpack in this region has unique characteristics, such as shorter duration and shallower depth relative to high latitudes. Winter soil temperature is also near the physical melting point and is sensitive to changes in snow cover (Li et al., 2017). The subalpine forests of southwestern China contain a large amount of soil organic C (Zhang et al., 2013), but most studies of global-change biology have only focused on responses during the growing season (e.g., Xu et al., 2012; 2015; Yin et al., 2013), even though warming is extremely pronounced and microbial activity is unexpectedly high during winter (Wang et al., 2016; Wang et al.

2012; Tan et al., 2014). Future soil frost will also likely affect the biological and environmental controls of soil C cycling in these forests, but the underlying mechanisms of such processes remain unknown.

We conducted a snow-manipulation experiment to investigate the immediate and carry-over effects of increased winter frost on soil C cycling in a spruce forest on the eastern Tibetan Plateau. Specifically, we hypothesized that (1) More intense frost in the soil as a result of exclusion of snow would decrease microbial activity and soil respiration in winter; (2) frost-reduced biological processes would carry over into the subsequent snow-free growing season.

2.2. Materials and methods

2.2.1. Site description

The field manipulation experiment was conducted in a dragon spruce (*Picea asperata* Mast.) stand at the Long-term Research Station of Alpine Forest Ecosystems of Sichuan Agricultural University on the eastern Tibetan Plateau of China (31°15′N, 102°53′E; 3021 m a.s.l.). The mean annual precipitation and temperature are 850 mm and 3.0 °C, respectively. Snow generally begins to accumulate in late November and melts in late March the following year. The soil is classified as a Cambic Umbrisol (IUSS Working Group WRB, 2007). The soil (0-15 cm) contains 88.5 g kg^{-1} organic C and 5.4 g kg^{-1} nitrogen (N) and has a pH of 6.4 (Li et al., 2017).

2.2.2. Experimental design

Winter snowfall was excluded using shelters to intensify soil frost. Shelters are

considered to be a useful tool for studying the responses of soil processes to winter climate change because they can effectively reduce snow cover and minimize the changes in other unwanted environmental conditions (Li et al., 2016). In early November 2015, six wooden roofs were set up in the spruce forest to prevent the accumulation of snow on the ground. One control plot was established in the vicinity of each roof. The roofs were 2 m in height with a ground area of 3 × 3 m. The snow manipulation began in mid-November and ended in late March the following year.

2.2.3. Soil sampling

Soil samples were collected from the topsoil (0-15 cm) in the frost period (FP, late January), early thawing period (ETP, early April) and the middle of the growing season (MGS, mid-August) in the year of 2016 and 2017, respectively. Three cores (5 cm in diameter, 15 cm in depth) were collected in each plot at each sampling. The three cores from each plot were combined to form one composite sample. Each composite sample was passed through a 2-mm sieve, and any visible living plant material was manually removed. The sieved soil was used for biochemical analysis.

2.2.4. Soil CO_2 efflux

Two PVC collars (20 cm in diameter, 12 cm in height) were permanently installed in each plot for measuring soil respiration. Soil CO_2 efflux was measured using a portable infrared gas analyzer (Li-8100, Li-Cor Inc., Lincoln, USA) between 10:00 and 14:00 (Beijing time, China Standard Time) approximately every two weeks during the experimental period. Simultaneously, soil temperature and volumetric moisture at a depth of 5 cm were measured nearby each collar using an auxiliary soil temperature

probe (Omega Engineering Inc., USA) and a Theta probe (Delta-T Devices, Cambridge, UK), respectively. Small red flags were attached to the PVC collars in the control plots to minimize disturbance during the period of snow cover. During the winters of 2015/2016 (four times) and 2016/2017 (once), the surface snow was removed carefully from the top of the collars when the snowpack was thicker than the height of the collar. We then waited 5 min to allow the system to equilibrate before measuring the CO_2 efflux. The removed snow was gently backfilled after the measurements. We measured CO_2 efflux under the natural snowpack (~10 cm) in the winter of 2015/2016 in the same forest stand adjacent to the snow manipulation site. CO_2 efflux was also measured after removing the snow within and around the collars. CO_2 efflux did not differ significantly before and after snow removal (unpublished data). Snow removal therefore likely negligibly affected the quantification of immediate CO_2 efflux, at least within an interval of a few minutes.

2.2.5. Microclimate, extractable N and microbial respiration

Air temperature 2 m above the ground in the forest stand was measured using Thermochron iButton DS1923–F5 Recorders (Maxim Dallas Semiconductor Corp., USA) every 2 h during the experimental period. Meanwhile, soil temperatures 5 cm below the surface were recorded in the snow-exclusion and control plots, respectively. Snow depth in the control plots was measured by a metal ruler approximately every two weeks during winter.

Soil extractable N (nitrate, NO_3^--N, and ammonium, NH_4^+-N) was extracted with 2 M KCl (1:5 soil:solution). The extracts were shaken for 1 h and filtered with a filter paper. The concentrations of NO_3^--N and NH_4^+-N in the extracts were determined by

colorimetry (Li et al., 2017).

The rate of soil microbial respiration was estimated using alkali absorption (Anderson et al., 1982). Soil samples (50 g) were incubated in 1-L jars at 20 °C for 2 weeks. Empty jars without soil were used as controls. The CO_2 produced was captured with 0.5 M NaOH in a beaker suspended inside each jar. The NaOH solution was removed and titrated with 0.25 M HCl solution to determine the amount of CO_2 produced. Microbial respiration was reported as mg CO_2-C kg^{-1} soil d^{-1}.

2.2.6. Aggregate fraction and fine-root biomass

Aggregates were isolated as described by Kristiansen et al. (2006). Two soil cores from each plot were collected from the 0-15 cm layer using an auger 10 cm in diameter in the early thawing periods of 2015/2016 and 2016/2017 winters. Soil samples were air-dried to optimal moisture (~10-15%) that would allow limited mechanical stress to maximize brittle failure along natural planes of weakness, and the samples were then gently manually crumbled to < 8 mm. The recovered samples were transferred to a nest of sieves (2 and 0.25 mm) and shaken at 100 min^{-1} for 2 min. All visible roots and stones were removed, and aggregates >2 mm (large macroaggregates) were collected. The same procedure was used for the material retained on the 0.25 mm sieve, isolating an aggregate size class 0.25-2 mm (small macroaggregates). The remaining material passing through the 0.25 mm sieve was identified as aggregate class < 0.25 mm (microaggregates).

Two soil cores were collected from each plot using an auger (15 cm long and 10 cm in diameter) in the ETPs of 2015/2016 and 2016/2017 winters. Root samples were washed in the laboratory on sieves (mesh size 0.1 mm) and dried to constant weight at

65 °C. Fine roots (< 2 mm in diameter) were separated into live and dead components based on their colour and mechanical consistency.

2.2.7. Assays of soil phospholipid fatty acids and enzyme activities

Microbial biomass was estimated as the total extractable phospholipid fatty acids (PLFAs) with a modified method described by White et al., (1996). Lipids from 2 g of fresh soil were extracted in a chloroform-methanol-phosphate buffer mixture (1:2:0.8). The phospholipids in the extracts were transformed by alkaline methanolysis into fatty acid methyl esters (FAMEs), which were identified by gas chromatography/mass spectrometry (GC/MS-QP2010 Series, Shimadzu, Japan). Fatty acids were quantified by comparisons of the peak areas from the sample with the peak areas of internal standards at 19:0 (nonadecanoic methyl ester) of the known concentration. The areas were used to estimate the abundance of PLFA markers, which were expressed as nmole g^{-1} dry soil.

We assessed the activities of four enzymes involved in soil C cycling: two hydrolytic enzymes, β-glucosidase (BG) that catalyzes one of the later steps of cellulose degradation and β-N-acetyl-glucosaminidase (NAG) involved in the breakdown of chitin and fungal cell walls, and two oxidases, polyphenol oxidase (PPO) that breaks down recalcitrant polymers such as lignin and humic compounds and peroxidase (POD), a nonspecific enzyme that oxidizes and depolymerizes lignin. The activities were measured using assay techniques described by Allison and Jastrow (2006). Substrate solutions were 5 mM pNP-β-glucopyranoside for BG, 50 mM pyrogallol and 50 mM EDTA for PPO, 2 mM pNP-β-N-acetylglucosaminide for NAG and 5 mM L-DOPA and 10 μL of 0.3% H_2O_2 for POD. Activities were determined using a microplate

spectrophotometer and expressed as μmol of substrate produced or consumed h^{-1} g^{-1} dry soil.

2.2.8. Data analysis

A repeated-measures ANOVA was used to test the effects of treatment, sampling date and their interactions on all response variables. A Bonferroni post hoc test was used to examine the treatment effect on the variables on a given sampling date when the interaction of treatment and sampling date was significant ($P < 0.05$), and a paired *t*-test was used when the interaction was not significant. All data were tested for the assumptions of an ANOVA before analysis. Heterogeneous data were ln-transformed before analysis. An exponential regression model was used to describe the relationship between CO_2 efflux and soil temperature during specific periods (winter, growing season and entire year). All data from two winters or growing seasons were used for the analyses due to the limited number of measurements. Winter was defined as the period between the first day in autumn and the last day in spring when soil temperature was continually below 5 °C for 5 d in the control plots. The temperature sensitivity (Q_{10}) of soil respiration was estimated using van't Hoff equation (Van's Hoff, 1898). $R = \alpha \times e^{\beta \times T}$, Where R is the soil respiration rate (μmol m^{-2} s^{-1}), T is the soil temperature at 5 cm (°C), α and β are parameters. The Q_{10} values are calculated as: $Q_{10} = e^{10 \times \beta}$. All statistical tests were performed using the Software Statistical Package for the Social Sciences (SPSS) version 17.0 (IBM SPSS Statistics Inc., Chicago, IL, USA).

2.3. Results

2.3.1. Treatment effect on winter soil conditions

The mean and minimum air temperatures were -2.1 and -14.1 °C during the winter of 2015/2016 and -0.9 °C and -6.4 °C during the winter of 2016/2017, respectively (Figure 1 a). The maximum snow depth was 40 cm in the winter of 2015/2016 but only 23 cm in the winter of 2016/2017. The mean air temperature in the winter of 2015/2016 (-2.1 °C) was comparable to the seven-year average of -2.4 °C for 2010-2016. The mean air temperature in the winter of 2016/2017 (-0.9 °C), however, was the highest in the last seven winters and 1.5 °C higher than the mean.

The snow-exclusion treatment successfully created a more intense frost regime in both winters (Figure 1 a). The minimum daily mean soil temperatures were -2.2 °C (2015/2016) and -2.4 °C (2016/2017) in the snow-exclusion plots but were only -0.5 °C (2015/2016) and -1.3 °C (2016/2017) in the control plots. The numbers of days with differences in soil temperature ≥0.5 °C between the treatment and control plots were 42 and 56 for the winters of 2015/2016 and 2016/2017, respectively. Such differences were mainly during mid- and late winter when snow cover was >10 cm. Soil temperature fluctuated more in the treatment than the control plots. Volumetric soil moisture was similar between the treatment and control plots across the two years (F=3.364, P = 0.116, Figure 1 b).

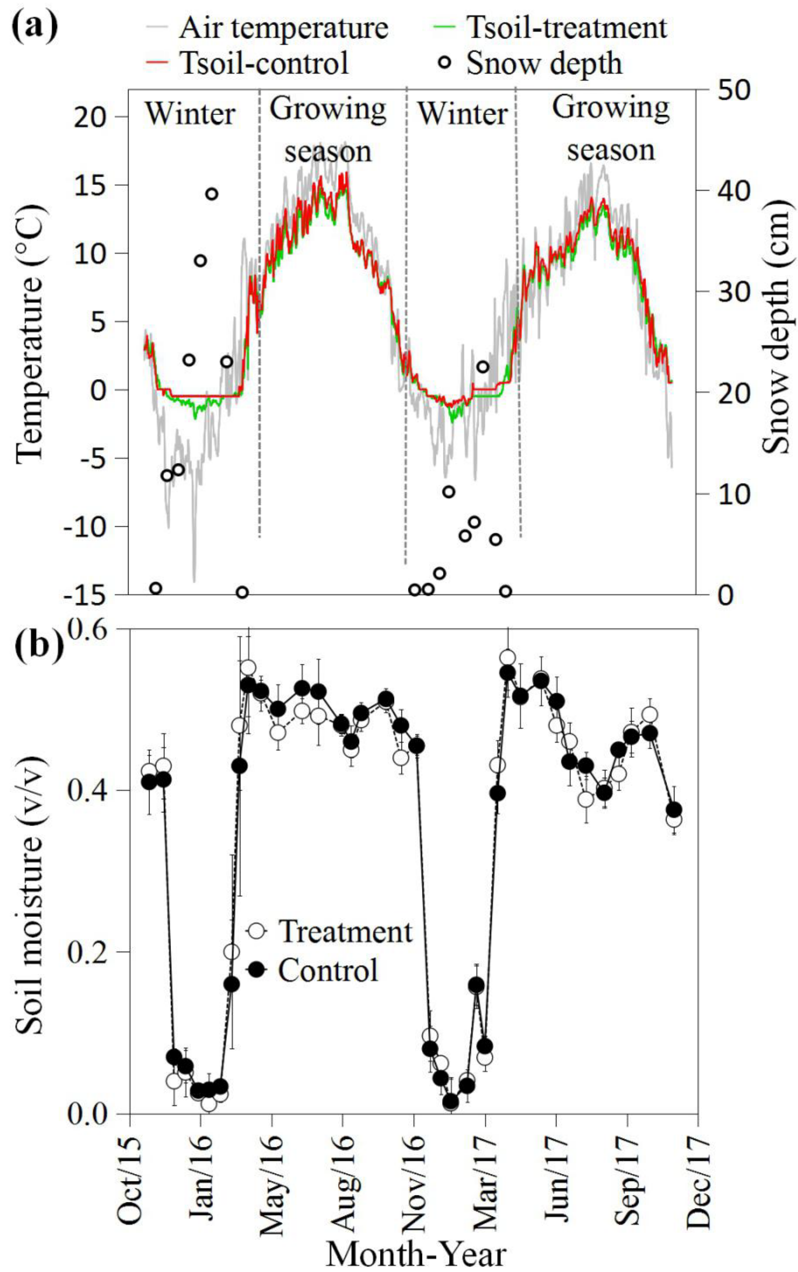

Figure 1. Air temperature, soil temperature and snow depth (a) and soil moisture (b) in the snow-exclusion treatment and control plots during the experimental period.

2.3.2. Soil CO_2 efflux

The snow exclusion lowered CO_2 efflux early in the winter of 2015/2016 and in mid-winter of 2016/2017. The snow exclusion reduced CO_2 efflux by averages of 15% and 19% in the winters of 2015/2016 and 2016/2017, respectively, and these reductions

were statistically significant (Fig. 2. $F = 11.13$, $P < 0.01$ for the winter of 2015/2016; $F = 9.143$, $P < 0.05$ for the winter of 2016/2017). The snow-exclusion manipulation, however, did not affect CO_2 efflux during the snow-free growing seasons ($F = 1.065$, $P = 0.323$ for 2016; $F = 1.354$, $P = 0.305$ for 2017). Mean CO_2 efflux differed marginally between the frost regimes in the winter of 2015/2016 ($t = 2.006$, $P = 0.076$; Table 1), but differed significantly between the regimes in the winter of 2016/2017 ($t = 3.909$, $P < 0.01$). Mean CO_2 efflux nevertheless did not differ significantly between the treatment and control plots in either growing season ($t = -1.584$, $P = 0.335$ for 2016; $t = -0.285$, $P = 0.465$ for 2017).

Table 1. Mean soil CO_2 efflux (μmol m^{-2} s^{-1}, means ± SEs) during the winter and growing season.

Year	Period	Treatment	Control
2016	Winter	0.47 ± 0.08	0.55 ± 0.08
	Growing season	2.07 ± 0.28	2.19 ± 0.29
2017	Winter	0.43 ± 0.06	0.53 ± 0.07
	Growing season	2.23 ± 0.26	2.25 ± 0.30

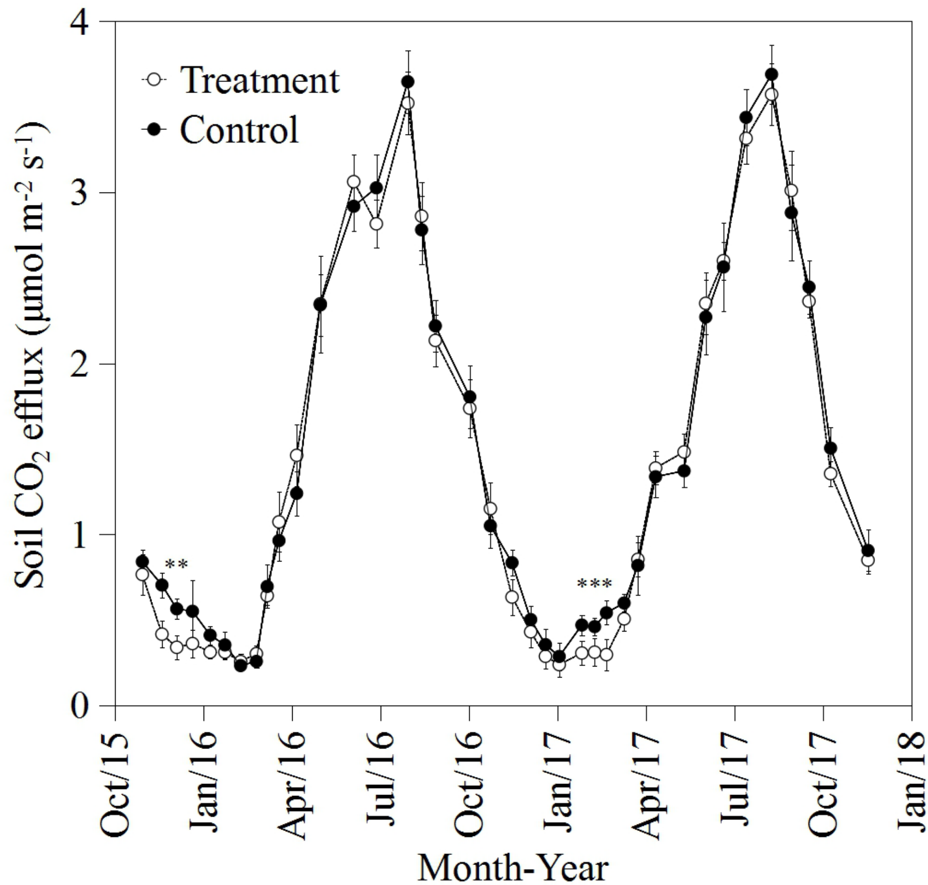

Figure 2. Soil CO_2 efflux (means ± SEs) in the snow-exclusion treatment and control plots during the experimental period. Significant differences between the control and treatment on a given date are indicated by asterisks ($P < 0.05$).

Soil CO_2 efflux increased exponentially with soil temperature throughout the study period (Figure 3a-c). Soil temperature explained 82-83% of the variation in CO_2 efflux during the growing seasons (Figure 3b) but explained only 52-53% of the variations in the winters (Figure 3a). Soil temperature explained 90-91% of the variance in CO_2 effluxes when the data for the two years were pooled (Figure 3c). The temperature sensitivity (Q_{10}) of the CO_2 efflux was 23.3, 3.2 and 4.4 in the snow-exclusion treatment plots and 22.6, 3.3 and 4.7 in the control plots for winter, growing season and the entire year, respectively. Q_{10} did not differ significantly between the frost regimes for each period modeled (all $P > 0.05$).

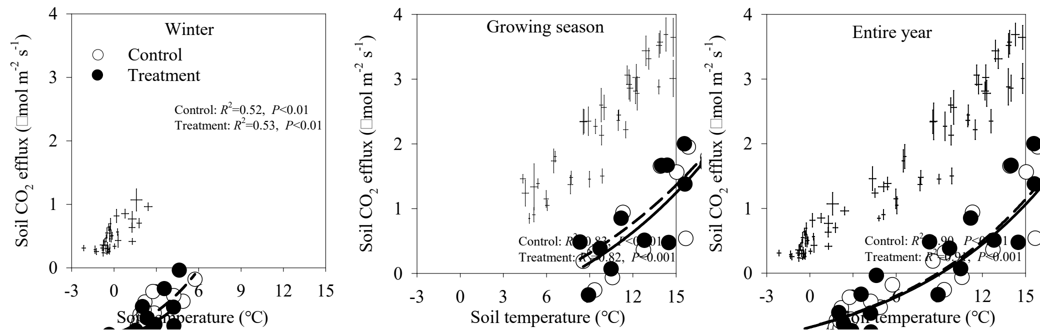

Figure 3. Exponential relationships between soil CO_2 efflux and soil temperature (means ± SEs) in the treatment and control plots for winter, growing season and entire year, respectively.

2.3.3. Soil PLFAs and microbial respiration

The intensified frost tended to decrease the soil PLFAs biomarkers. PLFA content was lower in the snow-exclusion treatment than in the control plots in the FPs of 2015/2016 (t = -2.072, $P < 0.05$) and 2016/2017 (t = -3.686, $P < 0.05$; Figure 4) but did not differ significantly in the MGSs of 2016 (t = 1.368, P = 0.245) or 2017 (t = 0.035, P = 0.895).

Microbial activity, measured as basal respiration without roots, was estimated by determining CO_2 emission. The intensified frost tended to decrease soil microbial respiration in the winter. The snow-exclusion treatment negatively affected soil microbial respiration in the FP of 2015/2016 (t = -0.918, $P < 0.05$; Figure 5) and in the ETP of 2016/2017 (t = -5.821, $P < 0.01$) but had no effect in the MGSs of 2016 and 2017 (both $P > 0.05$).

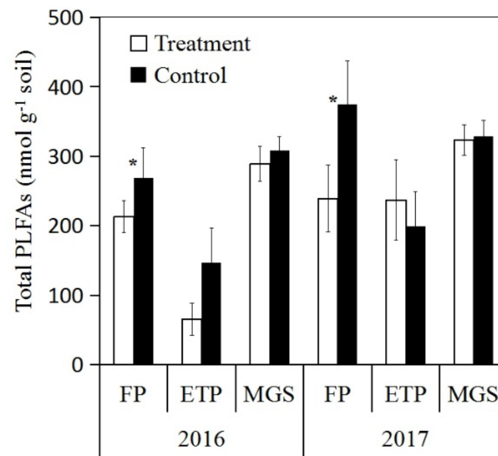

Figure 4. Total soil phospholipid fatty acids (means ± SEs) in the snow-exclusion treatment and control plots. Significant differences between the control and treatment on a given date are indicated by asterisks ($P < 0.05$). FP, frost period; ETP, early thawing period; MGS, middle of the growing season.

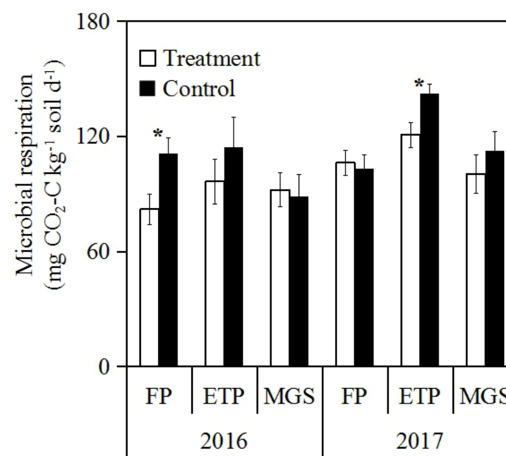

Figure 5. Soil microbial respiration (means ± SEs) in the snow-exclusion treatment and control plots. Significant differences between the control and treatment on a given date are indicated by asterisks ($P < 0.05$). FP, frost period; ETP, early thawing period; MGS, middle of the growing season.

2.3.4. Soil enzymes

The activities of the soil enzymes varied significantly with sampling date (all $P < 0.01$,

Figure 6a-d). The snow-exclusion treatment tended to reduce the enzyme activities in the winter. Activity was significantly lower in the treatment than in the control plots for BG in the ETP of 2015/2016 (t = -1.975, $P < 0.05$; Figure 6a) and for PPO in the ETP of 2016/2017 (t = -2.643, $P < 0.05$; Figure 6b). The intensified frost decreased POD activity in the FPs of 2016 and 2017 (both $P < 0.05$, Figure 6c) and decreased NAG activity in the ETPs of 2016 and 2017 (both $P < 0.05$, Figure 6d) but did not significantly affect the activities of the enzymes in the MGSs of 2016 or 2017.

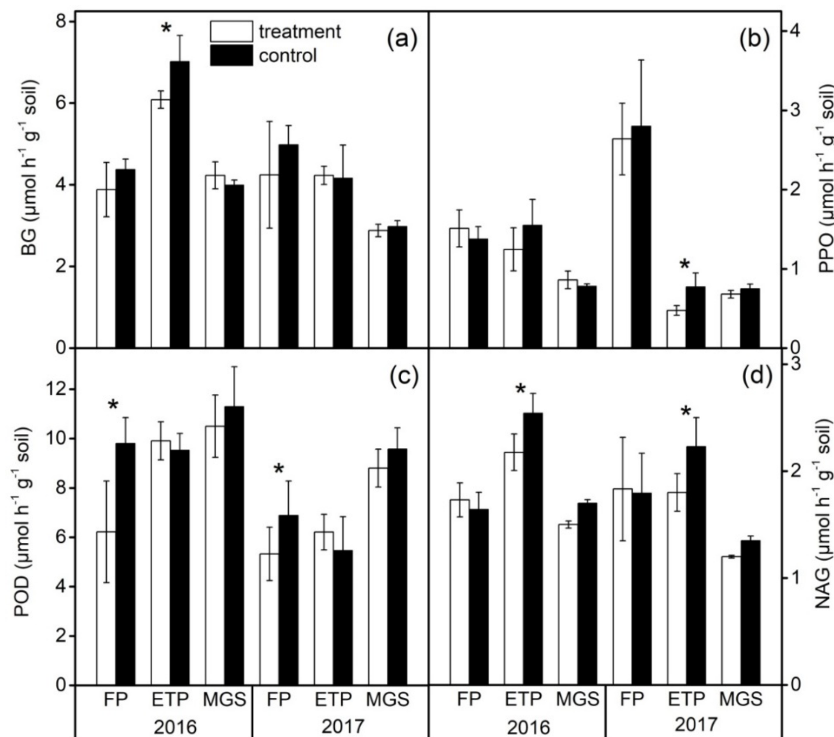

Figure 6. Activities of (a) β-glucosidase, (b) polyphenol oxidase, (c) peroxidase and (d) β-N-acetyl-glucosaminidase (means ± SEs) in the snow-exclusion treatment and control plots. Significant differences between the control and treatment on a given date are indicated by asterisks ($P < 0.05$). FP, frost period; ETP, early thawing period; MGS, middle of the growing season.

2.3.5. Soil extractable N

Frost treatment, sampling date and their interaction all significantly affected soil NH_4^+-N concentration (all $P < 0.05$, Figure 7a). The snow-exclusion treatment increased NH_4^+-N concentrations in the FP and ETP of 2015/2016 (all $P < 0.01$) but not in the winter of 2016/2017 (both $P > 0.05$). Likewise, the intensified frost increased NO_3^--N concentrations in both winters ($F = 16.575$, $P < 0.01$; Figure 7b). NO_3^--N concentrations were significantly higher in the treatment than the control plots in the ETP of 2015/2016 ($t = 2.309$, $P < 0.05$) and in the FP of 2016/2017 ($t = 5.017$, $P < 0.01$). Neither NH_4^+-N nor NO_3^--N concentration, however, differed between the frost regimes in the MGS of 2016 and 2017 (both $P > 0.05$).

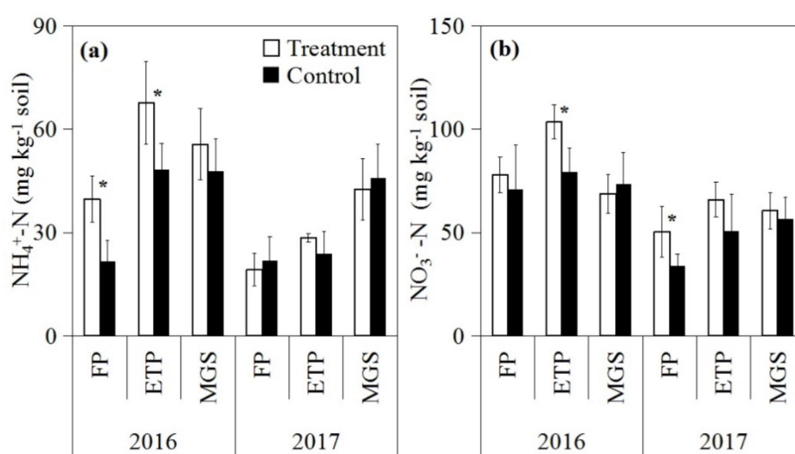

Figure 7. Soil ammonium (a) and nitrate (b) concentrations (means ± SEs) in the snow-exclusion treatment and control plots. Significant differences between the control and treatment on a given date are indicated by asterisks ($P < 0.05$). FP, frost period; ETP, early thawing period; MGS, middle of the growing season.

2.3.6. Aggregate fraction and fine-root biomass

The relative distribution of the aggregate-size classes of the bulk soil was in the order

small macroaggregates (0.25-2 mm) > large macroaggregates (>2 mm) > microaggregates (<0.25 mm) irrespective of frost regime ($F = 221.75$, $P < 0.001$; Table 2). The snow-exclusion treatment did not affect the distribution of aggregates in the size classes ($F = 0.159$, $P = 0.897$), the live fine-root biomass ($F = 0.202$, $P = 0.663$; Table 3) or the dead fine-root biomass ($F = 0.171$, $P = 0.688$) in the ETP.

Table 2. Relative distribution of the aggregate size classes (%, means ± SEs) in the snow-exclusion treatment and control plots in the early thawing period of 2016 and 2017.

Year	Size	Treatment	Control
2016	>2 mm	34.9 ± 5.7	32.9 ± 6.9
	0.25-2 mm	59.9 ± 4.7	58.6 ± 4.2
	<0.25 mm	5.2 ± 2.0	8.5 ± 2.8
2017	>2 mm	35.3 ± 6.4	36.7 ± 4.5
	0.25-2 mm	53.9 ± 3.8	50.7 ± 2.2
	<0.25 mm	10.8 ± 3.6	12.6 ± 2.8

Table 3. Live and dead fine-root biomasses (g m^{-3}, means ± SEs) in the treatment and control plots in the early thawing period of 2016 and 2017.

Year	Fine root pool	Treatment	Control
2016	Live	243.7 ± 45.6	251.7 ± 35.6
	Dead	92.8 ± 14.4	79.4 ± 26.3
2017	Live	221.2 ± 38.5	215.6 ± 28.9
	Dead	94.3 ± 22.6	88.1 ± 16.4

2.4. Discussion

We investigated the impact of intensified soil frost on soil C cycling in a Tibetan subalpine spruce forest using a field snow-manipulation experiment. Our main objective was to determine whether differences in winter frost conditions induced

immediate and carry-over effects on soil CO_2 efflux. Snow exclusion resulted in more intensive soil frost which, in line with the first hypothesis, decreased soil respiration in the winter season. However, contrary to what we expected, increased winter frost did not have carry-over effects on soil respiration in the subsequent growing season. Several potential mechanisms will be tested to account for the underlying changes of CO_2 efflux during the snow-covered and snow-free seasons.

Firstly, snow exclusion could decrease winter CO_2 efflux, in part, by the direct effect of temperature, because snow exclusion during the winter decreased soil temperature. A slight reduction in soil temperature over the lower range is especially important to CO_2 production, because the temperature sensitivity of microbial processes is extremely high at low temperatures (Davidson and Janssens, 2006; Schütt et al., 2014). Soil temperature in our study accounted for only 52-53% of the variance in winter CO_2 efflux. CO_2 efflux was very sensitive to small changes in soil temperature throughout the winter, particularly near 0 °C. Previous studies assumed a "critical point" to divide winter soil temperature into two periods (freeze-thaw conditions) resulting two distinct Q_{10}, which revealed that other factors (e.g., freeze-thaw, soil moisture, snow depth) than soil temperature also affect Q_{10} value obviously in winter (Du et al., 2013). Snow depth, soil moisture, microbial activity and freeze-thaw cycles, are directly affecting soil temperature or are affected by soil temperature, all of those are markedly impact on soil respiration in winter, which therefore, is very sensitive to soil temperature fluctuation. Conversely, temperature-induced environmental factor changing could cause both positively and negatively impact on winter soil respiration. So, only temperature could not well explain the variation of soil CO_2 in winter.

Q_{10} was much higher in the winter than in the growing season regardless of frost regime. Relatively high *Q*10 value of winter soil respiration is also found in temperate,

boreal and subalpine forests (Wang et al., 2010; Du et al., 2013). The higher Q_{10} value in winter can be explained partly by the great temperature sensitivity of microbial respiration at low temperatures. In agreement with Lipson et al (2009), which demonstrated that $Q10$ value was linked to change in the composition of soil microbial communities, and soil microbial communities from snow-covered period had high Q_{10} values. Uchida et al. (2005) studied the rates of microbial respiration in leaf litter sampled under the snow pack in the cool-temperate forest and showed that from -2 to +7 °C microbial respirations exhibited a high Q_{10} value of 5.1. Otherwise, Q_{10}, however, was estimated over a narrow temperature span (~5-6 °C), which could result higher Q_{10} in same soil respiration fluctuation trend with narrower temperature span. Moreover, the temperature-CO_2 relationship was as weak as in temperate forests (Schindlbacher et al., 2007; Wang et al., 2010; Schindlbacher et al., 2014).

Other factors co-varying with soil temperature may also likely regulate winter CO_2 efflux. The intensified frost may have produced a stronger 'freezing drought', which would likely limit microbial activity and the extracellular diffusion of substrates (Rivkina et al., 2000). Soil moisture did not differ significantly between the treatment and control plots during the winters, suggesting that soil moisture was not likely responsible for the decreased winter CO_2 efflux. The lack of significant differences in CO_2 efflux during the snow-free seasons was likewise partially attributed to the lack of significant differences in both soil temperature and moisture between the treatment and control plots during the growing season.

Secondly, root activity is extremely low during dormant seasons, and winter soil respiration in cold ecosystems is primarily derived from microbial decomposition (Muhr et al., 2009; Wang et al., 2010). Winter soil respiration is thus largely determined by the biomass and activity of soil microbes (Lipson et al., 2002; Moorhead et al., 2014).

Soil microbes are very susceptible to soil frost (Monson et al., 2006; Aanderud et al., 2013), which can kill a substantial proportion of the organisms by the rupture of cell membranes by ice crystals (Sulkava and Huhta, 2003; Jusselme et al., 2016; Gavazov et al., 2017). We also found that the increased frost significantly reduced soil microbial PLFAs, implying a lowered potential for the microbial community to metabolize soil C in the winters. A significant decline in the cross-winter microbial PLFAs also implied that seasonal frost would kill the soil microbes in the spruce forest irrespective of frost manipulation. Our measurements of microbial basal respiration, excluding plant roots, also indicated a similar decline with winter frost, consistent with *in situ* soil CO_2 efflux. Frost-induced decreases in winter soil respiration may thus largely be attributed to the lower microbial biomass and activity. Soil PLFAs and basal respiration in the middle of the growing season nevertheless did not differ significantly between frost regimes. These observations may partially account for the neutral effect of increased frost on CO_2 efflux during the snow-free growing seasons.

Soil enzymes play very important roles in the cycling of soil C and nutrients. Little attention has been paid to enzymatic activities in studies of winter climate change, despite the importance of soil enzymes in soil C cycling. A recent study found that enzymatic activities were negatively correlated with the intensity of soil frost in mixed-hardwood forests (Sorensen et al., 2016), and another experiment also found that snow removal decreased the activity of soil invertase in an alpine spruce-fir forest (Tan et al., 2014). We assayed the activities of four enzymes involved in soil C cycling to further assess the functional capacity of soil. The intensified frost tended to reduce soil enzymatic activities. Soil enzymatic activities are strongly temperature-dependent (Tabatabai, 1982), so a decrease in soil temperature caused by snow-exclusion may, to some extent, reduce soil enzymatic activities directly. The lower activities may also

partly be attributed to the smaller population size of the microbes, which are an important source of enzyme synthesis. Soil enzymes, as proximate agents of the decomposition of soil organic C, can break down plant and microbial cell walls and catabolize macromolecules into soluble substrates for microbial assimilation (Sinsabaugh et al., 2008). Frost-induced decreases in enzymatic activities may thus constrain this decomposition, which could also partly account for the lower winter CO_2 efflux. Conversely, intensified frost did not affect activities in the snow-free growing seasons, which may account for the lack of significant responses during the subsequent growing season.

Thirdly, soil frost may also have affected the decomposition of soil C in winter by altering nutrient availability. Intensified frost can increase the mortality of roots and microbes (Henry, 2007; Repo et al., 2014; Blume-Werry et al., 2016), which are important substrates for soil microbial metabolism during winter (Schimel et al., 2004). Dead roots and microbes are also main N sources during winter in cold systems (Chapin III et al., 1988; Tierney et al., 2001). In an earlier study we observed that soil at -5 °C could release considerable extractable N in the soils of this spruce forest, possibly due to the effect of freezing on microbial mortality (Xu et al., 2014). The snow-exclusion treatment in the present study stimulated the production of soil extractable N in the two winters, likely due mainly to the increased microbial mortality. An increase in N availability but a decrease in soil PLFAs throughout the winter, irrespective of the frost regime, may also support this conclusion. Live and dead fine-root biomass did not differ significantly between frost regimes later in the winter, further suggesting that the increased N availability was mainly attributable to microbial mortality rather than to root injury. An increase in N availability coincided with a decrease in CO_2 efflux, implying that the cycling of soil C could be decoupled from N availability during winter

under intensified frost.

In addition to microbial and root mortality, substrate availability could have been affected by the physically disruptive effects of frost on soil aggregates (Chai et al., 2014). Freezing can break down macroaggregates into microaggregates (Oztas and Fayetorbay, 2003). Microaggregates with a larger surface area have more contact points, which can potentially increase the amount of substrate decomposed by microorganisms (Grogan et al., 2004). Snow removal increased the fraction of microaggregates in a northern hardwood forest, implying that soil substrate could become more accessible to soil microorganisms (Steinweg et al., 2008). Our observations, however, did not provide further evidence that more intense frost could disrupt aggregates in the soil of this Tibetan spruce forest. The intensified frost did not affect the distribution of aggregates among the size classes, suggesting that frost-associated changes to aggregates may not importantly affect soil respiration in the spruce forest during the winter and growing season.

Lastly, the flux of CO_2 derived from decaying litter accounts for a considerable part of total soil respiration during winter (Uchida et al., 2005). CO_2 flux derived from aboveground litter accounts for an average of 14.2% of total soil respiration in this spruce-forest stand (Xiong et al., 2015). In a previous study we also found that the mass loss of spruce needles over the winter constituted 18.3-28.8% of the net loss rates for the entire year (Xu et al., 2016). The lack of snow cover at this experimental site decreased the temperature of the surface soil by an average of 1.4 °C during the winter (Li et al., 2017), implying that litter decomposition was most likely inhibited by the lower temperatures. A growing number of studies have documented that thick snow covers can provide relatively stable conditions for biological activity, favoring the decomposition of plant litter (Christenson et al., 2010; Bokhorst et al., 2013; Saccone

et al., 2013). The rates of decomposition of litter from subalpine tree species in this area similarly decrease with decreasing snow depth (Ni et al., 2014; He et al., 2015). The lower rate of litter decomposition due to the lack of snow cover may therefore also have contributed to the lower winter soil respiration in the snow-exclusion plots, but further supporting evidence is needed.

2.5. Implications

The climate on the Tibetan Plateau has changed considerably in recent decades, especially in winter (Wang et al., 2016). Winter snowfall has tended to decrease substantially due to strong winter warming (Xu et al., 2017). The decrease or absence of insulating snow cover associated with climate change may thus increase the duration and intensity of soil frost in the future in this special region. The importance of soil frost, large storage of soil C and sensitivity of snow cover to winter warming indicate that understanding the potential effects of projected frost increases on soil C cycling in the subalpine forests of western China is essential. To our knowledge, our study is the first to identify the effects of changes in soil frost on soil C cycling in a Tibetan forest. Our results generally indicate that more intense soil frost decreases winter soil respiration and biological activities. Winter soil CO_2 emission was lower in the snow-exclusion than in the control plots during the two winters of the study. Intensified soil frost, however, did not affect soil CO_2 efflux and biological activities during the subsequent growing season, suggesting that a short-term change in snow cover does not produce large carry-over effects in snow-free periods. If the observed effects apply to natural conditions, intensified soil frost would decrease the amount of soil C released to the atmosphere from subalpine forests during winter, but additional supporting

evidence is needed.

This study was conducted during two contrasting winters (cold winter and thick snow cover in 2015/2016 and mild winter and thin snow cover in 2016/2017) so offered a good opportunity for determining the effect of the lack of snow cover in winters with different weather on soil C cycling in the Tibetan spruce forest. The decrease in soil respiration due to frost in the first winter occurred early but then disappeared, suggesting that soil biological processes may begin to acclimate to the frost late in the winter. Soil respiration early in the mild winter of 2016/2017 did not differ significantly between the treatment and control plots, mainly due to the absence of an insulating snow cover. CO_2 effluxes, however, were lower in the treatment plots after the formation of a steady snow cover (>10 cm). In addition to variable snowfall, extreme winter events (e.g., warm weather and snow storms) may become more frequent and likely under scenarios of future climate, indicating the complexity and uncertainty of winter climate change in this specific region. The comparably strong climate change and variable winter snowfall on the Tibetan Plateau bring great challenges and opportunities for studying winter climate change and its impacts on the structure and function of Tibetan ecosystems. Long-term monitoring is strongly needed for exploring the natural winter variations and underlying mechanisms of the observed phenomena to help developing models for providing more realistic predictions of future winter conditions.

The frost intensity due to the lack of snow cover was low at our experimental forest site, unlike in temperate and boreal forests (e.g., Groffman et al., 2006; Muhr et al., 2009; Sorensen et al., 2016), but the difference in temperature minima was nearly 2 °C, likely due to the site-specific characteristics, such as winter snowfall, air temperature, properties of soil heat transfer and albedo. Such soil frost, however, had large impacts

on soil respiration, microbial PLAFs, enzymatic activity and N availability, suggesting that Tibetan forest soils will be sensitive to changing soil frost in the future. The direction and magnitude of the response of soil respiration to intensified soil frost may largely depend on the interaction between less snowfall and warmer temperature in winter. Winter warming may offset the negative effects induced by frost to some extent. Seasonal snow cover in cold regions plays a key role in decoupling soil from cold winter weather, but soil temperature is often insensitive to a small change in air temperature. Changes in snow cover will thus likely have a stronger influence on soil biological processes than winter warming itself (Groffman et al., 2001a), and long-term changes in soil frost may also have carry-over effects on soil C dynamics during the subsequent growing season in cold systems (Zhao et al., 2017). More research is warranted to integrate potential factors and separate their relative importance for a better understanding and ability to predict potential C feedbacks in snowy regions under a warmer future.

2.6. Conclusions

This study explored the immediate and carry-over effects of intensified soil frost on soil C cycling in a subalpine spruce forest on the Tibetan Plateau of China. Our results suggested that the lack of snow cover increased the intensity of soil frost, which decreased soil respiration in the winters but did not affect it during the subsequent growing seasons. Frost decreased microbial biomass and activities in the winters but not in the snow-free growing seasons. More intense soil frost did not affect the size distribution of soil aggregates or the fine-root biomass. Predicted soil frost due to winter climate change may thus decrease winter soil respiration by direct environmental

effects (e.g., soil temperatures) and indirect biological processes (e.g. microbial biomass and activities) in the subalpine forests on the Tibetan Plateau. Intensified soil frost did not cause cross-seasonal effects on soil CO_2 efflux or biological activities in the subsequent growing seasons. Our observations underscore the ecological importance of seasonal snowpack and microbe-associated C processes in subalpine forest soils. These findings improve our understanding of the response of soil C dynamics to winter climate change in this region experiencing large decreases in winter snowfall.

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Competing interests

The authors declare no competing financial interests.

References

Aanderud, Z.T., Jones, S.E., Schoolmaster Jr, D.R., Fierer, N., Lennon, J.T., 2013. Sensitivity of soil respiration and microbial communities to altered snowfall. Soil Biol. Biochem. 57, 217–227.

Anderson, J.P.E., 1982. Soil Respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy-Soil Science Society of America, Madison, 831–871.

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem. 38, 3245–3256.

Blume-Werry, G., Kreyling, J., Laudon, H., Milbau, A., 2016. Short-term climate change manipulation effects do not scale up to long-term legacies: effects of an absent snow cover on boreal forest plants. J. Ecol. 104, 1638–1648.

Bokhorst, S., Metcalfe, D.B., Wardle, D.A., 2013. Reduction in snow depth negatively affects decomposers but impact on decomposition rates is substrate dependent. Soil Biol. Biochem. 62, 157–164.

Brooks, P.D., Mcknight, D., Elder, K., 2004. Carbon limitation of soil respiration under winter snowpacks: potential feedbacks between growing season and winter carbon fluxes. Glob. Chang. Biol. 11, 231–238.

Chai, Y.J., Zeng, X.B., S, Z.E., Bai, L.Y., Su, S.M., Huang, T., 2014. Effects of freeze-thaw on aggregate stability and the organic carbon and nitrogen enrichment ratios in aggregate fractions. Soil Use Manage. 30, 507–516.

Chapin III, F.S., Fetcher, N., Kielland, K., Everett, K.R., Linkins, A.E., 1988. Productivity and nutrient cycling of Alaskan tundra: enhancement by flowing soil water. Ecology 69, 693–702.

Chen, H., Zhu, Q.A., Peng, C.H., Wu, N., Wang Y.F., Fang, X.Q., Gao, Y.H., Zhu, D., Yang, G., Tian, J.Q., Kang, X., Piao, S.L., Ouyang, H., Xiang, W.H., Luo, Z.B., Jiang, H., Song, X.Z., Zhang, Y., Yu, G.R., Zhao, X.Q., Gong, P., Yao, T.D., Wu, J.H., 2013. The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. Glob. Chang. Biol. 19, 2940–2955.

Christenson, L.M., Mitchell, M.J., Groffman, P.M., Lovett, G.M., 2010. Winter climate change implications for decomposition in northeastern forests: comparisons of sugar maple litter with herbivore fecal inputs. Glob. Chang. Biol. 16, 2589–2601.

Comerford, D.P., Schaberg, P.G., Templer, P.H., Socci, A.M., Campbell, J.L., Wallin K.F., 2013. Influence of experimental snow removal on root and canopy physiology of sugar maple trees in a northern hardwood forest. Oecologia 171, 261–269.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Du, E., Zhou, Z., Li, P., Jiang, L., Hu, X., Fang, J., 2013. Winter soil respiration during soil-freezing process in a boreal forest in Northeast China. J. Plant Ecol. 6, 349–357.

Gavazov, K., Ingrisch, J., Hasibeder, R., Mills, R.T.E., Buttler, A., Gleixner, G., Pumpanen, J., Bahn, M., 2017. Winter ecology of a subalpine grassland: Effects of snow removal on soil respiration, microbial structure and function. Sci. Total Environ. 590, 316–324.

Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L., 2001a. Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. Biogeochemistry 56, 135–150.

Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L.,

2001b. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. Biogeochemistry 56, 191–213.

Groffman, P.M. , Hardy, J.P., Driscoll, C.T., Fahey, T.J., 2006. Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. Glob. Chang. Biol. 12, 1748–1760.

Grogan, P., Michelsen, A., Ambus, P., Jonasson, S., 2004. Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biol. Biochem. 36, 641–654.

Haei, M., Laudon, H., 2015. Carbon dynamics and changing winter conditions: a review of current understanding and future research directions. Biogeosciences Discu.12, 15763–15808.

Henry, H.A.L., 2007. Soil freeze-thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biol. Biochem. 39, 977–986

He, W., Wu, F.Z., Zhang, D.J., Yang, W.Q., Tan, B., Zhao, Y.Y., Wu, Q.Q., 2015. The effects of forest gaps on cellulose degradation in the foliar litter of two shrub species in an alpine fir forest. Plant Soil 393, 109–122.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY.

Janssens, I.A., Pilegaard, K., 2003. Large seasonal changes in Q_{10} of soil respiration in a beech forest. Glob. Chang. Biol. 9, 911–918.

Jusselme, M.D., Saccone, P., Zinger, L., Faure, M., Le Roux X., Guillaumaud, N., Bernard,L., Clement, J. C., Poly, F., 2016. Variations in snow depth modify N-related soil microbial abundances and functioning during winter in subalpine

grassland. Soil Biol. Biochem. 92, 27–37.

Kristiansen, S.M., Schjnning, P., Thomsen, I.K., Olesen, J.E., Kristensen, K., Christensen, B.T., 2006. Similarity of differently sized macro-aggregates in arable soils of different texture. Geoderma 137, 147–154.

Lipson, D.A., Schadt, C.W., Schmidt, S.K., 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microb. Ecol. 43, 307–314.

Liu, J.P., Curry, J. A., Wang, H.J., Song, M.R., Horton, R. M., 2012. Impact of declining Arctic sea ice on winter snowfall. PANS 109, 6781–6783.

Li, W.B., Wu, J.B., Bai, E., Jin, C.J., Wang, A.Z., Yuan, F.H., Guan, D.X., 2016. Response of terrestrial carbon dynamics to snow cover change: A meta-analysis of experimental manipulation (II). Soil Biol. Biochem. 103, 388–393.

Li, Z.J., Yang, W.Q., Yue, K., Justine, M.F., He, R.Y., Yang, K.J., Zhuang, L.Y., Wu, F.Z., Tan, B., Zhang, L., Xu, Z.F., 2017. Effects of snow absence on winter soil nitrogen dynamics in a subalpine spruce forest of southwestern China. Geoderma, 307, 107–113.

Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., Schmidt, S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. Nature 439, 711–714.

Moorhead, D., Lashermes, G., Recous, S., Bertrand, I., 2014. Interacting microbe and litter quality controls on litter decomposition: a modeling analysis. PLOS ONE 9: e108769.

Muhr, J., Borken, W., Matzner, E., 2009. Effects of soil frost on soil respiration and its radiocarbon signature in a Norway spruce forest soil. Glob. Chang. Biol. 15, 782–793.

Ni, X.Y., Yang, W.Q., Li, H., Xu, L.Y., He, J., Tan, B., Wu, F.Z., 2014. The responses of early foliar litter humification to reduced snow cover during winter in an alpine forest. Canad. J Soil Sci. 94, 453–461.

Oquist, M.G., Laudon, H., 2008. Winter soil frost conditions in boreal forests control growing season soil CO_2 concentration and its atmospheric exchange. Glob. Chang. Biol. 14, 2839–2847.

Oztas, T., Fayetorbay, F., 2003. Effect of freezing and thawing processes on soil aggregate stability. Catena 52, 1–8.

Repo, T., Sirki, S., Heinonen, J., Lavigne, A., Roitto, M., Koljonen, E., Koljonen, E., Sutinen, S., Finer, L., 2014. Effects of frozen soil on growth and longevity of fine roots of Norway spruce. For. Ecol. Manag.313, 112–122.

Rivkina, E.M., Friedmann, E.I., McKay, C.P., Gilichinsky, D.A., 2000. Metabolic activity of permafrost bacteria below the freezing point. Appl. Environ. Microbiol. 66, 3230–3233.

Saccone, P., Morin, S., Baptist, F., Bonneville, J.M., Colace, M.P., Domine, F., Faure, M., Geremia, R., Lochet, J., Poly, F., Lavorel, S., Clement, J.C., 2013. The effects of snowpack properties and plant strategies on litter decomposition during winter in subalpine meadows. Plant Soil 363, 215–229.

Sanders-DeMott, R., Templer, P.H., 2017. What about winter? Integrating the missing season into climate change experiments in seasonally snow covered ecosystems. Methods Ecol. Evol. 8, 1183–1191.

Schimel, J.P., Bilbrough, C., Welker, J. A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biol. Biochem. 36, 217–227.

Schindlbacher, A., Jandl, R., Schindlbacher, S., 2014. Natural variations in snow cover

do not affect the annual soil CO_2 efflux from a mid-elevation temperate forest. Glob. Chang. Biol. 20, 622–632.

Schindlbacher, A., Zechmeister-Boltenstern, S., Glatzel, G., Jandl, R., 2007. Winter soil respiration from an Austrian mountain forest. Agric. For. Meteorol. 146, 205–215.

Schutt, M., Borken, W., Spott, O., Stange, C.F., Matzner, E., 2014. Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biol. Biochem. 69, 320–327.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo M.E., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.

Song, Y., Zou, Y.C., Wang, G.P., Yu, X.F., 2017. Altered soil carbon and nitrogen cycles due to the freeze-thaw effect: A meta-analysis. Soil Biol. Biochem. 109, 35–49.

Sorensen, P.O., Templer, P.H., Finzi, A.C., 2016. Contrasting effects of winter snowpack and soil frost on growing season microbial biomass and enzyme activity in two mixed-hardwood forests. Biogeochemistry 128, 141–154.

Steinweg, J.M., Fisk, M.C., McAlexander, B., Groffman, P.M., Hardy, J.P., 2008. Experimental snowpack reduction alters organic matter and net N mineralization potential of soil macroaggregates in a northern hardwood forest. Biol. Fertil. Soils 45, 1–10

Sulkava, P., Huhta, V., 2003. Effects of hard frost and freeze–thaw cycles on decomposer communities and N mineralisation in boreal forest soil. Appl. Soil Ecol. 22, 225–239.

Tabatabai, M.A., 1982. Soil Enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.),

Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. American Society of Agronomy—Soil Science Society of America, Madison, WI, pp. 903–947.

Tan, B., Wu, F.Z., Yang, W.Q., He, X.H., 2014. Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest. Appl. Soil Ecol. 76, 34–41.

Tierney, G.L., Fahey, T.J., Groffman, P.M., Hardy, J.P., Fitzhugh, R.D., Driscoll, C.T., 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. Biogeochemistry 56, 175–190.

Uchida, M., Mo, W., Nakatsubo, T., Tsuchiya, Y., Horikoshi, T., Koizumi, H., 2005. Microbial activity and litter decomposition under snow cover in a cool-temperate broad-leaved deciduous forest. Agric. For. Meteorol. 134, 102–109.Van's Hoff, J.H., 1898. Lectures on Theoretical and Physical Chemistry. Part 1: Chemical Dynamics. Edward Arnold, London, pp. 224–229.

Wang, A., Wu, F.Z., Yang, W.Q., Wu, Z.C., Wang, X.X., Tan, B., 2012. Abundance and composition dynamics of ammonia-oxidizing archaea in alpine fir forest on the eastern Tibetan Plateau of China. Can. J. Microbiol. 58, 572–580.

Wang, J., Zhang, M.J., Wang, S.J., Ren, Z.G., Che, Y.J., Qiang, F., Qu, D.Y., 2016. Decrease in snowfall/rainfall ratio in the Tibetan Plateau from 1961 to 2013. J. Geogr. Sci. 26, 1277–1288.

Wang, W., Peng, S.S., Wang, T., Fang, J.Y., 2010. Winter soil CO_2 efflux and its contribution to annual soil respiration in different ecosystems of a forest-steppe ecotone, north China. Soil Biol. Biochem. 42, 451–458.

White, D.C., Stair, J.O., Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. J. Ind. Microbiol. 17, 185–

196.

Wubs, E.R.J., Woodin, S.J., Stutter, M.I., Wipf, S., Sommerkorn, M., Wal, R.V.D., 2018. Two decades of altered snow cover does not affect soil microbial ability to catabolize carbon compounds in an oceanic alpine heath. Soil Biol. Biochem. 124, 101–104.

Xiong, L., Xu, Z.F., Yang, W.Q., Yin, R., Tang, S.S., Wang, B., Xu, L.Y., Chang, C.H., 2015. Aboveground litter contribution to soil respiration in a subalpine dragon spruce plantation of western Siehuan. Sheng Tai Xue Bao 35, 4678–4686.

Xu, M., Qi, Y., 2001. Spatial and seasonal variations of Q_{10} determined by soil respiration measurements at a Sierra Nevadan forest. Glob. Biogeochem. Cycles 15, 687–696.

Xu, W.F., Ma, L.J., Ma, M.N., Zhang, H.C., Yuan, W.P., 2017. Spatial–temporal variability of snow cover and depth in the Qinghai-Tibetan Plateau. J. Clim. 30, 1521–1533.

Xu, Z.F., Liu, Q., Yin, H,J., 2014. Effects of temperature on soil net nitrogen mineralisation in two contrasting forests on the eastern Tibetan Plateau, China. Soil Res. 52, 562–567.

Xu, Z.F., Pu, X.Z., Yin, H.J., Zhao, C.Z., Liu, Q., Wu, F.Z., 2012. Warming effects on the early decomposition of three litter types, Eastern Tibetan Plateau, China. Eur. J. Soil Sci. 63, 360–367.

Xu, Z.F., Wan, C.A., Xiong, P., Tang, Z., Hu, R., Cao, G., Liu, Q., 2010. Initial responses of soil CO_2 efflux and C, N pools to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China. Plant Soil 336, 183–195.

Xu, Z.F., Zhao, C.Z., Yin, H.J., Liu, Q., 2015. Warming and forest management

interactively affect the decomposition of subalpine forests on the eastern Tibetan Plateau: A four-year experiment. Geoderma 239, 223–228.

Xu, Z.F., Zhu, J.X., Wu, F.Z., Liu, Y., Tan, B., Yang, W.Q., 2016. Effects of litter quality and climate change along an elevational gradient on litter decomposition of subalpine forests, Eastern Tibetan Plateau, China. J. For. Res. 27, 505–511.

Yin, H.J., Li, Y.F., Xiao, J., Xu, Z.F., Cheng, X.Y., Liu, Q., 2013. Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. Glob. Chang. Biol. 19, 2158–2167.

Zhang, Y.D., Gu, F.X., Liu, S.R., Liu, Y.C., Li, C., 2013. Variations of carbon stock with forest types in subalpine region of southwestern China. For. Ecol. Manag. 300, 88–95.

Zhao, J.B., Peichl, M., Nilsson, M.B., 2017. Long-term enhanced winter soil frost alters growing season CO_2 fluxes through its impact on vegetation development in a boreal peatland. Glob. Chang. Biol. 23, 3139–3153.

CHAPTER 3. Exchange of volatile organic compounds between the atmosphere and the soil: A review

Kaijun Yang, Joan Llusià, Catherine Preece, Yu Tan, Josep Peñuelas

Abstract

Volatile organic compounds (VOCs) are crucial in ecology and atmospheric chemistry, and recent studies have highlighted their significance in soil systems, where they can serve as both sources and sinks of VOCs. The soil system is one of the main places for the generation, emission, and capture of VOCs, but these processes are not sufficiently understood. We aim to provide a comprehensive review of the exchange of VOCs between soils and the atmosphere. We classed the main sources of VOCs in soil and discuss these sources connections via VOCs specificity and common, and then give an overview of main mechanisms of how VOCs returned to soil system. Lastly, summarized how climate change affects the balance of VOCs exchange between the soil and the atmosphere. The composition and emission rates of VOCs exhibit variability across different ecosystem soils, with isoprenoids being predominantly emitted by forest soils, while non-isoprenoids dominate in tundra and cropland soils. Soil, although often overlooked, acts as an important sink for VOCs via key mechanisms, including deposition, adsorption, and microbial degradation of VOCs. Climate change exerts an impact on the soil carbon cycle, potentially leading to increased soil VOC emissions if warming and nitrogen enrichment enhance soil organic matter decomposition. Conversely, the soil's VOC sink capacity may expand if drought-resistant soil microbes capable of consuming VOCs are present. In summary, soil represents a vital yet often neglected component in the VOC exchange between the atmosphere and the soil.

Keywords: Volatile organic compounds; Bidirectional exchange; Source; Sink; Climate change.

3.1. Introduction

Volatile organic compounds (VOCs) play an important role in atmospheric chemistry and climate, and they are involved in producing secondary organic aerosols and photochemical ozone (Andreae, 1997; Atkinson, 2000; Williams, 2004; Tunved et al., 2006; Lelieveld et al., 2008). VOCs are predominantly released from terrestrial vegetation and anthropogenically produced activities (Guenther et al., 1993), but emissions of VOCs from soil sources have received considerably less attention (Peñuelas et al., 2014; Llusia et al., 2021; Tang et al., 2019).

Although soil VOC emissions has typically been reported to be 1-2 orders of magnitude lower than those from plant canopies (Peñuelas et al., 2014), recent studies have revealed that soil emissions can indeed be significant, depending on factors such as habitat and season (Bourtsoukidis et al., 2018; Llusia et al., 2021). Accurately estimating soil VOCs is crucial for predicting global VOC emissions, yet global models currently exclude soil VOC fluxes due to insufficient data (Guenther et al., 2012). Therefore, there is an urgent need to measure soil VOC emissions across various ecosystems, especially in soils rich in soil organic carbon (SOC) and microorganisms, as these soils are likely to be substantial VOC sources but remain understudied (Guenther et al., 1993; Peñuelas et al., 2014; Bourtsoukidis et al., 2018; Llusia et al., 2021).

In soil, the primary contributors to VOC emissions include plant litter, roots, soil organic matter (SOM), and microorganisms (Leff and Fierer, 2008; Insam and Seewald, 2010; Peñuelas et al., 2014). Additionally, accumulating evidence suggests that soil has the potential to act as a VOC sink (Spielmann et al., 2016; Albers et al., 2018; Trowbridge et al., 2020), although the magnitude of this sink capability remains

uncertain (Cleveland and Yavitt, 1997; McGenity et al., 2018; Rinnan and Albers, 2020).

Soil releases a variety of VOCs, including acids, alkanes, alkenes, alcohols, aldehydes, benzenoids, ketones, esters, ethers, and terpenes (Peñuelas et al., 2014). In this review, we classify them into four main classes: isoprene, monoterpenes, sesquiterpenes, and other VOCs. We provide an overview of recent advances in soil VOC research, estimate potential VOC emissions and uptake in natural soils, and focus on describing the characteristics of soil VOCs in different ecosystems, the sources of VOCs in soil ecosystems, the main mechanisms of atmospheric VOCs returning to soil, and the effects of climate change on the exchange of soil VOCs.

3.2. Materials and methods

3.2.1. Data collation

We collected data related to VOCs from soil-related sources by searching Web of Science and Google Scholar for peer-reviewed journal articles published before and including 2021. The search terms used in the online database were (volatile organic compound OR VOC(s) OR isoprenoid(s) OR monoterpene OR sesquiterpene) AND soil. Each article was cross-checked to determine whether the studies met the following criteria: (1) the flux of VOC was studied in field conditions; (2) the VOC flux could be extracted directly from the text, table, figure and published supplementary material. We used Engauge Digitizer version 12.1 (http://markum mitchell.github.io/engauge-digitizer) to digitally extract data from figures when the results were graphically reported. According to the aforementioned criteria, 30 papers could be used for the data analysis, representing three ecosystems (forest, tundra and cropland). For the forest

ecosystem, five different vegetation types are included (boreal forest, subalpine forest, Mediterranean forest, subtropical forest and tropical forest).

Before doing the statistical analysis, conversions were made of all necessary data so that they were fully comparable. VOC exchange rates reported with other units in the literature were converted to ug m^{-2} h^{-1}. For the comparison of soil VOC exchange in various ecosystems, the maximum, minimum, mean and median flux rates of the five different VOC classes (total VOCs, isoprene, monoterpenes, sesquiterpenes, and other VOCs) were calculated from the raw data, including both emission and uptake, for all soil sources and all treatments. Additionally, to highlight the uptake capacity of the soil, studies that found uptake rates are shown separately. The relative abundance of VOCs from the different soil sources was summarised in pie charts as the ratio between the number of VOCs in that chemical class and the total number of VOCs in all classes. An additional graphical representation was made (Cytoscape 3.9.1) to highlight the associations between groups of VOCs and their sources. We then identified which VOCs were specific and common to bare soil, plant root, plant litter and soil microbes.

3.3. Main detectable VOCs emitted from soil

According to the Model of Emissions of Gases and Aerosols from Nature (MEGAN), global VOC emissions from terrestrial vegetation are estimated to be 500, 89 and 36 Tg C y^{-1} for isoprene, monoterpenes and sesquiterpenes, respectively (Guenther et al., 2012; Acosta Navarro et al., 2014), but their emissions from soil have still not been estimated at a global scale (Figure 1). This section presents a summary of current information on the different classes of VOC emission.

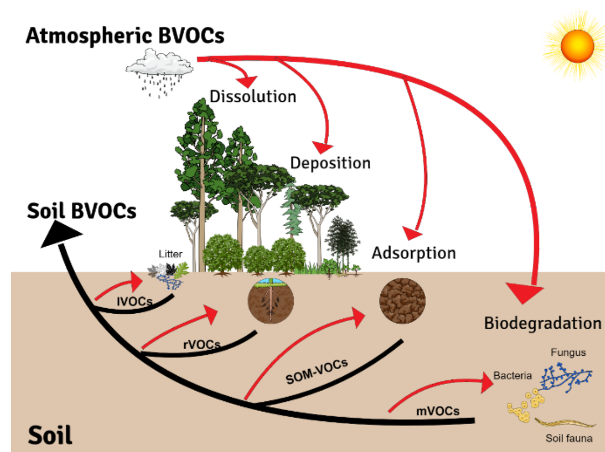


Figure 1. Schematic of the main flows and processes that determine levels of biogenetic volatile organic compounds (BVOCs) between the atmosphere and the soil ecosystem. The black arrow represents soil BVOCs emitted by related soil sources (litter, root, SOM, and microbes), and the red arrow represents atmospheric VOCs that are absorbed by soil by related (biotic and abiotic) mechanisms. lVOCs, VOCs derived from litter; rVOCs, VOCs derived from roots; SOM-VOCs, VOCs derived from SOM; mVOCs, VOCs derived from microbes.

3.3.1. Isoprene

Isoprene is the most globally abundant VOC emitted from aboveground sources (Guenther et al., 1993), and an important secondary metabolite of plants. Compared to aboveground sources, isoprene emissions from soil have been reported to be relatively low (Gray et al., 2014; Mäki et al., 2019), with the highest rate observed in cropland is 5.7 μg m^{-2} h^{-1} (Figure 2 and Table 1). Other ecosystems, such as forests and tundra, have shown low emission rates and concentrations in the soil horizon (< 1 μg m^{3}) (Asensio et al., 2007b; Mäki et al., 2017; Mäki et al., 2019). Moreover, isoprene emission is not always detectable in soil samples because it depends heavily on the litter species and microbes present (Veres et al., 2014).

Table 1. Ranges of soil VOC exchange rates (mean, median) (μg m^{-2} h^{-1}) in different ecosystems based on the data in the appendix (positive and negative data are emissions and uptakes, respectively).

Ecosystem	Total VOCs	Isoprene	Monoterpenes	Sesquiterpenes	Other VOCs
Tundra	31, 118 (66, 58)	0.4, 3 (2.0, 2.4)	0.2, 2.1 (1.2,1.2)	0–0.1 (0.1,0.1)	31–113 (59, 46)
Boreal forest	50, 5191 (1287, 439)	1	13, 4548 (546, 49)	0.4–18 (7.4, 3.1)	(110, 110)
Mediterranean forest	-37, 395 (94, 7.6)	-4.4, 0.4 (-1.8, -1.6)	-2.5, 20 (1.7, 0.2)	-0.4–2.4 (0.6, 0.1)	-33, 373 (91, 8.8)
Subalpine forest	0.2, 2.7 (1.5, 1.4)	-0.1 [b]	0.1, 0.7 (0.5, 0.7) [b]	NA	1.6, 4.0 (2.5, 1.9)
Subtropical forest	-44, 103 (29, 29)	-0.8, 0.7 (-0.1, -0.1)	36, 89 (63, 63)	NA	-133, 66 (-25, -15)
Tropical forest	NA	NA	-5.57, 406	-282, 100 (45, 22)	NA
Cropland	37.1 [a]	5.7 [a]	0.5 [a]	NA	-9, 200 (64, 33)

Note: NA, not available; [a], data from only one study; [b], mean cited from original study.

Plant litter and soil microbes serve as the primary sources of isoprene in soil (Guenther et al., 1993; Gray et al., 2010; Insam and Seewald, 2010; Mancuso et al., 2015; Svendsen et al., 2018). In contrast, plant roots do not appear to produce isoprene, as shown by a laboratory study in which no isoprene emissions were detected from the bare roots of 15 different tree species (Tsuruta et al., 2018). Isoprene emissions from litter occur mainly during the initial phase of decomposition (Gray et al., 2010; Svendsen et al., 2018). This can be attributed to the ability of cells in fresh leaf litter to maintain isoprene synthesis while still living. For soil microorganisms, certain bacteria and fungi, such as *Bacillus sp*., *Burkholderia sp*. and *Tuber borchii* have been found to release isoprene as a means of defence and communication (Lemfack et al., 2017). However, the role of soil microbes in isoprene emission may be insignificant, possibly due to the consumption of soil isoprene-degrading bacteria that can exceed its production in soil ecosystems (Cleveland and Yavitt, 1998; Carrion et al., 2020; Trowbridge et al., 2020). This phenomenon will be discussed further in a subsequent

section.

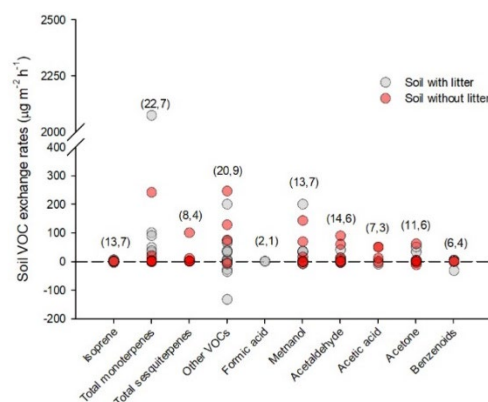


Figure 2. Soil VOC exchange rates reported in different ecosystems in Table 1. Data includes measurements in soil with litter (grey points) and without litter (red points). Treatments such as warming and N addition are not included. The number of data points and the number of supporting references for each compound are shown in parentheses.

3.3.2. Monoterpenes

Monoterpenes tend to have high emissions from soil and constitute a substantial proportion of soil VOCs, particularly in forest soils (Bourtsoukidis et al., 2018; Hayward et al., 2001; Mäki et al., 2019; Staudt et al., 2019). Extensive research has been conducted on the emissions of monoterpenes in boreal forest soils, revealing considerable variation in rates ranging from 13.0 to 4548.1 µg m^{-2} h^{-1}, with the variability influenced by seasons, tree species, and soil characteristics (Hayward et al., 2001; Aaltonen et al., 2011; Aaltonen et al., 2012; Staudt et al., 2019). For example, Hayward et al. (2001) reported normalised soil monoterpene emissions of 33.6 µg m^{-2} h^{-1}, and Mäki et al. (2017) found an average of 48.4 µg m^{-2} h^{-1} during the growing season at the SMEAR II station in Finland after removing the understorey. However, Staudt et al. (2019) measured significantly higher soil monoterpenes emissions (558.3−4548.1 µg m^{-2} h^{-1}) during summer in a Maritime pine (Pinus pinaster) forest in

France. The high heterogeneity in the emission of monoterpenes among various boreal forest sites is partially caused by variation in the quantity and traits of litter present in each site (Leff et al., 2008; Viros et al., 2020). Litter is a strong emitter of monoterpenes, and so the amount of litter biomass has a strong effect on the emission rate of monoterpenes. For instance, the emission rate of monoterpenes reported by Staudt et al. (2019) is significantly higher than that reported by Mäki et al. (2017). This may be attributable to the fact that litter biomass in the former study (740 g dry weight m^{-2}) was around 60 times higher than that in the latter study (12 g dry weight m^{-2}), which likely contributed to the high monoterpene emissions. However, the soil in the study by Staudt et al. (2019) still maintained a high rate of monoterpene emission (245 μg m^{-2} h^{-1}) after litter removal, indicating that other soil characteristics (e.g., SOM and soil microbes), as well as litter, are important sources of monoterpenes (Bourtsoukidis et al., 2018).

Compared to boreal forests, the emission rates of soil monoterpenes are relatively low in tundra, subalpine and Mediterranean forests (Table 1). According to a study by Kramshøj et al. (2016), the mean emission rate of monoterpenes in tundra soil during the growing season was 1.73 μg m^{-2} h^{-1}, and α-terpineol was found to be the primary compound. The low emission of monoterpenes in tundra soil may be attributed to the absence of litter cover iin combination with the low temperature, which can reduce microbial decomposition. Similarly, subalpine forest soils also are weak emitters of monoterpenes with emission rates ranging from 0.05 to 0.70 μg m^{-2} h^{-1} due to low temperatures and slow microbial activity (Greenberg et al., 2012; Gray et al., 2014; Trowbridge et al., 2020). However, in Mediterranean soils, the low monoterpene emissions seem to be limited by soil moisture content and a strong uptake by soil was observed (Asensio et al., 2007b; Asensio et al., 2007c).

Lastly, tropical forest soils are considerable sources of monoterpenes due to their abundant litter cover, high root density in soil, and a relatively high temperature (Bourtsoukidis et al., 2018; Llusia et al., 2021).

3.3.3. Sesquiterpenes

The boreal forest soil horizons host a variety of sesquiterpenes, such as α-gurjunene, α-humulene, and β-farnesene. However, their emission rates to the atmosphere are typically low, ranging from 0.4 to 17.5 μg m^{-2} h^{-1} (Mäki et al., 2017, 2019), due to their low volatility. Table 1 shows that other ecosystems, such as tundra and Mediterranean forests, also exhibit low emissions of sesquiterpenes. For instance, a study on tundra soil detected only one sesquiterpene during the growing season, with an emission rate of 0.14 μg m^{-2} h^{-1} (Kramshøj et al., 2016). Similarly, an experiment in a Mediterranean forest soil found the highest rate of soil sesquiterpenes to be 2.40 μg m^{-2} h^{-1} (Asensio et al., 2007b). Compared with those low emission of sesquiterpenes, tropical forest soils were found to have high sesquiterpene emissions (a maximum of 210 μg m^{-2} h^{-1}) in recent studies (Bourtsoukidis et al., 2018; Llusia et al., 2021), which highlights the potential source of sesquiterpenes inherent in this soil type. The substantial variation in sesquiterpene emissions among different ecosystem soils may be attributed, in part, to differences in soil microorganism communities, which are the primary contributors of sesquiterpenes in soil (Horvath et al., 2011, 2012; Weikl et al., 2016). Additionally, some studies have suggested that a high microbial biomass in soil may result in higher emissions of sesquiterpenes (Weikl et al., 2016; Bourtsoukidis et al., 2018; Kramshøj et al., 2018).

3.3.4. Other VOCs

Most of the research on soil VOC exchanges has focused on terpenoids (isoprene, monoterpenes and sesquiterpenes). Nevertheless, other VOCs, such as methanol (Asensio et al., 2007a), carbonyl compounds (e.g., acetone, acetaldehyde, and acetic acid) (Asensio et al., 2008; Gray et al., 2014; Mielnik et al., 2018), benzenoids (Zheng et al., 2015), sulphurous compounds (e.g., dimethyl sulphide, carbon disulphide, and dimethyl disulphide) (Yi et al., 2010), and formaldehyde (Gray et al., 2014; Li et al., 2016) have also been reported as being emitted from soils, but are rarely studied. The annual global emissions of methanol, acetone, formic and acetic acids are estimated to be 187, 95, 57 and 85 Tg y^{-1} (Jacob et al., 2002; Paulot et al., 2011; Stavrakou et al., 2011), respectively. Some of these compounds have high emission rates in a specific type of soil (Kramshøj et al., 2016; Bourtsoukidis et al., 2018; Mäki et al., 2019). For example, aldehydes (octanal, nonanal, and hexanal) are found in tundra soil, and account for about 75% of the total VOC emissions in that ecosystem (78.4 μg m^{-2} h^{-1}) (Kramshøj et al., 2016), and methanol is predominant in cropland soil (Bachy et al., 2018).

The emission of methanol is generally positively correlated with temperature (Schade and Goldstein 2001), and a recent experiment suggested that this relationship could be affected by soil moisture (Bachy et al., 2018). Methanol is mainly emitted by soil bacteria and the decomposition of residual organic matter, so methanol synthesis is likely driven by temperature-dependent enzymatic activity, microbial community structure and soil moisture. Methanol emissions under dry conditions have been strongly positively correlated with soil temperature, but methanol was taken up under wet conditions in the same cropland, and the amount taken up increased with temperature (Bachy et al., 2018). Similarly, in a Mediterranean forest soil, methanol emission was high during a dry summer and uptake was strong in a wet autumn

(Asensio et al., 2008).

3.4. Sources of VOCs in soil

3.3.1. VOC emissions in decomposing litters

Plant litter produces abundant VOCs during its decomposition process, and the emission rate and diversity of these VOCs varies with changes in the environment (Gray et al., 2010; Isidorov et al., 2016; Svendsen et al., 2018; Viros et al., 2021. While early field studies suggested that litter may be a minor source of VOCs compared to aboveground plant emitters (Faubert et al., 2010; Greenberg et al., 2012), a recent study emphasized that a thick litter layer above the soil may be a large potential source, with the removal of that litter layer substantially reducing (by 81%) total soil VOC emissions in that system (Staudt et al., 2019). Results from laboratory litter incubation experiments show that litter emission rates range from 0.1 to 265.5 μg g (dw)$^{-1}$ h^{-1} (Table 2), depending on litter types, degree of decomposition and incubation conditions (Leff and Fierer 2008; Gray et al., 2010; Isidorov et al., 2016; Svendsen et al., 2018).

Table 2. Average VOC emission rates from various litter sources measured in laboratory studies.

Species	Litter type	Condition	Main VOCs and their compositions	Overall rate[1]	Reference
Centaurea maculosa	Grass	22 °C/20 days	Isoprene, 0.05%; monoterpenes, 0.01%; other VOCs, 99.94%; methanol, 96.69%	19.7	Gray, 2010
*Centaurea maculosa**	Grass	22 °C/20 days	Isoprene, 3.53%; monoterpenes, 0.29%; other VOCs, 96.19%; methanol, 43.40%	10.8	Gray, 2010
Eucalyptus sp.	Broadleaf	22 °C/20 days	Isoprene, 0.16%; monoterpenes, 22.16%; other VOCs, 77.96%; methanol, 70.83%	265.5	Gray, 2010
Eucalyptus sp.*	Broadleaf	22 °C/20 days	Isoprene, 0.35%; monoterpenes, 85.72%; other VOCs, 14.72%; methanol, 7.56%	58.4	Gray, 2010
Fraxinus pennsylvanica	Broadleaf	22 °C/20 days	Isoprene, 0.07%; monoterpenes, 0.01%; other VOCs, 99.92%; methanol, 98.71%	142.9	Gray, 2010
*Fraxinus pennsylvanica**	Broadleaf	22 °C/20 days	Isoprene, 1.9%; monoterpenes, 0.32%; other VOCs, 97.79%; methanol, 65.80%	14.3	Gray, 2010
Miscanthus sp.*	Grass	22 °C/20 days	Isoprene, 1.16%; monoterpenes, 0.34%; other VOCs, 98.49%; methanol, 35.29%	5.0	Gray, 2010
Miscanthus sp.	Grass	22 °C/20 days	Isoprene, 0.06%; monoterpenes, 0.05%; other VOCs, 99.36%; methanol, 85.35%	4.3	Gray, 2010

Pinus contorta	Pine	22 °C/20 days	Isoprene, 0.15%; monoterpenes, 9.74%; other VOCs, 90.19%; methanol, 85.15%	23.0	Gray, 2010
*Pinus contorta**	Pine	22 °C/20 days	Isoprene, 1.50%; monoterpenes, 16.04%; other VOCs, 82.56%; methanol, 40%	14.4	Gray, 2010
Pinus ponderosa	Pine	22 °C/20 days	Isoprene, 0.23%; monoterpenes, 4.65%; other VOCs, 95.24%; methanol, 90.41%	89.9	Gray, 2010
*Pinus ponderosa**	Pine	22 °C/20 days	Isoprene, 1.14%; monoterpenes, 33%; other VOCs, 66.22%; methanol, 32.51%	13.6	Gray, 2010
Populus deltoides	Broadleaf	22 °C/20 days	Isoprene, 0.04%; monoterpenes, 0.04%; other VOCs, 99.92%; methanol, 98.30%	226.8	Gray, 2010
*Populus deltoides**	Broadleaf	22 °C/20 days	Isoprene, 0.66%; monoterpenes, 0.41%; other VOCs, 90.94%; methanol, 60.1%	10.5	Gray, 2010
Populus tremuloides	Broadleaf	22 °C/20 days	Isoprene, 0.09%; monoterpenes, 0.02%; other VOCs, 99.89%; methanol, 97.86%	78.5	Gray, 2010
*Populus tremuloides**	Broadleaf	22 °C/20 days	Isoprene, 0.67%; monoterpenes, 0.40%; other VOCs, 98.95%; methanol, 60.86%	7.5	Gray, 2010
Quercus macrocarpa	Broadleaf	22 °C/20 days	Isoprene, 0.62%; monoterpenes, 0.77%; other VOCs, 98.64%; methanol, 70.72%	4.7	Gray, 2010
*Quercus macrocarpa**	Broadleaf	22 °C/20 days	Isoprene, 0.71%; monoterpenes, 0.88%; other VOCs, 98.42%; methanol, 58.13%	5.4	Gray, 2010
Quercus rubra	Broadleaf	22 °C/20 days	Isoprene, 0.5%; monoterpenes, 0.31%; other VOCs, 99.21%; methanol, 85.11%	7.4	Gray, 2010
*Quercus rubra**	Broadleaf	22 °C/20 days	Isoprene, 0.83%; monoterpenes, 0.86%; other VOCs, 98.32%; methanol, 35.63%	9.2	Gray, 2010
Rhododendron maximum	Shrub	22 °C/20 days	Isoprene, 0.05%; monoterpenes, 0.03%; other VOCs, 99.92%; methanol, 97.53%	52.2	Gray, 2010
*Rhododendron maximum**	Shrub	22 °C/20 days	Isoprene, 0.35%; monoterpenes, 0.06%; other VOCs, 99.59%; methanol, 71.77%	7.1	Gray, 2010
Thinopyrum intermedia	Grass	22 °C/20 days	Isoprene, 0.20%; monoterpenes, 0.03%; other VOCs, 99.78%; methanol, 89.33%	5.8	Gray, 2010
*Thinopyrum intermedia**	Grass	22 °C/20 days	Isoprene, 0.94%; monoterpenes, 0.61%; other VOCs, 98.44%; methanol, 46.90%	4.3	Gray, 2010
Pinus sylvestris	Pine	20 °C/0 days	Monoterpenes	2.0	Isidorov, 2010
Pinus sylvestris	Pine	20 °C/77 days	Monoterpenes	7.5	Isidorov, 2010
Pinus sylvestris	Pine	20 °C/165 days	Monoterpenes	1.6	Isidorov, 2010
Pinus sylvestris	Pine	20 °C/282 days	Monoterpenes	0.3	Isidorov, 2010
Pinus sylvestris	Pine	20 °C/490 days	Monoterpenes	0.2	Isidorov, 2010
Norway spruce	Pine	20 °C/0 days	Monoterpenes	0.2	Isidorov, 2010
Norway spruce	Pine	20 °C/77 days	Monoterpenes	1.5	Isidorov, 2010
Norway spruce	Pine	20 °C/165 days	Monoterpenes	1.3	Isidorov, 2010
Norway spruce	Pine	20 °C/282 days	Monoterpenes	0.5	Isidorov, 2010
Norway spruce	Pine	20 °C/490 days	Monoterpenes	0.2	Isidorov, 2010
Acer rubrum	Broadleaf	21 °C/73 days	Monoterpenes, 0.19%; other VOCs, 95.55%; methanol, 89.40%	0.6	Ramirez,2010
Pinus taeda	Pine	21 °C/73 days	Monoterpenes, 34.90%; other VOCs, 54.58%; methanol, 31.50%	0.3	Ramirez,2010
Cassiope tetragona	Shrub	5 °C/14 days	Monoterpenes, 53.1%; sesquiterpenes, 2.6%; other VOCs, 44.3%	0.2	Svendsen, 2018
Cassiope tetragona	Shrub	12 °C/14 days	Isoprene, 1.0%; monoterpenes, 62.4%; sesquiterpenes, 6.7%; other VOCs, 29.9%	0.3	Svendsen, 2018
Cassiope tetragona	Shrub	16 °C/14 days	Isoprene, 0.1%; monoterpenes, 58.2%; sesquiterpenes, 24.3%; other VOCs, 17.5%	0.4	Svendsen, 2018
Cassiope tetragona	Shrub	19 °C/14 days	Isoprene, 0.2%; monoterpenes, 76.4%; sesquiterpenes, 9.7%; other VOCs, 13.8%	0.7	Svendsen, 2018
Salix glauca	Shrub	5 °C/14 days	Isoprene, 0%; monoterpenes, 24.18%; other VOCs, 75.82%	0.4	Svendsen, 2018
Salix glauca	Shrub	12 °C/14 days	Isoprene, 0%; monoterpenes, 32.6%; sesquiterpenes, 1.1%; other VOCs, 66.35%	0.1	Svendsen, 2018
Salix glauca	Shrub	16 °C/14 days	Isoprene, 0%; monoterpenes, 8.92%; sesquiterpenes, 27.46%; other VOCs, 63.62%	0.1	Svendsen, 2018
Salix glauca	Shrub	19 °C/14 days	Isoprene, 0%; monoterpenes, 10.78%; sesquiterpenes, 14.56%; other VOCs, 74.66%	0.1	Svendsen, 2018

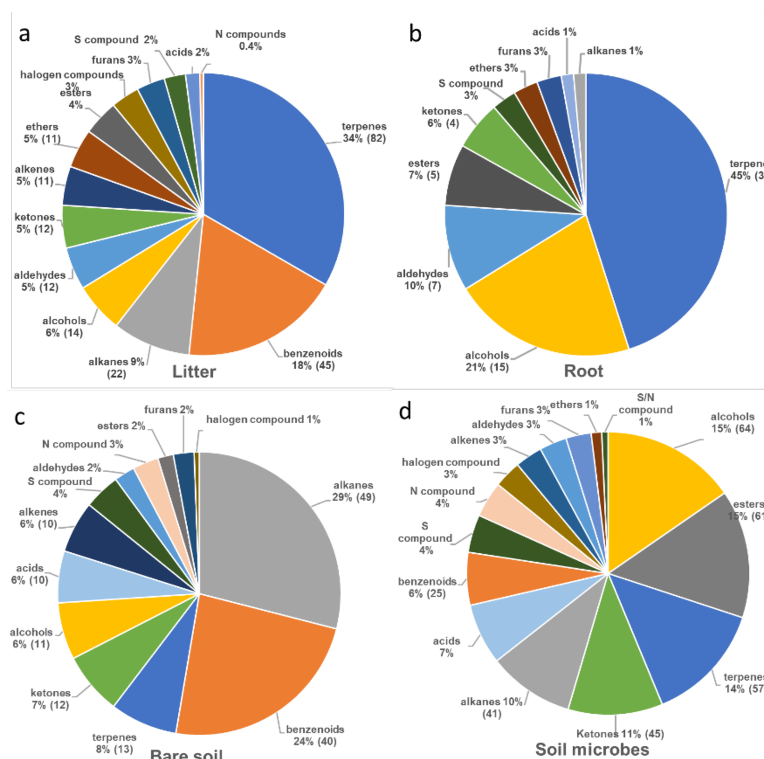
*Litter samples were sterilized in the study. [1]Rates were converted to μg g $(dw)^{-1}$ h^{-1} where

necessary.

In general, the decomposition of broadleaf litter is faster than other types of litter, thus, the emission rate of VOCs may be higher in broadleaf forest soils than in others. For instance, the emission rates of broadleaf litters (e.g., Eucalyptus sp., Fraxinus sp. and Populus sp.) are shown to be higher than from litters of pine (e.g., *Pinus contorta*), grasses (e.g., *Miscanthus sp*.) and shrubs (e.g., *Cassiope sp*.) (Table 2), and that is partly attributed to litter quality. Incubation experiments have shown that the VOC emission rate in the early stage of decomposition is positively related to the amount of labile C in the litter and the presence of a structure (that some leaves have) for storing terpenoids (Leff and Fierer, 2008; Ramirez et al., 2009; Gray et al., 2010; Svendsen et al., 2018). Overall, the VOC emission rate of litter decreases with time of decomposition (Ramirez et al., 2009), but increased temperature can stimulate emission rates, especially for alkenes and terpenoids (Svendsen et al., 2018). The profiles of litter VOCs are also highly associated with the community structure of microbes in the litter (Isidorov et al., 2016; Svendsen et al., 2018), with litter in different decomposition stages being dominated by different microorganisms, causing the differences in emitted VOCs that are found (Isidorov et al., 2016).

The full range of VOCs that can be emitted by litter is still unclear. Here, we collected references of VOCs emitted from litter from 34 plant species, which encompassed a total of 244 different VOCs belonging to twelve chemical groups (Figure 3a). These VOCs included 81 terpenes (34% of those described), 45 benzenoids (18%), 23 alkanes (9%), 14 alcohols (6%), 12 aldehydes (5%), 12 ketones (5%), 11 alkenes (5%), 11 ethers (5%), eight halogenated compounds (3%), eight furans (3%), six sulphur (S) compounds (2%), four acids (2%) and one nitrogen (N) compound

(0.4%). The first four classes, which make up 67% of the measured VOCs, represented the major classes of litter VOCs. After comparing VOCs from litter with VOCs emitted from other sources (root, SOC and microbes), our Venn diagram revealed that litter emitted 122 unique VOCs not shared by any other sources (Figure 4), with most of them belonging to terpenes (41 VOCs), benzenoids (22 VOCs), alkanes (17 VOCs), ethers (8 VOCs), halogenated compounds (7 VOCs) and S compounds (5 VOCs) as shown in Figure 3e.



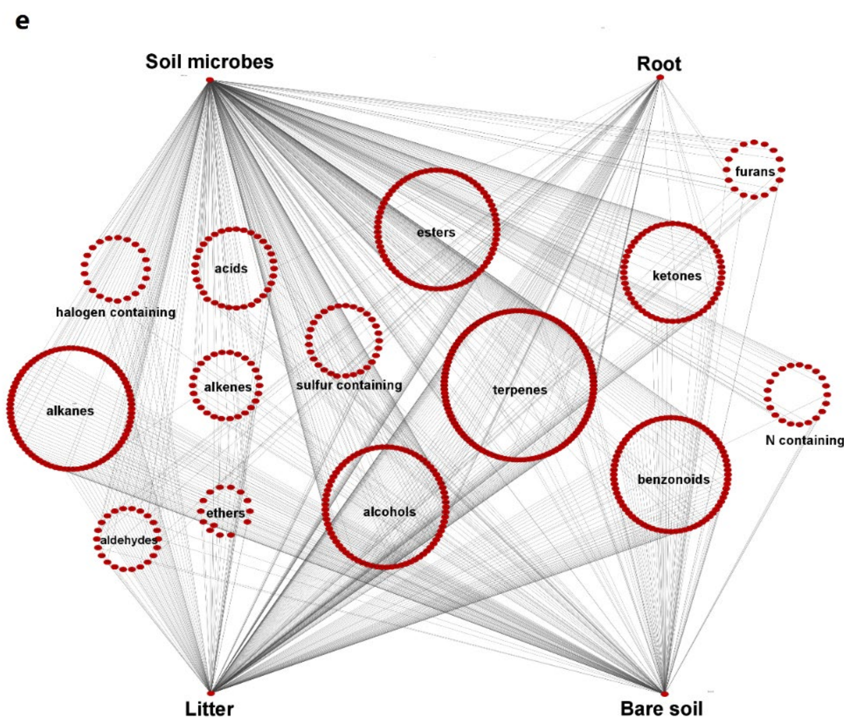


Figure 3. Classes and diversity of VOCs emitted by soil sources (litter, root, bare soil, and microbes). (a) Relative abundance of litter VOC chemical classes, given by the ratio between the number of VOCs in a chemical class and the number of VOCs in all classes, a total of 244 volatiles released from 35 plant litter types in 6 articles (Isidorov and Jdanova, 2002; Leff and Fierer, 2008; Ramirez *et al*., 2009; Gray *et al*., 2010; Isidorov *et al*., 2010; Svendsen *et al*., 2018); (b) Relative abundance of plant VOC chemical classes, a total of 71 volatiles released from the roots of 15 species (only bare root without mycorrhizae considered) in 6 articles (Steeghs *et al*., 2004; Lin *et al*., 2007; Jassbi *et al*., 2010; Erb *et al*., 2011; Gfeller *et al*., 2013; Tsuruta *et al*., 2018); (c) Relative abundance of bare soil VOC chemical classes, a total of 168 volatiles released from 35 samples of bare soil in 5 articles (Leff and Fierer, 2008; Mancuso *et al*., 2015; Raza *et al*. 2017; Kramshoj *et al*., 2019; Gulati *et al*., 2020). (d) Relative abundance of soil microbial VOC chemical classes, a total of 416 volatiles released from 36 species in 22 articles (Garbeva *et al*., 2014a, 2004b; Bitas *et al*., 2015; Cordovez et al. 2015; Giorgio et *al*., 2015; Park *et al*., 2015; Raza *et al*., 2015; Schulz-Bohm *et al*., 2015; Wu *et al*., 2015; Isidorov *et al*., 2016; Raza *et al*., 2016a; Raza *et al*., 2016b; Che *et al*., 2017; Cordovez *et al*., 2017; Gao *et al*., 2017; Perez-Flores *et al*., 2017; Rajer *et al*., 2017; Tahir *et al*., 2017a; Tahir *et al*., 2017b; Yuan *et al*., 2017; Cordovez *et al*., 2018; Moisan *et al*., 2019; Gulati *et al*., 2020). Each line represents one hit for a given VOC association; the diameters of the circles that represent each chemical class are proportional to the number of different VOCs within that class.

3.4.2. Contribution of roots

Plants allocate 40−73% of the photosynthesised C for root metabolism and growth and root-associated (rhizosphere) microbes (Grayston et al., 1997). Roots are known to produce VOCs for defence and communication (Schenkel et al., 2015), but little is known about the production and emission rates (Lin et al., 2007). Furthermore, recent evidence has suggested that the rate of production of root VOCs might correlate with morpho-anatomical traits of roots, such as root taxonomy and mycorrhizal type (Tsuruta et al., 2018). Otherwise, the presence of roots in soil has an inconsistent effect on soil VOC emissions (Rinnan et al., 2013; Gray et al., 2014), with positive, negative and no impacts, which are related to individual VOCs (Asensio et al., 2007a; Mäki et al., 2017).

The low number of papers that have been published on root VOCs is likely due to the technical difficulties in sampling VOCs in soil matrices (Peñuelas et al., 2014; Tsuruta et al., 2018). We collected six papers focused on root VOCs and found a total of 71 different compounds produced by the roots of 20 plant species (Figure 4). The majority (89%) of the identified VOCs were terpenes (32 VOCs), alcohols (15 VOCs), aldehydes (seven VOCs), esters (five VOCs) and ketones (four VOCs) (Figure 3b), and the remaining 11% was composed of minor groups (S compounds, ethers, furans, acids and alkanes). Moreover, most VOCs from roots are shared with VOCs measured from belowground microbes (Figure 3e), with the remaining 25 unique VOCs including 11 terpenes (all sesquiterpenes), seven alcohols, four esters, two aldehydes and one furan (Figure 4).

Some studies have tried to quantify the rate of VOC emission by roots (Lin et al., 2007; Tsuruta et al., 2018). Lin et al. (2007) reported a mean emission rate of 24.3 μg g^{-1} h^{-1} for eight monoterpenes (mainly α-pinene, β-pinene, and limonene) emitted from

the washed roots of Pinus spp. in a pot experiment. Another study using a similar method with *Pinus densiflora* roots found the same monoterpenes but a higher emission rate (122.6 µg g^{-1} h^{-1}) (Tsuruta et al., 2018). This difference may be associated with root traits such as oleoresin content and pools of stored terpenoids (Tsuruta et al., 2018). Oleoresin consists of a mixture of terpenoids and is produced by specialised secretory tissues of coniferous tree roots (Lewinsohn et al., 1991). Large differences were also found amongst the emission rates of monoterpenes and sesquiterpenes from roots of 15 tree species, with the roots of gymnosperms associated with ectomycorrhiza having a strong potential for releasing monoterpenes, whereas angiosperm roots emitted low amounts of monoterpenes regardless of mycorrhizal type Tsuruta et al. (2018).

A field trenching experiment in a forest dominated by Pinus contorta estimated that roots contributed to 53% of total soil VOC emissions. However, the removal of roots did not affect the rate of emission of soil monoterpenes (Gray et al., 2014), which could be indicate that the roots of the study species are a minor emitter of monoterpenes. Currently, the assessment of the contribution of root emissions in situ to overall soil VOC fluxes is difficult because of their linkages with soil organisms, which inevitably impact the potential soil sink and source of VOCs (Trowbridge et al., 2020).

3.4.3. Contributions of SOC

Although SOC could emit a series of VOCs naturally, given that the strengths and composition of VOCs emissions are always associated with microbial activities, it is a challenge for researchers to verify the source of VOCs derived from SOC or soil microbes (Bourtsoukidis et al., 2018). SOC-derived VOCs are emitted during the processes of SOM breakdown and decomposition, which are dominated by different pathways of microbial metabolism *such as aerobic decomposition, fermentation, and*

terpenoid biosynthesis (Tang et al., 2019). Therefore, the community and function of microbes in soil play a vital role in determining the production and emission of SOM-derived VOCs (Leff and Fierer 2008; Mancuso et al., 2015). Additionally, soil microbes are an important contributor to the diversity of VOCs, which are summarised in a microbial VOC database *(mVOC 2.0)* that has documented about 2000 *microbial VOCs* emitted by almost 1000 microbial species (Lemfack et al., 2017).

Use of the experimental method combining litter removal and root trenching could potentially estimate VOC emissions from field soil, although it appears that the emission rates of VOCs derived from SOM only are small compared to the rates from litter and roots (Lin et al., 2007; Leff and Fierer 2008). For example, one study detected an obvious emission of formic acid from sterilized soil with the average rate of $\sim 6\times10^{-3}$ nmolm^{-2} s^{-1} (Li et al., 2019). Rossabi et al., (2018) incubated three distinct ecosystem soils (agricultural soil, grassland soil and subalpine soil) at a room temperature and measured the rates of VOCs emission ranging from 25 to 190 ng g^{-1} dry soil h^{-1}. These rates are lower than the emission of litter that in the same region, which varied from 81 to 960 ng g^{-1} (dry soil h^{-1}, which caused by the litter decomposition periods and incubation temperature (Svendsen et al., 2018). The amount of VOC production from bare soil was 10-100 folds lower than from litter emitters (Leff and Fierer 2008).

The profiles of VOCs from bare soil were also dramatically changed by incubation temperature and soil moisture. One incubation study suggested that higher temperature can increase the emission of soil VOCs emission, but soil moisture had the opposite effect (Raza et al., 2017). Kramshøj et al., (2019) incubated permafrost soil with different water conditions (drained and non-drained) under two temperature levels (10 ºC and 20 ºC) and measured the total VOCs with the rate ranging from 2.5 to 42.7 ng g^{-1} dry soil h^{-1}, also demonstrating that higher temperature and drained soil produced

more VOCs. Apart from soil temperature and moisture, the quantity of VOCs emitted from bare soil is also positively correlated with SOC and microbial biomass (Mancuso et al., 2015), for example, ethanol and methanol are strongly emitted from soil with high SOM content and active microbes (Kramshøj et al., 2018).

In this review, a total of 168 VOCs were found emitted from bare soil, with the main chemical classes comprising alkanes (29%), benzenoids (24%), terpenes (8%), ketones (7%), alcohols (6%), acids (6%), alkenes (6%), totalling 86% of total VOCs (Figure 3c). The remaining 14% was composed of minor groups (S compounds, aldehydes, N compounds, furans, and halogen compounds) each represented by a few compounds. Compared with other sources, bare soils have 80 unique volatiles not shared by other sources (Figure 4), and these VOCs include alkanes (35 VOCs), benzenoids (19 VOCs), ketones (five VOCs), *S* compounds (five VOCs), terpenes (three VOCs), acids (three VOCs), N compounds (three VOCs), alcohols (two VOCs), esters (two VOCs) and one halogen compound.

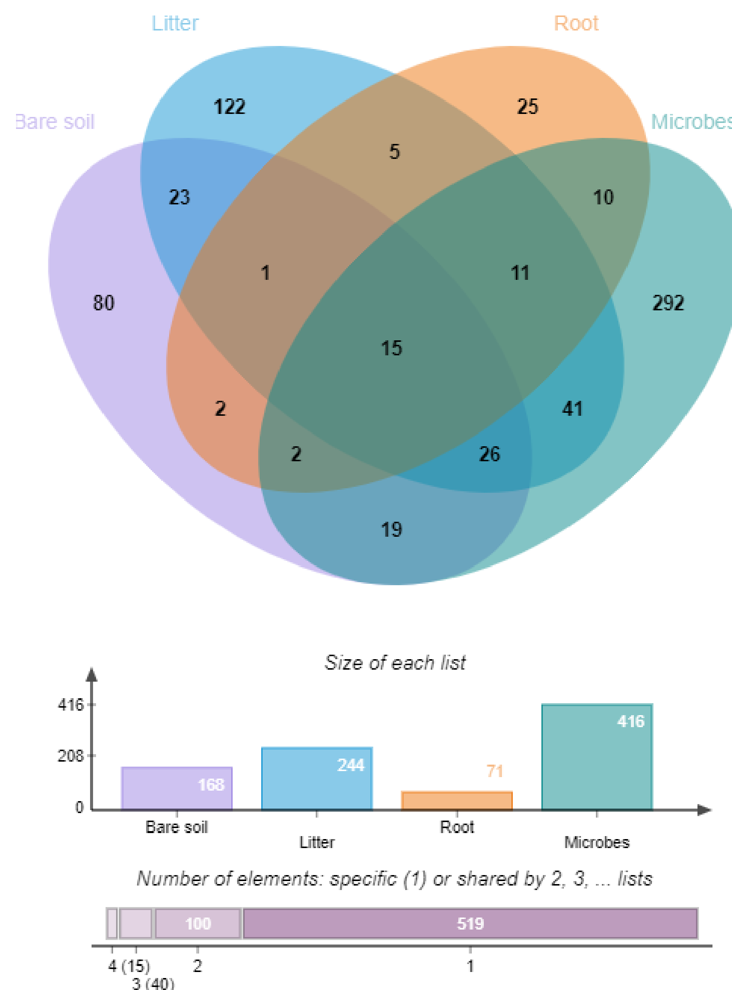


Figure 4. Number of VOC specific and common to bare soil, plant root, plant litter, and soil microbes. A total of 675 VOCs emitted by soil was grouped based on their sources (litter, root, bare soil and soil microbe) in the four categories and were displayed in a Venn diagram. The numbers of VOCs specific and shared among categories were displayed below.

3.3.4. Contributions of soil microorganisms

Microbial VOCs are produced by a wide array of microorganisms ranging from bacteria to fungi, and VOC profiles emitted by microorganisms are usually consistent, relating to microbial traits, culture conditions, and environments (Back et al., 2010). A laboratory experiment found parallel changes in soil VOC emissions and the compositions of fungal communities with the application of different types of fertilisers

(Seewald et al., 2010). Moreover, the emission of sesquiterpenes by fungi (e.g., *Alternaria alternata*) was strongly related to fungal growth phases rather than its biomass, with fungi in the early stages of growth being able to emit much higher amounts of sesquiterpenes than in their mature stages, with higher biomass (Weikl et al., 2016). Another study noted that bacteria may be as important as fungi in releasing sesquiterpenes in Amazonian soils (Bourtsoukidis et al., 2018).

The mVOC 3.0 database, established in 2013, has summarized 2061 VOCs emitted by microbes including human pathogens, plant pathogens and soil microorganisms (Lemfack et al., 2014; Lemfack et al., 2017), identifying more than 841 VOCs that are known to be produced by soil microbes from different soil habitats (e.g., rhizosphere, bulk soil) (Schenkel et al., 2015). High amounts of VOCs are released from microbes as end and intermediate products of primary metabolism, and some are emitted as secondary metabolites (Insam and Seewald 2010). From the recently updated database of mVOC 3.0, the profiles of bacterial VOCs are dominated by (in descending order) alkenes, alcohols, ketones, terpenes, benenoids, pyrazines, acids and esters. The profiles of fungal VOCs are dominated by alcohols, benzenoids, aldehydes, alkenes, acids, esters, and ketones (https://bioinformatics.charite.de/mvoc). We summarised 22 papers related to soil microbe only incubations and found a total of 416 VOCs produced by 36 microbial species. Our data show that soil microbes have higher production of alcohols (64 VOCs), esters (61 VOCs), terpenes (57 VOCs), ketones (45 VOCs) and alkanes (41 VOCs), which comprised 64% of total VOCs (Figure 3d). Moreover, there are 292 unique VOCs, shared with neither litter, roots or bare soil (Figure 4), with most of them belonging to esters (54 VOCs), alcohols (47 VOCs), ketones (37 VOCs), alkanes (26 VOCs) and terpenes (22 VOCs).

3.5. Capacity for uptake of VOCs by soils

Field chamber and continuous-flow studies have demonstrated that soil is a potential biological sink for VOCs at environmentally relevant concentrations (McGenity et al., 2018; Abis et al., 2020, Trowbridge et al., 2020). VOCs can also be taken up by soils and subjected to biotic and abiotic processes including microbial degradation (Cleveland and Yavitt 1997; Gray et al., 2015), chemical oxidation (Insam and Seewald 2010), and physical desorption (Ruiz et al., 1998). Recent studies have highlighted that the capacity of soils to act as a sink for VOCs is affected by the diversity, community structure, biomass and growth phases of soil microbes (Abis et al., 2020, Trowbridge et al., 2020). Microorganisms can metabolize and utilize VOCs as carbon and energy sources (Cleveland and Yavitt, 1998; Owen et al., 2007; Albers et al., 2018), and soil microbes have been shown to consume the majority of VOCs released by other sources (Ramirez et al., 2009; Bachy et al., 2018). Moreover, soils containing high SOC may have a stronger potential ability to take up VOCs than mineral soil (Kramshøj et al., 2018). A recent study demonstrated that VOC metabolism (e.g., of methanol and acetone) in soil may be a previously unrecognized carbon sequestration pathway by contributing to the accumulation of soil labile C and increasing the immobilization of *N* (McBride et al., 2019).

An initial attempt to quantify the rate of isoprene uptake in a laboratory setting soil led to an estimate of −97.3 μg m^{-2} h^{-1} and global isoprene sink strength was calculated as 20.4 Tg C y^{-1} under a given isoprene concentration (385 ppb), which is higher than the current atmospheric concentration (Cleveland and Yavitt 1997). Much higher estimates of rates of isoprene uptake have since been calculated (−987.7 to −4168.9 μg m^{-2} h^{-1}) using higher concentrations of isoprene under mesocosm conditions (up to 1000 ppb) (Pegoraro et al., 2005). The isoprene concentrations used

in that study, however, greatly exceeded those in the atmosphere, which is generally lower than 10 ppb. The clear positive correlation between air isoprene concentration and rate of isoprene deposition suggests that an increasing isoprene gradient can increase the rate of deposition of isoprene in soil (Pegoraro et al., 2005; Spielmann et al., 2016). The highest reported rate of isoprene uptake measured under ambient conditions was 4.4 μg m^{-2} h^{-1} in a Mediterranean forest (Asensio et al., 2007b), which is much lower than the estimates by Cleveland et al. (1997) and Pegoraro et al. (2005). The capacity of soil to take up isoprene has, therefore likely been overestimated (Table 3).

Table 3. Mean net rates of soil VOC uptake (μg m^{-2} h^{-1}) among various ecosystems based on the data in the appendix.

Reference	Ecosystem	Source	Season	Isoprene	Total monoterpenes	Total sesquiterpenes	Other VOCs	Methanol
Asensio, 2007	Mediterranean forest	Soil	Seasonal variation	0.31-4.42	2.5	0.05	1.29-32.89	NA
Asensio, 2008	Mediterranean shrub	nLsoil	Seasonal variation	NA	0.04-0.97	0.39	1.99-26.8	6.9
Gray, 2014	Subalpine forest	Soil	Summer	0.14	NA	NA	0.28	NA
Gray, 2014	Subalpine forest	nRsoil	Summer	0.13	NA	NA	0.31	NA
Zhang, 2017	Subtropical forest	Soil	Spring	0.81	32.82	NA	133.40	NA
Bachy, 2016	Cropland	Soil	Spring	NA	NA	NA	13.20	NA
Bachy, 2018	Cropland	Soil	Spring	NA	NA	NA	NA	9.00
Staudt, 2019	Boreal forest	Soil	Summer	NA	9.96	NA	NA	NA
Staudt, 2019	Boreal forest	nLsoil	Summer	NA	1.44	NA	NA	NA
Trowbridge, 2020	Subalpine forest	nLsoil	Spring-Autumn	0.03	NA	NA	NA	1.71

nLsoil, no litter soil; nRsoil, no root soil; NA, not available

Although monoterpenes can be degraded or adsorbed in soil (Kleinheinz et al., 1999; Ramirez et al., 2009; Albers et al., 2018), the magnitude of uptake rate in the field has rarely been measured. The highest reported uptake rate of monoterpenes in soil was 28.4 μg m^{-2} h^{-1} in a boreal forest, equivalent to 2% of total soil VOC emissions at that site (Staudt et al., 2019). Net uptake of monoterpenes by soil has

been demonstrated in Mediterranean forest soil, where the rate of uptake was highest (12.5 μg m^{-2} h^{-1}) in summer (Asensio et al., 2007b), and a simulated drought treatment in the same type of habitat generally shifted the soil from being a source of monoterpenes to a weak sink (average −0.5 μg m^{-2} h^{-1}) (Asensio et al., 2007c). Uptake of sesquiterpenes and whether they can be directly consumed by microbes is unclear as they are easily oxidized into other compounds in a short time (Tang et al., 2019).

Other VOCs, such as acetaldehyde and methanol are readily taken up by soil (Asensio et al., 2007b; Albers et al., 2018; Bachy et al., 2018). Field studies showed that cropland soil takes up methanol at high rates and that the net uptake is high in summer (306.0 μg m^{-2} h^{-1}) and autumn (243.0 μg m^{-2} h^{-1}) (Bachy et al., 2018). The differences in uptake ability between seasons may be due to environmental factors, especially soil-moisture content. For example, soil can switch between releasing methanol when dry and taking up methanol when wet (Bachy et al., 2018; Bourtsoukidis et al., 2018). Regarding other compounds, a study in a subtropical pine forest detected high uptakes of undecane (−66.2 μg m^{-2} h^{-1}) and dodecane (−36.2 μg m^{-2} h^{-1}) by soil during sampling in spring (Zhang et al., 2017). Additionally, 2-hexenal was taken up in the highest amounts in a Mediterranean forest in autumn (−26.0 μg m^{-2} h^{-1}), even under drought conditions, but was not stored in the soil in other seasons (Asensio et al., 2007b; Asensio et al., 2007c).

3.6. Main pathways of atmospheric VOC uptake by soil

The main pathways of how the soil can act as a sink can be divided into abiotic processes (e.g., adsorption and deposition) and biotic processes (e.g., microbial consumption and mineralization). The mechanisms of the uptake of VOCs to soil

likely differ between compounds due to their high heterogeneity and specificity (Cleveland and Yavitt, 1997; Ruiz et al., 1998; Asensio et al., 2007a; Albers et al., 2018; Li et al., 2019). The abiotic processes are primarily driven by concentration gradients and soil properties, which generally do not change the VOCs, and balance is established with the surrounding air concentrations over time (Rinnan and Albers 2020). The biotic processes, however, are mostly the result of microbial activity and VOCs are ultimately degraded to CO_2 (Albers et al., 2018; Carrion et al., 2020).

3.6.1. VOC deposition

An estimated 130−270 Tg C y^{-1} of atmospheric VOCs are deposited on vegetation and soil surfaces (Goldstein and Galbally 2007), which accounts for 17−36% of annual terrestrial vegetation emissions of VOCs (760 Tg C y^{-1}) (Sindelarova et al., 2014). The deposition of VOCs to vegetation is ubiquitous, but it has become clear that deposition onto soil also plays a more important role than previously thought (Goldstein and Galbally, 2007; Hallquist et al., 2009; Laffineur et al., 2012; Park et al., 2013; Spielmann et al., 2016). Atmospheric VOC deposition includes wet deposition (hydrophilic VOCs dissolved in precipitation) and dry deposition (VOCs deposited onto the soil surface) (Nguyen et al., 2015; Rinnan and Albers, 2020). It has been suggested that the process of wet deposition of VOCs is dependent on the physico-chemical properties of atmospheric water and surrounding air temperatures (Šoštarić et al., 2017; Stojić et al., 2019), whereas dry deposition of VOCs is affected by the aboveground plant species, surrounding VOC concentrations and light (Spielmann et al., 2016; Staudt et al., 2019). There are reports of several chemical classes of VOC, including aldehydes, ketones, aromatic hydrocarbons and isoprenoids, being deposited on various types of soil (Asensio et al., 2008; Gray et al., 2014).

3.6.2. Adsorption and dissolution

VOCs can be adsorbed to SOM or dissolved in the vapour and water phases of soil (English and Loehr 1991; Tang et al., 2019). They can be adsorbed by soil directly, or indirectly by first reacting with ozone and hydroxyl radicals on the soil surface (Li et al., 2016). The soil surface area and other properties regulate the capacity of soil to adsorb VOCs (Petersen et al., 1994; Ruiz et al., 1998). A soil surface that is completely covered with water molecules can increase VOCs adsorption and dissolution, and characteristic hydrophilic VOCs such as methanol, ethanol, formaldehyde, and acetone can be adsorbed onto soil at high levels and can then diffuse into the soil water (Li et al., 2016; Bachy et al., 2018). Under dry conditions, however, soil properties such as aggregate structure and content of organic matter are important for affecting the strength of VOCs adsorption (Ruiz et al., 1998; Hamamoto et al., 2009). For example, in dry soil, soil macroaggregates and a high SOC content can increase the adsorption of VOCs (e.g., isohexane) (Van Roon et al., 2005; Hamamoto et al., 2009). Also, the capacity of VOCs to be adsorbed can be an order of magnitude higher for clay than for sand and two orders of magnitude higher than for limestone (Ruiz et al., 1998).

3.6.3. Biodegradation of VOCs by soil microbes

The microbial sink of VOCs in soil is of potentially high importance to both carbon cycling and the atmospheric concentrations of these gases (Owen et al., 2007; Albers et al., 2018). A number of isoprene-degrading bacteria have been detected in various ecosystems (Cleveland and Yavitt 1997; Cleveland and Yavitt 1998; Carrion et al., 2020). For example, the following genera have been linked to isoprene degradation:

Rhodococcus, *Nocardia*, *Arthrobacter*, *Gordonia*, *Mycobacterium*, Leifsonia, *Alcaligenes*, *Alcanivorax*, *Pseudomonas*, *Alcaligenes*, and *Klebsiella* (Gray et al., 2015; El Khawand et al., 2016; Carrion et al., 2020). However, there seem to be no anaerobes, archaea or fungi that have been isolated from soil that can grow on isoprene as a C source (McGenity et al., 2018).

Soil microorganisms seem to be more important for the biodegradation of small compounds with lower reactivity in the atmosphere, such as methanol and methane (Jacob et al., 2005). Methylotrophs are microorganisms that can consume these small compounds as their sole source of C (Kolb, 2009), and soil methylotrophic communities can vary with ecosystem type and soil pH (Stacheter et al., 2013). Methanol uptake in a Mediterranean forest was higher in rhizosphere soil than in bare soil, indicating that rhizosphere microorganisms can consume methanol (Asensio et al., 2007a). A later study reported that the capacity of methylotrophs to oxidise methanol was higher in soil containing roots than in root-free soil (Stacheter et al., 2013), suggesting that methanol uptake may be strong in root-rich soil. Moreover, a recent study by Trowbridge et al. (2020) has uncovered that soil parameters related to different mycorrhizal communities are important for affecting exchanges of VOC, and ectomycorrhizal (EMC) soils exhibited greater uptake of methanol than arbuscular mycorrhiza dominated soil due to high methylotrophic taxa in EMC soil. Although the microbial degradation of VOCs in soil is widely recognized to occur (Albers et al., 2018), it is challenging to estimate the degradation rate due to the variability of rates based on compound specificity and various soil type.

3.7. Role of ecosystem type and seasonality on soil VOCs

Although most studies relating to soil VOCs have been conducted laboratory experiments, soil VOC fluxes have been quantified in the field for various ecosystems including boreal, temperate, Mediterranean, tropical forests and Arctic regions (Zheng et al., 2015; Bachy et al., 2016; Kramshøj et al., 2016; Bourtsoukidis et al., 2018; Mäki et al., 2019; Staudt et al., 2019). Some general patterns are found (Table 1) with boreal and subtropical forest soils being strong emitters of monoterpenes (Zhang et al., 2017; Staudt et al., 2019), whereas tropical forest soils appear to emit more sesquiterpenes (Bourtsoukidis et al., 2018). Cropland, Mediterranean forest and tundra region soils seem to have a high emission of other (non-isoprenoid) VOCs (Asensio et al., 2008; Kramshøj et al., 2016; Bachy et al., 2018), and subalpine forest soil seems to be a low emitter of VOCs in general, neither being a high emitter of isoprenoids nor of other VOCs (Greenberg et al., 2012; Gray et al., 2014; Trowbridge et al., 2020).

The effects of abiotic factors may differ between ecosystems, for example, temperature is a critical environmental parameter that is positively correlated with soil VOC emissions in forest ecosystems (Mäki et al., 2019; Staudt et al., 2019; Trowbridge et al., 2020). Temperature, however, may be less important in cold regions since the only available study measuring the effect of warming on soil VOCs, performed in the Arctic, indicated that an increase in soil temperature did not stimulate soil VOC emissions but decreased them (Kramshøj et al., 2016). That decreasing trend was due to the saturation of the soil caused by the slow draining of meltwater in the soil layer, which slows the diffusion of gases (Kramshøj et al., 2019).

Soil moisture content can have a direct effect on soil VOC fluxes via regulating gas diffusion, microbial enzyme activities, and water availability of roots (Mäki et al., 2019; Kramshoj et al., 2019; Llusia et al.,2021). However, the effect of soil moisture

on soil VOC fluxes varied in different ecosystems. For example, in boreal forest, increasing soil moisture decreased monoterpene fluxes from forest soil as the result of stimulation of microbial VOC uptake in soil and also by increasing VOC dissolution into soil water (Mäki et al., 2019). Conversely, in Mediterranean forest, increases of soil moisture shifted the soil from being a sink of VOCs to a source, and this shift seems significantly related to the activities of plant root and microbes, which boosted the emission of VOCs due to increases in the decomposition of SOM and root activities (Asensio et al., 2007b; Mu et al., 2022).

The emission of VOCs from aboveground sources shows obvious seasonal dynamics (Aaltonen et al., 2012; Mäki et al., 2019), however, the seasonal variations of soil VOCs remain unknown because many studies are short-term investigations, and field fluxes tend to be measured during the growing season (Gray et al., 2014; Bourtsoukidis et al., 2018 Gray et al., 2015; Mäki et al., 2017). The available information suggests that soils may alter between being a sink or a source of VOCs depending on the season (Asensio et al., 2007b; Asensio et al., 2007c; Asensio et al., 2008). Further studies should seek to collect long term measurements, to confirm whether soil switches between being a source and a sink of VOCs in different seasons, in different ecosystem types. These data would also help to determine how much soils contribute to global VOC emissions and to predict how these emissions will change in a warming climate.

3.8. Soil VOCs measurements

Experimental studies typically employ three primary approaches to investigate the composition and flux of VOCs in soil: direct thermal desorption (TD), proton transfer

reaction mass spectrometry (PTR-MS) and soil-phase microextraction (SPME).

To quantitate the flux of soil VOCs, chamber-based techniques coupled to a PTR-MS or adsorbed tubes with a thermal desorption gas chromatography mass spectrometer (TD-GC-MS) were applied in most current studies (Bourtsoukidis et al., 2018; Mäki et al., 2019; Trowbridge et al., 2020). The PTR-MS has high sensitivity and can perform real-time measurements of VOC rate from samples, which have the benefits of allowing us to understand the dynamic of VOCs (Bourtsoukidis et al., 2018). In addition, PTR-MS can distinguish between VOCs of different molecular weights by using quadrupole or time of flight (TOF) mass filters. The TOF mass filter has the advantage of mass resolution as it allows the estimation of the molecular formula of the detected ions. Unfortunately, this method cannot discern compounds with same mass, like monoterpene and sesquiterpene. Therefore, additional sampling method, such as TD-GC-MS, is necessary. GC-MS can identify compounds with same masses (Mäki et al., 2019a, 2019b; Ghirardo et al., 2020; Llusia et al., 2021). The TD-GC-MS use adsorbent tubes and then thermally desorbs them into a GC-MS for subsequent off-line analysis (Ghirardo et al., 2020).

The SPME is easy to use for sampling VOCs in various sources (including soil) for composition analysis. There are four polymers commercially used as SPME stationary phases, including divinylbenzene (DVB), polydimethylsiloxane (PDMS), polyacrylate (PA) and carbowax-polyethylene glycol (PEG), based on the research purposes. One shortcoming of SPME is that it is difficult to quantify the flux of VOCs, therefore, to overcome this, this method can be used in combination with another quantitative method.

3.9. The pattern of soil VOCs under climate change

3.9.1. Warming

Although the emissions of VOCs from plants seem to exhibit a high sensitivity to rising temperatures, studies examining the fluxes of soil VOC emissions from warming are scarce and have shown mixed responses, particularly in the Arctic region (Kramshoj et al., 2016; Peñuelas and Staudt., 2010).

Cold regions are particularly sensitive to climate warming and will likely experience large changes in climate and ecosystem types (Lindwall et al., 2016; Kramshøj et al., 2019). A robust effect of warming increasing the release of aboveground VOCs from vegetation has been reported in many studies, especially in arctic regions (Faubert et al., 2010; Rinnan et al., 2013; Lindwall et al., 2016; Kramshøj et al., 2018), where VOC emissions from understorey vegetation are strong under simulated warming (Faubert et al., 2010; Kramshøj et al., 2016; Lindwall et al., 2016). However, responses in soils may be more complex, as warming combined with high vegetation cover increased fine-root biomass and levels of dissolved organic C in the soil of a healthy subarctic ecosystem (Faubert et al., 2010; Valolahti et al., 2015), which in turn may increase both the microbial synthesis and consumption of belowground VOCs. Warming also directly stimulates soil microorganisms, further decreasing the emission of soil VOCs due to consumption by microbes (Kramshøj et al., 2018). Warming affects soil VOC dynamics by adsorbing VOCs onto clay particles and SOM during freeze-thaw cycles, which can reduce VOC emissions (Insam and Seewald, 2010; Kramshøj et al., 2016; Kramshøj et al., 2018; Kramshøj et al., 2019). Experimental soil warming in the tundra found that warming did not increase the emission of VOCs from bare soil but slightly decreased total VOCs (Kramshøj et al., 2016). Another study also found no increase in belowground VOCs under 2 °C of warming in a pot experiment

(Tiiva et al., 2019). Therefore, the warming response of VOCs from soil may not be in accordance with patterns shown by aboveground sources.

3.9.2. Drought

Drought negatively affects the rates of litter decomposition, microbial activity, and plant growth, which in turn directly and indirectly affects the diversity and size of VOC fluxes between the soil and the atmosphere (Lindwall et al., 2016; de Vries et al., 2019; Tiiva et al., 2019). For example, the decreasing rate of litter decomposition and plant growth under drought will reduce the emission of VOCs, whereas the effect of decreasing soil microbial activity on VOC emission depend on the capacity of that microbial community to produce or consume VOCs in that soil. A decrease in soil-moisture content of bare soil in a lab incubation showed a strong increase of the VOCs emission, such as terpenoids (Raza et al., 2017). Field experiments, however, have suggested that the response of soil VOC fluxes to drought vary over seasons due to changes in soil biotic and abiotic factors (Asensio et al., 2007c; Allison et al., 2013; Trowbridge et al., 2020). For example, more C is estimated to be released from plants into soil under drought conditions as exudates from roots (Preece et al., 2018). This may stimulate soil microbial activity and strengthen microbial respiration (de Vries et al., 2019), in contrast to the direct effect of low soil moisture on microbes. High microbial activity could thus increase the acquisition and consumption of soil VOCs from the atmosphere by microorganisms and thereby decrease emission rates from the soil (Asensio et al., 2007c). Lastly, VOC emissions from aboveground plant tissues have been shown to increase under drought (Peñuelas and Staudt., 2010), and this increase in the concentration of atmospheric VOCs may form a larger concentration gradient for specific VOCs between the atmosphere and the soil, impacting VOC

exchanges (Llusià et al., 2021). For instance, a large concentration gradient between the soil and atmosphere could increase the ability of soil to take up VOCs by organic adsorption and microbial biodegradation (Pegoraro et al., 2005, 2006; Spielmann et al., 2016). Thus, increasing future droughts may strengthen the capacity of soil to act as a sink of VOCs in some ecosystems, even though drought could accelerate VOC emissions from aboveground vegetation (Asensio et al., 2007c; Peñuelas and Staudt, 2010).

3.9.3. Nitrogen deposition

Atmospheric nitrogen elements, mainly in the forms of NH_x and NO_x, enter the soil through dry and wet deposition. An increase in nitrogen deposition could relieve nitrogen limitation and increase aboveground net primary productivity in most ecosystems but may also negatively affect soil ecosystems. A meta-analysis demonstrated that nitrogen addition negatively affected bacterial and fungal diversity and decreased soil microbial diversity (Wang et al., 2018). VOC sources in soil respond differently to nitrogen deposition, and the exchange of soil VOCs also differs depending on the ecosystem (Gray and Fierer, 2012; Zheng et al., 2015; Zhang et al., 2017; Tiiva et al., 2019). An incubation study reported that the effect of nitrogen addition on VOCs emitted from litter was determined by plant type and that nitrogen addition generally decreased litter-derived VOC emissions (Gray and Fierer 2012). In an example of subtropical forest, soil VOC exchanges of two distinct tree types showed different pattens under nitrogen addition; nitrogen addition significantly shifted a subtropical broadleaf forest from being a source of benzenoids (benzene and toluene) to a sink (Zheng et al., 2015), whereas a pine forest was a source of alkanes and a sink of alkenes under nitrogen addition (Zhang et al., 2017). Additionally, some certain compounds can

be released from soil due to nitrogen addition. For example, a study analyzing soil VOC emissions in a nitrogen fertilized soil detected that some specific VOCs (e.g., p-xylene, β-thujone, 1-hydroxy-2-propanone and 2-phenylindolizine) were only present in fertilized but not control soils (Christodoulou et al., 2021).

The effect of the interaction between nitrogen deposition and other factors on soil VOC emissions should not be ignored, as temperature, moisture content and pH may synergistically affect soil VOC emission (Llusia et al., 2021). For example, soil pH changes caused by nitrogen deposition could affect the sources and sinks of VOCs (Asensio et al., 2007b; Kieloaho et al., 2017). Soil shifted from being a source to a sink of dimethylamine and diethylamine as soil pH increased (Kieloaho et al., 2017). An investigation of the impacts of soil pH on the sorption of 25 VOCs found that alkaline soils adsorbed more compounds than acidic soils (Serrano and Gallego, 2006).

Nitrogen addition has been shown to impact soil respiration through the regulation of plant and microbial activities (Janssens et al., 2010). The effects of nitrogen addition on soil CO_2 emissions can be positive, negative, or neutral, suggesting that the responses of soil VOCs to nitrogen in different ecosystems are also diverse, given the direct association between CO_2 and VOCs in the soil with soil microorganisms and enzymatic reactions (Bourtsoukidis et al., 2018). The increase in soil CO_2 levels resulting from nitrogen addition, indicating an enhanced microbial biomass, may lead to a decrease in VOC emissions, as VOCs are consumed by soil microbial communities (Owen et al., 2007; Trowbridge et al., 2020).

3.10. Conclusions

The exchange of volatile organic compounds between the soil and the atmosphere is a

significant research topic that has been overlooked. Recent studies have demonstrated that soils can be both strong emitters and considerable sinks of VOCs, with fluxes varying across different soil ecosystems and seasons. It is crucial to gather more in-situ data in various soil ecosystems to better understand the source-sink balance of VOCs and the influence of environmental factors. The composition of VOCs in soil originates from different sources such as litter, roots, and soil microbes, and exhibits a diverse and rich mixture. While certain compounds are shared among sources, the majority of VOCs are unique to each source. Litter VOCs are primarily composed of terpenes and benzenoids, root VOCs consist mainly of terpenes and alcohols, and VOCs emitted from soil organic matter (SOM) and soil microbes are less dominated by terpenes, with esters, alcohols, and ketones being important VOC classes. To accurately estimate global VOC emissions and improve existing global BVOCs models, it is essential to further investigate the magnitude and function of soil as a source and sink of VOCs.

Declaration on interest

All authors declare that there are no conflicts of interest relevant to this work.

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References

Aaltonen, H., Aalto, J., Kolari, P., Pihlatie, M., Pumpanen, J., Kulmala, M., Nikinmaa, E., Vesala, T., Bäck, J., 2013. Continuous VOC flux measurements on boreal forest floor. Plant Soil 369, 241–256.

Aaltonen, H., Pumpanen, J., Pihlatie, M., Hakola, H., Hellén, H., Kulmala, L., Vesala, T., Bäck, J., 2011. Boreal pine forest floor biogenic volatile organic compound emissions peak in early summer and autumn. Agric. For. Meteorol. 151, 682–691.

Abis, L., Loubet, B., Ciuraru, R., Lafouge, F., Houot, S., Nowak, V., Tripied, J., Dequiedt, S., Maron, P.A., Sadet-Bourgeteau, S., 2020. Reduced microbial diversity induces larger volatile organic compound emissions from soils. Sci. Rep. 10, 6104.

Acosta Navarro, J.C., Smolander, S., Struthers, H., Zorita, E., Ekman, A.M., Kaplan, J.O., Guenther, A., Arneth, A., Riipinen, I., 2014. Global emissions of terpenoid VOCs from terrestrial vegetation in the last millennium. J. Geophys. Res: atmos. 119, 6867–6885.

Albers, C.N., Kramshøj, M., Rinnan, R., 2018. Rapid mineralization of biogenic volatile organic compounds in temperate and Arctic soils. Biogeosciences 15, 3591–3601.

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder KK, Martiny JB., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–25.

Andreae, M.O., Crutzen, P.J., 1997. Atmospheric Aerosols: Biogeochemical Sources and Role in Atmospheric Chemistry. Science 276, 1052–1058.

Asensio, D., Peñuelas, J., Filella, I., Llusià, J., 2007. On-line screening of soil VOCs exchange responses to moisture, temperature and root presence. Plant Soil 291,

249–261.

Asensio, D., Peñuelas, J., Llusià, J., Ogaya, R., Filella, I., 2007. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. Soil Biol. Biochem. 39, 2471–2484.

Asensio, D., Peñuelas, J., Ogaya, R., Llusia, J., 2007. Seasonal soil VOC exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmos. Environ. 41, 2456–2466.

Asensio, D., Peñuelas, J., Prieto, P., Estiarte, M., Filella, I., Llusià, J., 2008. Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland. Eur. J. Soil Sci. 59, 878–891.

Atkinson, R., 2000. Atmospheric chemistry of VOCs and NOx. Atmos. Environ. 34, 2063–2101.

Bachy, A., Aubinet, M., Amelynck, C., Schoon, N., Bodson, B., Moureaux, C., Delaplace, P., De Ligne, A., Heinesch, B., 2018. Methanol exchange dynamics between a temperate cropland soil and the atmosphere. Atmos. Environ. 176, 229–239.

Bachy, A., Aubinet, M., Schoon, N., Amelynck, C., Bodson, B., Moureaux, C., Heinesch, B., 2016. Are BVOC exchanges in agricultural ecosystems overestimated? Insights from fluxes measured in a maize field over a whole growing season. Atmos. Chem. Phys. 16, 5343–5356.

Bäck, J., Aaltonen, H., Hellén, H., Kajos, M.K., Patokoski, J., Taipale, R., Pumpanen, J., Heinonsalo, J., 2010. Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. Atmos. Environ. 44, 3651–3659.

Bourtsoukidis, E., Behrendt, T., Yañez-Serrano, A.M., Hellén, H., Diamantopoulos, E.,

Catão, E., Ashworth, K., Pozzer, A., Quesada, C.A., Martins, D.L., Sá, M., Araujo, A., Brito, J., Artaxo, P., Kesselmeier, J., Lelieveld, J., Williams, J., 2018. Strong sesquiterpene emissions from Amazonian soils. Nat. Commun. 9, 2226.

Carrión, O., Gibson, L., Elias, D.M.O., McNamara, N.P., van Alen, T.A., Op, den Camp H.J.M., Supramaniam, C.V., McGenity, T.J., Murrell, J.C., 2020. Diversity of isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation. Microbiome 8, 81.

Christodoulou, E., Agapiou, A., Omirou, M., Ioannides, I.M., 2021. Profiling soil volatile organic compounds after N fertilization in a soil grown with Rosmarinus officinalis. Appl. Soil. Ecol. 164, 103934.

Cleveland, C.C., Yavitt, J.B., 1997. Consumption of atmospheric isoprene in soil. Geophys. Res. Lett. 24, 2379–2382.

Cleveland, C.C., Yavitt, J.B., 1998. Microbial consumption of atmospheric isoprene in a temperate forest soil. Appl. Environ. Microbiol. 64, 172–177.

de Vries, F.T., Williams, A., Stringer, F., Willcocks, R., McEwing, R., Langridge, H., Straathof, A.L., 2019. Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. New Phytol. 224, 132–145.

Delory, B.M., Delaplace, P., Fauconnier, M.L., du Jardin, P., 2016. Root-emitted volatile organic compounds: can they mediate belowground plant-plant interactions? Plant Soil 402, 1–26.

El Khawand, M., Crombie, AT., Johnston, A., Vavlline, D.V., McAuliffe, J.C., Latone, J.A., Primak, Y.A., Lee, S.K., Whited, G.M., McGenity, T.J., Murrell, J.C., 2016. Isolation of isoprene degrading bacteria from soils, development of isoA gene probes and identification of the active isoprene-degrading soil community using

DNA-stable isotope probing. Environ. Microbiol. 18, 2743–2753.

English, C.W., Loehr, R.C., 1991. Degradation of organic vapors in unsaturated soils. J. Hazard. Mater. 28, 55–64.

Faubert, P., Tiiva, P., Rinnan, A., Michelsen, A., Holopainen, J.K, Rinnan, R., 2010. Doubled volatile organic compound emissions from subarctic tundra under simulated climate warming. New Phytol. 187, 199–208.

Ghirardo, A., Lindstein, F., Koch, K., Buegger, F., Schloter, M., Albert, A., Michelsen, A., Winkler, J.B., Schnitzler, J., Rinnan, R., 2020. Origin of volatile organic compound emissions from subarctic tundra under global warming. Glob. Chang. Biol. 26, 1908–1925.

Goldstein, A.H., Galbally, I.E., 2007. Known and Unexplored Organic Constituents in the Earth's Atmosphere. Environ. Sci. Technol. 41, 1514–1521.

Gray, C.M., Fierer, N., 2012. Impacts of nitrogen fertilization on volatile organic compound emissions from decomposing plant litter. Global. Chang. Biol. 18, 739–748.

Gray, C.M., Helmig, D., Fierer, N., 2015. Bacteria and fungi associated with isoprene consumption in soil. Elem. Sci. Anthr., 3, 000053.

Gray, C.M., Monson, R.K., Fierer, N., 2010. Emissions of volatile organic compounds during the decomposition of plant litter. J. Geophys. Res. 115, G03015.

Gray, C.M., Monson, R.K., Fierer, N., 2014. Biotic and abiotic controls on biogenic volatile organic compound fluxes from a subalpine forest floor: Controls on BVOC fluxes from forest soil. J. Geophys. Res: Biogeosciences 119, 547–556.

Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5, 29–56.

Greenberg, J.P., Asensio, D., Turnipseed, A., Guenther, A.B., Karl, T., Gochis, D., 2012. Contribution of leaf and needle litter to whole ecosystem BVOC fluxes. Atmos. Environ. 59, 302–311.

Guenther, A.B., Jiang, X., Heald, C.L., Sakulyanontvittaya, T., Duhl, T., Emmons, L.K., Wang, X., 2012. The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. Geosci. Model. Dev. 5, 1471–1492.

Guenther, A.B., Zimmerman, P.R., Harley, P.C., Monson, R.K., Fall, R., 1993. Isoprene and monoterpene emission rate variability: Model evaluations and sensitivity analyses. J. Geophys. Res. 98, 12609–12617.

Hallquist, M., Wenger, J.C., Baltensperger, U., Rudich, Y., Simpson, D., Claeys, M., Dommen, J., Donahue, N.M., George, C., Goldstein, A.H., Hamilton, J.F., Herrmann, H., Hoffmann, T., Iinuma, Y., Jang, M., Jenkin, M.E., Jimenez, J.L., Kiendler-Scharr, A., Maenhaut, W., McFiggans, G., Mentel, T.F., Monod, A., Prevot, A.S.H., Seinfeld, J.H., Surratt, J.D., Szmigielski, R., Wildt, J., 2009. The formation, properties and impact of secondary organic aerosol: current and emerging issues. Atmos. Chem. Phys. 9, 5155–5236.

Hamamoto, S., Seki, K., Miyazaki, T., 2009. Effect of aggregate structure on VOC gas adsorption onto volcanic ash soil. J. Hazard. Mater. 166, 207–212.

Hayward, S., Muncey, R.J., James, A.E., Halsall, C.J., Hewitt, C.N., 2001. Monoterpene emissions from soil in a Sitka spruce forest. Atmos. Environ. 35, 4081–4087.

Horváth, E., Hoffer, A., Sebők, F., Dobolyi, C., Szoboszlay, S., Kriszt, B., Gelencsér A., 2011. Microscopic fungi as significant sesquiterpene emission sources. J. Geophys. Res. 116, D16301.

Horváth, E., Hoffer, A., Sebők, F., Dobolyi, C., Szoboszlay, S., Kriszt, B., Gelencsér, A., 2012. Experimental evidence for direct sesquiterpene emission from soils. J. Geophys. Res: Atmos. 117, D15304.

Insam, H., Seewald, M.S.A., 2010. Volatile organic compounds (VOCs) in soils. Biol. Fertil. Soils 46, 199–213.

Isidorov, V., Tyszkiewicz, Z., Pirożnikow, E., 2016. Fungal succession in relation to volatile organic compounds emissions from Scots pine and Norway spruce leaf litter-decomposing fungi. Atmos. Environ. 131, 301–306.

Jacob, D.J., Field, B.D., Li, Q.B., Blake, D.R., de Gouw, J., Warneke, C., Hansel, A., Wisthaler, A., Singh, H.B., Guenther, A., 2005. Global budget of methanol: Constraints from atmospheric observations. J. Geophys. Res. 110, D08303.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3, 315–322.

Kainulainen, P., Holopainen, J.K., 2002. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. Soil Biol. Biochem. 34, 37–42.

Kieloaho, A.J., Pihlatie, M., Launiainen, S., Kulmala, M., Riekkola, M.L., Parshintsev, J., Mammarella, I., Vesala, T., Heinonsalo, J., 2017. Soil concentrations and soil–atmosphere exchange of alkylamines in a boreal Scots pine forest. Biogeosciences 14, 1075–1091.

Kleinheinz, G.T., Bagley, S.T., St John, W.P., Rughani, J.R., McGinnis, G.D., 1999. Characterization of alpha-pinene-degrading microorganisms and application to a bench-scale biofiltration system for VOC degradation. Arch. Environ. Contam.

Toxicol. 37, 151–157.

Kolb, S., 2009. Aerobic methanol-oxidizing bacteria in soil. Fems. Microbiol. Lett. 300, 1–10.

Kramshøj, M., Albers, C.N., Holst, T., Holzinger, R., Elberling, B., Rinnan, R., 2018. Biogenic volatile release from permafrost thaw is determined by the soil microbial sink. Nat. Commun. 9, 3412.

Kramshøj, M., Albers, C. N., Svendsen, S. H., Björkman, M. P., Lindwall, F., Björk, R. G., Rinnan, R., 2019. Volatile emissions from thawing permafrost soils are influenced by meltwater drainage conditions. Glob. Chang. Biol. 25(5), 1704–1716.

Kramshøj, M., Vedel-Petersen, I., Schollert, M., Rinnan, Å., Nymand, J., Ro-Poulsen, H., Rinnan, R., 2016. Large increases in Arctic biogenic volatile emissions are a direct effect of warming. Nat. Geosci. 9, 349–352.

Laffineur, Q., Aubinet, M., Schoon, N., Amelynck, C., Müller, J.F., Dewulf, J., Van Langenhove, H., Steppe, K., Heinesch, B., 2012. Abiotic and biotic control of methanol exchanges in a temperate mixed forest. Atmos Chem Phys 12, 577–590.

Leff, J.W., Fierer, N., 2008. Volatile organic compound (VOC) emissions from soil and litter samples. Soil Biol. Biochem. 40, 1629–1636.

Lelieveld, J., Butler, T.M., Crowley, J.N., Dillon, T.J., Fischer, H., Ganzeveld, L., Harder, H., Lawrence, M.G., Martinez, M., Taraborrelli, D., Williams, J., 2008. Atmospheric oxidation capacity sustained by a tropical forest. Nature 452, 737–740.

Lemfack, M.C., Gohlke, B.O., Toguem, S.M.T., Preissner, S., Piechulla, B., Preissner, R., 2017. mVOC 2.0: a database of microbial volatiles. Nucleic Acids Res. 46, 1261–1265.

Lemfack, M.C., Nickel, J., Dunkel, M., Preissner, R., Piechulla, B., 2014. mVOC: a

database of microbial volatiles. Nucleic Acids Res. 42, D744-748.

Lewinsohn, E., Gijzen, M., Croteau, R., 1991. Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. Plant Physiol. 96, 44–49.

Li, G., Cheng, Y., Kuhn, U., Xu, R., Yang, Y., Meusel, H., Wang, Z., Ma, N., Wu, Y., Li, M., Williams, J., Hoffmann, T., Ammann, M., Pöschl, U., Shao, M., Su, H., 2019. Physicochemical uptake and release of volatile organic compounds by soil in coated-wall flow tube experiments with ambient air. Atmos. Chem. Phys. 19, 2209–2232.

Li, G., Su, H., Li, X., Kuhn, U., Meusel, H., Hoffmann, T., Ammann, M., Pöschl, U., Shao, M., Cheng, Y., 2016. Uptake of gaseous formaldehyde by soil surfaces: a combination of adsorption/desorption equilibrium and chemical reactions. Atmos. Chem. Phys. 16, 10299–10311.

Lin, C., Owen, S., Peñuelas, J., 2007. Volatile organic compounds in the roots and rhizosphere of Pinus spp. Soil Biol. Biochem. 39, 951–960.

Lindwall, F., Schollert, M., Michelsen, A., Blok, D., Rinnan, R., 2016. Fourfold higher tundra volatile emissions due to arctic summer warming. J. Geophys. Res: Biogeosci 121, 895–902.

Llusià, J., Asensio, D., Sardans, J., Filella, I., Peguero, G., Grau, O., Ogaya, R., Gargallo-Garriga, A., Verryckt, L.T., Van Langenhove, L., Brechet, L.M., Courtois, E., Stahl, C., Janssens, I.A., Peñuelas, J., 2022. Contrasting nitrogen and phosphorus fertilization effects on soil terpene exchanges in a tropical forest. Sci. Total Environ. 802, 149769.

Mäki, M., Aalto, J., Hellén, H., Pihlatie, M., Bäck, J., 2019. Interannual and Seasonal Dynamics of Volatile Organic Compound Fluxes From the Boreal Forest Floor.

Front. Plant. Sci. 10, 191.

Mäki, M., Aaltonen, H., Heinonsalo, J., Hellén, H., Pumpanen, J., Bäck, J., 2019. Boreal forest soil is a significant and diverse source of volatile organic compounds. Plant Soil 441, 89–110.

Mäki, M., Heinonsalo, J., Hellén, H., Bäck, J., 2017. Contribution of understorey vegetation and soil processes to boreal forest isoprenoid exchange. Biogeosciences 14, 1055–1073.

Mancuso, S., Taiti, C., Bazihizina, N., Costa, C., Menesatti, P., Giagnoni, L., Arenella, M., Nannipieri, P., Renella, G., 2015. Soil volatile analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TOF-MS). Appl. Soil Ecol. 86, 182–191.

McBride, S.G., Osburn, E.D., Barrett, J.E., Strickland, M.S., 2019. Volatile methanol and acetone additions increase labile soil carbon and inhibit nitrification. Biogeochemistry 145, 127–140.

McGenity, T.J., Crombie, A.T., Murrell, J.C., 2018. Microbial cycling of isoprene, the most abundantly produced biological volatile organic compound on Earth. ISME J 12, 931–941.

Mielnik, A., Link, M., Mattila, J., Fulgham, S.R., Farmer, D.K., 2018. Emission of formic and acetic acids from two Colorado soils. Environ. Sci. Process. Impacts. 20, 1537–1545.

Nguyen, T,B., Crounse, J.D., Teng, A.P., St Clair, J.M., Paulot, F., Wolfe, G.M., Wennberg, P.O., 2015. Rapid deposition of oxidized biogenic compounds to a temperate forest. PNAS 112, E392-401.

Owen, S.M., Clark, S., Pompe, M., Semple, K.T., 2007. Biogenic volatile organic compounds as potential carbon sources for microbial communities in soil from the

rhizosphere of Populus tremula. Fems Microbio. Lett. 268, 34–39.

Park, J.H., Goldstein, A.H., Timkovsky, J., Fares, S., Weber, R., Karlik, J., Holzinger, R., 2013. Active atmosphere-ecosystem exchange of the vast majority of detected volatile organic compounds. Science 341, 643–7.

Pegoraro, E., Abrell, L., Van Haren, J., Barron-Gafford, G., Grieve, K.A., Malhi, Y., Murthy, R., Lin, G., 2005. The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm. Glob. Chang. Biol. 11, 1234–1246.

Peñuelas, J., Asensio, D., Tholl, D., Wenke, K., Rosenkranz, M., Piechulla, B., Schnitzler, J.P., 2014. Biogenic volatile emissions from the soil. Plant Cell Environ. 37, 1866–1891.

Peñuelas, J., Staudt, M., 2010. BVOCs and global change. Trends Plant Sci. 15, 133–44.

Petersen, L.W., Rolston, D.E., Moldrup, P., Yamaguchi, T., 1994. Volatile Organic Vapor Diffusion and Adsorption in Soils. J. Environ. Qual. 23, 799–805.

Preece, C., Farre-Armengol, G., Llusia, J., Peñuelas, J., 2018. Thirsty tree roots exude more carbon. Tree Physiol. 38, 690–695.

Ramirez, K.S., Lauber, C.L., Fierer, N., 2010. Microbial consumption and production of volatile organic compounds at the soil-litter interface. Biogeochemistry 99, 97–107.

Raza, W., Mei, X., Wei, Z., Ling, N., Yuan, J., Wang, J., Huang, Q., Shen, Q., 2017. Profiling of soil volatile organic compounds after long-term application of inorganic, organic and organic-inorganic mixed fertilizers and their effect on plant growth. Sci. Total Environ. 607–608, 326-338.

Rinnan, R., Albers, C.N., 2020. Soil uptake of volatile organic compounds: Ubiquitous

and underestimated? J. Geophys. Res. Biogeosci. 125(6), e2020JG005773.

Rinnan, R., Gierth, D., Bilde, M., Rosenørn, T., Michelsen, A., 2013. Off-season biogenic volatile organic compound emissions from heath mesocosms: responses to vegetation cutting. Front. Microbio., 4, 224.

Ruiz, J., Bilbao, R., Murillo, M.B., 1998. Adsorption of Different VOC onto Soil Minerals from Gas Phase: Influence of Mineral, Type of VOC, and Air Humidity. Environ. Sci. Technol. 32, 1079–1084.

Schade, G.W., Goldstein, A.H., 2001. Fluxes of oxygenated volatile organic compounds from a ponderosa pine plantation. J. Geophys. Res: Atmo. 106, 3111–3123.

Schenkel, D., Lemfack, M.C., Piechulla, B., Splivallo, R., 2015. A meta-analysis approach for assessing the diversity and specificity of belowground root and microbial volatiles. Front Plant Sci. 6, 707.

Seewald, M.S.A., Singer, W., Knapp, B.A., Franke-Whittle, I.H., Hansel, A., Insam, H., 2010. Substrate-induced volatile organic compound emissions from compost-amended soils. Biol. Fert. Soils 46, 371–382.

Serrano, A., Gallego, M., 2006. Sorption study of 25 volatile organic compounds in several Mediterranean soils using headspace-gas chromatography-mass spectrometry. J. Chromatogr. A., 1118, 261–270.

Sindelarova, K., Granier, C., Bouarar, I., Guenther, A., Tilmes, S., Stavrakou, T., Müller, J.F., Kuhn, U., Stefani, P., Knorr, W., 2014. Global data set of biogenic VOC emissions calculated by the MEGAN model over the last 30 years. Atmos. Chem. Phys. 14, 9317–9341.

Šoštarić, A., Stanišić Stojić, S., Vuković, G., Mijić, Z., Stojić, A., Gržetić, I., 2017. Rainwater capacities for BTEX scavenging from ambient air. Atmos. Environ. 168, 46–54.

Spielmann, F.M., Langebner, S., Ghirardo, A., Hansel, A., Schnitzler, J.P., Wohlfahrt, G., 2017. Isoprene and α-pinene deposition to grassland mesocosms. Plant Soil 410, 313–322.

Stacheter, A., Noll, M., Lee, C.K., Selzer, M., Glowik, B., Ebertsch, L., Mertel, R., Schulz, D., Lampert, N., Drake, H.L., Kolb, S., 2013. Methanol oxidation by temperate soils and environmental determinants of associated methylotrophs. ISME J 7, 1051–64.

Staudt, M., Byron, J., Piquemal, K., Williams, J., 2019. Compartment specific chiral pinene emissions identified in a Maritime pine forest. Sci. Total Environ. 654, 1158–1166.

Stojić, A., Stanić, N., Vuković, G., Stanišić, S., Perišić, M., Šoštarić, A., Lazić, L., 2019. Explainable extreme gradient boosting tree-based prediction of toluene, ethylbenzene and xylene wet deposition. Sci. Total Environ. 653, 140–147.

Svendsen, S.H., Priemé, A., Voriskova, J., Kramshøj, M., Schostag, M., Jacobsen, C.S., Rinnan, R., 2018. Emissions of biogenic volatile organic compounds from arctic shrub litter are coupled with changes in the bacterial community composition. Soil Biol. Biochem. 120, 80–90.

Tang, J., Schurgers, G., Rinnan, R., 2019. Process Understanding of Soil BVOC Fluxes in Natural Ecosystems: A Review. Rev. Geophys. 57, 966–986.

Tiiva, P., Julkunen-Tiitto, R., Häikiö, E., Kasurinen, A., 2019. Belowground responses of Scots pine (*Pinus sylvestris*) seedlings to experimental warming, moderate nitrogen addition, and bark herbivory. Can. J. For. Res. 49, 647–660.

Trowbridge, A.M., Stoy, P.C., Phillips, R.P., 2020. Soil Biogenic Volatile Organic Compound Flux in a Mixed Hardwood Forest: Net Uptake at Warmer Temperatures and the Importance of Mycorrhizal Associations. J. Geophys. Rer: Biogeosci. 125.

Tsuruta, J., Okumura, M., Makita, N., Kosugi, Y., Miyama, T., Kume, T., Tohno, S., 2018. A comparison of the biogenic volatile organic compound emissions from the fine roots of 15 tree species in Japan and Taiwan. J. For. Res. 23, 242–251.

Tunved, P., Hansson, H.C., Kerminen, V.M., Strom, J., Maso, M.D., Lihavainen, H., Viisanen, Y., Aalto, P.P., Komppula, M., Kulmala, M., 2006. High natural aerosol loading over boreal forests. Science 312, 261–3.

Valolahti, H., Kivimäenpää, M., Faubert, P., Michelsen, A., Rinnan, R., 2015. Climate change-induced vegetation change as a driver of increased subarctic biogenic volatile organic compound emissions. Glob. Chang. Biol. 21, 3478–3488.

Van Roon, A., Parsons, J.R., Krap, L., Govers, H.A., 2005. Fate and transport of monoterpenes through soils. Part II: calculation of the effect of soil temperature, water saturation and organic carbon content. Chemosphere 61, 129–138.

Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol. Biochem. 120, 126–133.

Warneke, C., Karl, T., Judmaier, H., Hansel, A., Jordan, A., Lindinger, W., Crutzen, PJ., 1999. Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: Significance for atmospheric HOxchemistry. Glob. Biogeochem. Cycles 13, 9–17.

Weikl, F., Ghirardo, A., Schnitzler, J.P., Pritsch, K., 2016. Sesquiterpene emissions from Alternaria alternata and Fusarium oxysporum: Effects of age, nutrient availability, and co-cultivation. Sci. Rep. 6.

Williams, J., 2004. Organic Trace Gases in the Atmosphere: An Overview. Environ. Chem. 1, 125–136.

Yi, Z., Wang, X., Ouyang, M., Zhang, D., Zhou, G., 2010. Air-soil exchange of dimethyl

sulfide, carbon disulfide, and dimethyl disulfide in three subtropical forests in south China. J. Geophys. Res. 115, D18302.

Zhang, L.L., Yin, L., Zheng, L.L., Huang, X.R., Yi, Z.G., 2017. Effect of simulated nitrogen deposition on alkane and alkene fluxed from forest floor at Dinghuashan. Chinese J Ecol. 36, 3462–3469.

Zheng, L.L., Guo, P.P., Yi, Z.G., 2015. Responses of Soil BTEX Fluxes to Simulated Nitrogen Deposition in Two Dominated Forests of Dinghushan, China. Ecol. Environ. Sci. 24, 396–401.

CHAPTER 4. Impacts of seasonality, drought, nitrogen fertilization, and litter on soil fluxes of biogenic volatile organic compounds in a Mediterranean forest

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Abstract

Biogenic volatile organic compounds (BVOCs) play critical roles in ecosystems at various scales, influencing above- and below-ground interactions and contributing to the atmospheric environment. Nonetheless, there is a lack of research on soil BVOC fluxes and their response to environmental changes. This study aimed to investigate the impact of drought, nitrogen (N) fertilization, and litter manipulation on soil BVOC fluxes in a Mediterranean forest. We assessed the effects of drought and N fertilization on soil BVOC exchanges and soil CO_2 fluxes over two consecutive years using a dynamic chamber method, and solid-phase microextraction was utilized to quantify soil BVOCs in one year. Our findings revealed that the soil acted as an annual net sink for isoprenoids (1.30-10.33 μg m^{-2} h^{-1}), with the highest uptake rates observed during summers (25.90 ± 9.36 μg m^{-2} h^{-1}). The increased summer uptake can be attributed to the significant concentration gradient of BVOCs between atmosphere and soil. However, strong seasonal dynamics were observed, as the soil acted as a source of BVOCs in spring and autumn. The uptake rate of isoprenoids exhibited a significant positive correlation with soil temperature and atmospheric isoprenoid concentrations, while displaying a negative correlation with soil moisture and soil CO_2 flux. The effects of drought and N fertilization on soil BVOCs were influenced by the type of VOCs, litter layer, and season. Specifically, drought significantly affected the exchange rate and quantities of sesquiterpenes. N fertilization led to increased emissions of specific BVOCs (α-pinene and camphene) due to the stimulation of litter emissions. These findings underscore the importance of the soil as a sink for atmospheric BVOCs in this dry Mediterranean ecosystem. Future drought conditions may significantly impact soil water content, resulting in drier soils throughout the year, which will profoundly affect the exchange of soil BVOCs between the soil and atmosphere.

Keywords: Dynamic chamber method; Solid-phase microextraction; Holm oak forest; Atmospheric BVOCs; Soil BVOCs exchange.

4.1. Introduction

Biogenic volatile organic compounds (BVOCs) are a diverse group of hydrocarbons of biological origin that strongly influence the chemical and physical properties of the atmosphere (Arneth et al., 2010; Guenther et al., 1995). BVOCs are naturally emitted by various sources, including terrestrial vegetation, soils, and sediments ((Guenther et al., 1995; Tang et al., 2019; Yang et al., 2021). Vegetation is responsible for over 90% of BVOC emissions, resulting in the release of 1-1.5 Pg of carbon into the atmosphere annually (Geron et al., 2006; Guenther et al., 1995). Isoprenoids, including isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$), and sesquiterpenes ($C_{15}H_{24}$), represent the most abundant BVOCs with significant implications for both the atmospheric and terrestrial environment, as well as ecological interactions. These compounds exhibit high reactivity to ozone and play vital roles in multiple interactions, such as plant-plant, plant-animal, plant-microbe, and microbe-microbe communication (Geron et al., 2006; Peñuelas et al., 2014; Sindelarova et al., 2014). Most research on BVOC emissions has focused on forest canopies and understoreys (Aaltonen et al., 2013; Aaltonen et al., 2011; Mäki et al., 2019a; Wang et al., 2018b). Emerging evidence, however, indicates that BVOC emissions from different types of soils vary and respond differently to climate change (Bourtsoukidis et al., 2018; Huang et al., 2021; Mäki et al., 2017, 2019b; Rasheed et al., 2021; Tiiva et al., 2019), which needs further investigation.

BVOCs in soil are primarily produced by roots (Kesselmeier and Staudt, 1999; Stotzky and Schenck, 1976), litter (Leff and Fierer, 2008; Staudt et al., 2019), and

microorganisms (Insam and Seewald, 2010; Peñuelas et al., 2014). A growing body of research has described the amount of emissions from soil and has indicated the significance of forest type, soil characteristics, and climatic factors (Asensio et al., 2007a; Bourtsoukidis et al., 2018; Gray et al., 2014; Staudt et al., 2019). Soil BVOC fluxes are bidirectional, although it remains uncertain whether soil primarily acts as a net sink or a net source (Asensio et al., 2007a; Asensio et al., 2007b; Gray et al., 2014; Llusià et al., 2022; Rinnan and Albers, 2020; Trowbridge et al., 2020). Soil can act as a sink of BVOCs through multiple processes (Tang et al., 2019), including biological processes (primarily that soil microorganisms utilize VOCs as energy sources; Albers et al., 2018; Asensio et al., 2012; Carrion et al., 2020; Ramirez et al., 2010), physical processes (BVOCs absorbed/dissolved by soil clay minerals and soil water; Bachy et al., 2018; Li et al., 2016), and chemical processes (reactions with other compounds; Insam and Seewald, 2010), but these processes are much less studied. Moreover, the balance between sources and sinks of BVOCs needs to be further understood (Rinnan and Albers, 2020).

The exchanges of soil BVOCs are dependent on several biological processes and physical environmental factors. For example, increases in atmospheric BVOC concentrations can create a larger gradient between the atmosphere and soil, thereby influencing BVOC exchanges (Asensio et al., 2007a; Llusià et al., 2022). Other studies have demonstrated that drought can significantly alter the structure of soil microbial communities (Abis et al., 2020; Pegoraro et al., 2006; Preece et al., 2019) and impact root traits (de Vries et al., 2019), both of which strongly affect soil BVOC emissions (Kleiber et al., 2017; Lin et al., 2007). Similarly, the increase in nitrogen (N) deposition in this nutrient-limited region can affect the growth of plant communities (Rasheed et al., 2021), soil microbes (Peguero et al., 2021), and litter properties (Vallicrosa et al.,

2021), consequently influencing the balance of BVOC cycles. Therefore, it is crucial to determine the exchange of BVOCs from soil under different conditions in a changing world.

During the last decades, these environmental conditions have been changing globally, especially in the Mediterranean region (Peñuelas et al., 2013). There has been an increase in the frequency and intensity of drought and N deposition in the Mediterranean region (Peñuelas et al., 2018). Research has established that drought is linked to decreases in plant productivity (Ogaya and Peñuelas, 2007), intensive plant mortality (Peñuelas et al., 2018) and alterations in soil biogeochemistry (Vallicrosa et al., 2021), which, in return, will affect the exchange of BVOCs between the soil and the atmosphere (Abis et al., 2020). There are also indications that drought affects BVOC emissions from Mediterranean soils, but with contrasting results depending on the specific BVOC and the study (Pegoraro et al., 2006, Asensio et al., 2008). Indeed, it has previously been shown that BVOC emissions from aboveground plant tissues increase under drought conditions as well as following N fertilization (Llusià et al., 2006; Mu et al., 2018; Mu et al., 2019).

This study aimed to elucidate the effects of long-term drought and short-term soil nitrogen (N) fertilization on the exchange of BVOCs between the soil and the atmosphere in a Mediterranean holm oak forest. The fluxes of soil BVOCs were quantified using an dynamic chamber system and solid-phase microextraction fibres (SPME) in a field experiment over two consecutive years. Building upon previous findings at the same site (Asensio et al., 2007a), we hypothesized that (1) low soil moisture either caused by long-term drought or seasonal climate changes would promote the uptake of soil BVOCs, and (2) short-term N fertilization would increase soil BVOC emissions by enhancing soil microbial activity.

4.2. Materials and methods

4.2.1 Study site

The study was performed in a natural holm oak forest in the Prades Mountains in southern Catalonia, northeastern Spain (41°21′N, 1°20′E, 930 m a.s.l.), on a south-facing slope. The 45-year (1975-2020) average annual precipitation at Prades is 658 mm, and the mean annual temperature is 12 °C (Vallicrosa et al., 2021). The annual and seasonal distribution of precipitation is irregular, with annual precipitation ranging from 376 to 926 mm (Peñuelas et al., 2018). Spring and autumn are typically the wettest seasons, and summer droughts coincide with the highest temperatures. Long-term meteorological conditions (rainfall, air and soil temperatures and soil moisture content) were recorded by an automatic meteorological station in the study area. The forest has not been disturbed for the last 70 years and the maximum height of the dominant species is ~6-10 m (Vallicrosa et al., 2021). The forest of the area is dominated by *Quercus ilex* L. and the tall shrubs *Phillyrea latifolia* L. and *Arbutus unedo* L. (Peñuelas et al., 2018), with occasional deciduous species (*Sorbus torminalis* L. *Crantz* and *Acer monspessulanum* L.). The three tree species (*Q. ilex*, *P. latifolia*, and *A. unedo*) represent 97% of the total aboveground biomass of the forest and frequently co-occur in Mediterranean maquis shrubland and evergreen *Q. ilex* forests (Ogaya and Peñuelas, 2007). The soil is a Dystric Cambisol over Palaeozoic schist.

4.2.2 Experimental design

Twelve 15 × 10 m plots were established at the same altitude in a random plot design. Drought was simulated in four plots (Figure S1), four plots received N fertilizer, and four plots were untreated (control). The long-term drought experiment began in 1999,

as described by Asensio et al. (2007a). Briefly, the plots were subjected to a drought treatment, where rainfall was partially excluded by suspending transparent PVC strips 0.5-0.8 m above the soil. The PVC strips covered approximately 30% of the plot surfaces and were installed below the canopy, so they do not intercept direct sunlight. A ditch (0.8 m deep) was also excavated along the entire top edge of the drought treatment to intercept runoff water. The water intercepted by the strips and ditches was conducted around the plots to below their bottom edges. For the short-term N fertilization, a total fertilization of 60 kg N ha^{-1} y^{-1} (at a rate of 15 kg N ha^{-1} each season each year) was applied every year from 2015 and 2021 as a solution of ammonium nitrate (NH_4NO_3, Fluka, Buchs, Switzerland) with a sprayer in each plot. In each year, this fertilizer was sprayed three different times per season (5 kg N ha^{-1} each application), with at least one week between applications. The fertilizer was consistently applied to the canopy as a humid deposition from the top of the tallest tree in each plot. This study was conducted from 2019 to 2020 for two consecutive years although, unfortunately, one sampling campaign of 2019 autumn was not possible because of a device problem.

4.2.3 Environmental variables

The flux of soil CO_2 was monitored during BVOC sampling using an EGM-4 IRGA non-dispersive CO_2 analyser (PP Systems, Hitchin, UK). Soil temperature and soil moisture were measured at the same time as BVOC sampling with the soil chamber. Soil temperature was measured at a depth of 5 cm (using a soil digital thermometer, TO 15, Jules Richard instruments, Argenteuil, France), and volumetric soil water content averaged over the top 5 cm of soil was also measured (using ML2x portable Theta

probes, Delta-T Devices, Cambridge, UK). Both variables were measured in triplicate in each plot. Litter dry biomass was measured, with litter inside the chamber being carefully removed manually after the soil BVOC samples were collected and then dried at 60 °C until a constant weight was reached.

4.2.4 Sampling BVOCs

4.2.4.1. Dynamic chamber

The exchange rate of soil BVOCs was quantified in µg m^{-2} h^{-1} using an open sampling system. The system consisted of a Teflon chamber with the following dimensions: 14 cm internal diameter, 0.5 cm thickness, 17 cm height, and a valid volume of 2 L (Llusià et al., 2022). Two holes were present in the chamber at different heights, located at 5 cm and 13.5 cm from the ground level. To ensure air homogenization and to prevent condensation accumulation, a small fan was positioned inside the upper central part of the chamber. During the measurement process, the sampling air was extracted from the upper hole of the chamber using a Q-MAX air-sampling pump (Supelco, Bellefonte, USA). The pump was connected to the chamber via Teflon tubes. The air was then circulated through stainless steel cartridges (Markes International Inc., Wilmington, USA) containing adsorbents (115 mg of Tenax TA and 230 mg of SulfiCarb; Markes International Inc.). The flow rate was maintained at 200-300 mL min^{-1}, and the flow rate was recorded using a Bios Defender 510 flowmeter (Bios International Corporation, Butler, USA) for calibration purposes. Simultaneously, unfiltered ambient air was permitted to enter the enclosure through small holes in the chamber walls via a Teflon tube. This was done to compensate for the air flow that was withdrawn from the enclosure for sampling purposes. The continuous circulation of air inside the chamber

was maintained by the small fan throughout the sampling period. Each sampling session lasted for 20 minutes, after which the adsorbent cartridges were sealed using Teflon-coated brass caps. The sealed cartridges were then stored in a cool chamber at 4 °C until analysis (typically within one week of sampling).

BVOCs were initially measured from the soil, both with the natural litter layer intact and then with the litter layer removed in each plot. Notably, unlike tundra or boreal forest soils, which are typically covered by moss or grass, the soil in our Mediterranean study area is relatively dry and has minimal grass coverage. As a result, no aboveground plant organs were present in the soil samples collected using our chamber method during the sampling period.

To determine the contribution of litter to the BVOCs flux, after the initial soil BVOCs samples were collected, the chamber lid was carefully removed. The top layers of litter and organic residues inside the chamber were then gently removed to avoid any disturbance to the roots of ground vegetation, as this could significantly impact BVOC emissions (Hayward et al., 2001). Subsequently, the chamber lid was securely replaced, and further BVOCs samples were collected using the same chamber sampling method.

Before conducting each measurement of soil BVOCs, the concentrations of atmospheric BVOCs were measured in each plot using a similar approach. In this case, a Tedlar PVF film was placed between the soil and the chamber to prevent the emission or uptake of BVOCs from the soil. The sampling air for atmospheric BVOCs was collected at a height of 40 cm above the ground surface using a Teflon tube connected to the lower hole of the soil chamber. These samples served as a blank for calculating the exchange rate of BVOCs between the soil and the atmosphere. In each campaign (one per season), a total of 36 BVOCs samples were collected, comprising initial soil,

soil after litter removal, and atmosphere, equally distributed.

4.2.4.2 SPME

To avoid severe background interference and evaluate the quantity of BVOCs in soil pores, the SPME (Solid-Phase Microextraction) technique provided complimentary data about BVOCs in the soil. In each plot, a pre-cleaned SPME fibre (PDMS, film thickness 100 μm and length 10 mm, PDMS/DVB, 65 μm, Torion Technologies Inc., Utah, USA) was used to analyse the BVOCs present in the topsoil. The fibre was subjected to a cleaning process at 250 °C for 20 minutes prior to use. The SPME fibres were utilized in conjunction with syringes for improved and safe handling. These syringes were equipped with a push-button trigger mechanism and a screw-on/off cap, providing protection for the SPME fibre during the sampling and analysis process. To protect the fibre, it was encased within a stainless steel needle and attached to a metal shaft, while a plastic button was situated at the opposite end to facilitate the extraction of the fibre from the needle, exposing it to the environment.

For sampling, a hole was initially created in the soil adjacent to the chamber in each plot using a stainless-steel stick with a thickness of 2 mm and a length of 10 cm. Subsequently, a SPME fibre was inserted into the hole. The fibre was left exposed beneath the soil surface for a duration of 60 minutes (Barreira et al., 2017). After sampling, the fibres were sealed with a Teflon-coated cap, custom-made by the researchers, and placed in glass tubes. These tubes were then stored at 4 °C until analysis in the laboratory. Due to there being a limited number of SPME available, only nine plots were selected following an equal distribution of control, drought, and N fertilization treatments. Subsequently, one sample was collected from each plot,

resulting in a total of nine samples per campaign (n = 3 per treatment). SPME analysis was completed on the second day after being brought back to the laboratory.

4.2.5 Quality assurance and quality control

Prior to sampling, blank soil BVOC measurements were performed using cartridges that had been conditioned twice at a flow rate of 100 ml min^{-1} of purified helium for 30 min at 350 °C. To ensure the cleanliness of the cartridges of each sampling campaign, one of them was extracted randomly and analysed in the same way as the field samples with TD-GC/MS. If the target compounds were higher than the detection limits, re-cleaning of this group of cartridges was performed.

The results showed that the target compounds were either undetectable or below the method detection limits. Furthermore, a one-point calibration was performed daily to ensure that the retention times of the target compounds remained consistent. The terpenes that were sampled in our study remained chemically unaltered within the cartridges. This was determined by comparing them to reference standards (α-pinene, Δ3-carene, limonene, α-humulene, and dodecane) that were also trapped in the cartridges. Additionally, we assessed the trapping and desorption efficiency of standards such as α-pinene, β-pinene, and limonene, which demonstrated a highly efficient rate of 99%.

4.2.6 BVOC analysis

The BVOCs sampled with the Teflon chamber and the SPME fibres were analysed using a 7890A gas chromatograph–coupled to a 5975C mass spectrometer inert

MSD/DS Performance Turbo EI System (Agilent Technologies, Santa Clara, USA). The desorption of BVOCs from the metal tubes was carried out by thermal desorption (Ultra 2 and Unity 2; Markes International Ltd, Llantrisant, UK). The SPME fibres were desorbed by the 7890A gas chromatograph–mass spectrometer.

For the chromatographic analysis, helium was used as the carrier gas. The oven temperature was initially set at 35 °C for 5 minutes. It was then increased to 150 °C at a rate of 15 °C/min and held for 5 minutes. Subsequently, the temperature was raised to 250 °C at a rate of 15 °C/min for an additional 3 minutes. Finally, the temperature was increased to 280 °C at a rate of 30 °C/min and maintained for 5 minutes.

To ensure accuracy, a new standard solution was prepared before each analysis, following the detailed process outlined in our recent paper (Llusià et al., 2022). BVOCs were identified using pure standards and their mass spectra in the NIST 05a and Wiley 275 mass spectral data, then quantified by pure standards solutions for α-pinene, sabinene, β-pinene, limonene and α-caryophyllene based on selected ion counts (SIC) (Fluka Chemie AG, Buchs, Switzerland). Chromatograms were analysed using Enhanced ChemStation software (Agilent Technologies). More detail about the process has been described in the paper of Llusià et al. (2022). The exchange rates measured using the chamber were calculated by comparing the concentrations of BVOCs between the background atmosphere and the soil. The exchange rate (E, μg m^{-2} h^{-1}) of each BVOC was determined using the following equation:

$$E = ((C_{soil} \times F_{soil} \times 60) / (1000 \times A)) - ((C_{air} \times F_{air} \times 60) / (1000 \times A))$$

In this equation:

- C_{soil} represents the concentration of BVOCs in the air sampled from the soil (μg m^{-3})
- C_{air} represents the concentration of BVOCs in the background atmospheric sample (μg m^{-3})
- F_{soil} and F_{air} represent the air flow rates during the sampling of soil and the corresponding atmosphere, respectively (L min^{-1})
- A represents the surface area of the soil (m^2)

The exchange rate is referred to as an 'emission rate' when the value is positive (indicating BVOCs moving from the soil to the atmosphere) and as a 'rate of uptake' when the value is negative (indicating BVOCs moving from the atmosphere into the soil).

To determine the amount of top-soil BVOCs adsorbed to the SPME fibres, a calibration was performed using a mixture of standards. Pentane was used as our solution for mixing standard compounds. The standards used for calibration included compounds such as α-pinene, Δ3-carene, limonene, and α-caryophyllene. These standards are commonly used as representative BVOCs for calibration purposes. By analyzing the response of the SPME fibres to the known concentrations of these standards, a calibration curve or equation can be established. This calibration curve or equation is then used to quantify the amount of BVOCs adsorbed to the SPME fibres in the soil samples.

4.2.7 Statistical analyses

The statistical analyses were conducted using R Studio (Version 1.4.1103) (R Core Team, 2013). We performed log-transformation on the data when needed to achieve normal distributions and equal variances. Tukey's multiple comparisons were carried out to determine significance at a = 0.05. We performed two-way analyses of variance (ANOVAs) to examine the effects of treatments (drought and N fertilization), season, litter manipulation, and their interactions on the concentration of atmospheric BVOCs, soil CO_2 flux, the fluxes of soil BVOC (measured with the chamber method) and the amounts of soil BVOC (measured with the SPME method). Pearson correlations were calculated to examine the relationships between the total and individual soil BVOC flux variables and litter mass and atmospheric BVOCs concentration.

To examine how the treatments and seasons collectively influenced the soil biotic factors, soil CO_2 efflux, and soil BVOC amounts were subjected to principal component analysis (PCA). The PCA was performed using the 'ggplot2' and 'factoextra' packages (Kassambara, 2015; Wickham, 2016). For statistical tests, *P*-values between 0.1 and 0.05 were reported as marginally significant, while *P*-values less than 0.05 were reported as significant effects.

The data used for multivariate analyses were visualized as a heatmap using the hiplot 0.1.0 software (https://hiplot.com.cn). The heatmap was constructed using standardized raw values, which allows for better comparison and interpretation of the data. The relationships between soil CO_2 efflux and abiotic factors (soil temperature, soil water content, and litter mass) were analysed using linear regressions. The relationships of soil BVOCs flux with soil water content and soil CO_2 efflux were modelled using nonlinear regression, utilizing SigmaPlot 12.5 software (Systat Software, San Jose, CA, USA).

4.3. Results

4.3.1. Soil environmental variation

Based on the continuous recordings obtained from the meteorological station located in the Prades Forest, the annual rainfall for the years 2019 and 2020 was 789.4 mm and 776.2 mm, respectively (Figure S2a). The distribution of rainfall exhibited a pattern where significant amounts of rain occurred from August to December 2019 (607.3 mm) and from January to May 2020 (466.1 mm), which accounted for approximately 77% and 60% of the total annual precipitation, respectively. It is worth noting that an extreme rainfall event of 287.4 mm took place on October 22nd, 2019. Throughout the study period, the daily average air temperature ranged from -0.4 to 32.0 °C, with an average of 13.1 °C. The soil temperature at a depth of 5 cm remained relatively consistent across all treatments, fluctuating between -0.2 and 24.1 °C, with an average of 12.2 °C. In terms of soil moisture, the topsoil layer exhibited a range of 3.0 to 64.9% water content (Figure S2b), with an average of 15.0%, 12.5%, and 13.6% for the control, drought, and N fertilization treatments, respectively. Notably, during the wet seasons (spring and autumn), the soil water content in the drought treatment was on average 40% lower compared to the control treatments, indicating a significant reduction ($P < 0.001$). No significant differences were observed in soil temperature and moisture content between the fertilization and control treatments.

4.3.2. Soil CO_2 flux

Soil CO_2 flux varied significantly between seasons and was highest in spring and lowest in summer ($P < 0.001$, Figure 1). The average fluxes of soil CO_2 were 3.24 ± 0.52, 2.18 ± 0.32, and 3.01 ± 0.45 μmol m^{-2} s^{-1} for the control, drought, and N fertilization treatments, respectively. The drought treatment resulted in reduced soil CO_2 fluxes during the winter and spring of both sampling years, and significant reductions were observed during both spring seasons compared to the control treatment ($P < 0.05$). On the other hand, N fertilization had variable effects on soil CO_2 flux, with significantly higher fluxes in summer 2019 and significantly lower fluxes in spring 2020 compared to the control treatment.

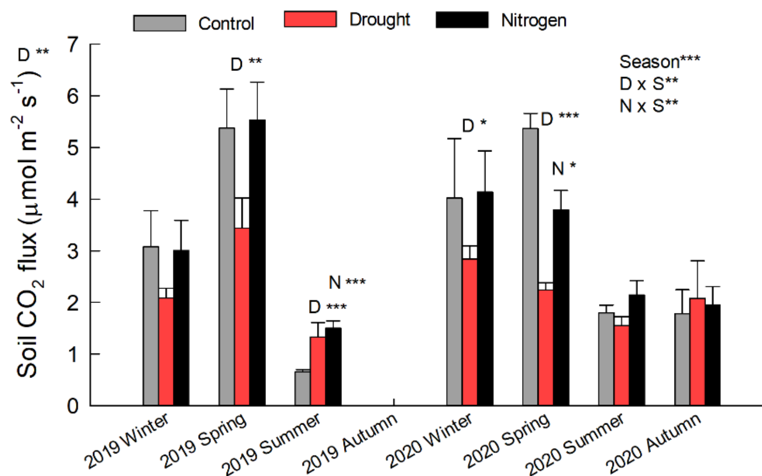

Figure 1. Seasonal dynamic of soil CO_2 flux. Error bars indicate standard errors of the means (n = 4). Significant main effects of drought (D) and nitrogen fertilization (N) for two-way ANOVAs are indicated by †$P < 0.1$, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ within a season. The data in the 2019 autumn was missing because of a device problem.

The flux of soil CO_2 was negatively correlated with soil temperature ($r^2 = 0.45$, $P < 0.001$, Figure 2a), and the drought treatment altered the slope of this relationship, indicating a reduced sensitivity of CO_2 flux to soil temperature. In contrast, soil moisture content showed a significant positive correlation with CO_2 flux in all

treatments ($r^2 = 0.56$, $P < 0.001$, Figure 2b). The drought and N fertilization treatments did not significantly affect the correlation between CO_2 flux and soil moisture content. Additionally, litter mass demonstrated a positive correlation with CO_2 flux, except in the drought treatment where the flux was not influenced by litter mass ($r^2 = 0.22$, $P < 0.05$, Figure 2c).

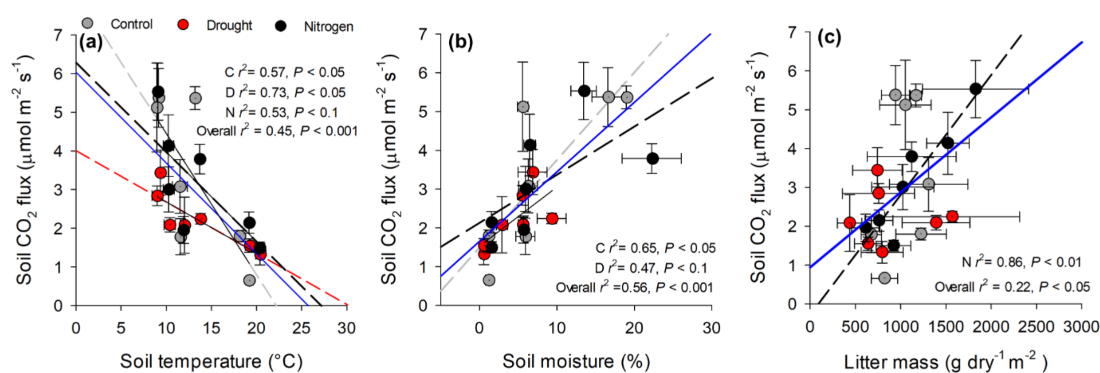

Figure 2. Relationships of the seasonal means of soil CO_2 flux with the seasonal means of soil temperature (a), soil-moisture content (b), and litter mass (c) amongst different treatments. Each point represents the mean of different seasonal data (mean ± SE, n = 4). The grey dashed line indicated the regression of control; the red dashed line indicated the regression of drought; the black dashed line indicated the regression of N fertilization; the blue line indicated the regression of overall data. C refers to control, D refers to plots with drought treatment and N refers to plots with nitrogen fertilization.

4.3.3. Atmospheric isoprenoid concentrations

The seasonal variation of atmospheric isoprenoid concentrations is presented in Table S1, indicating the highest concentration of total VOCs observed during both summers. The impact of drought and N fertilization on atmospheric isoprenoid concentrations was found to be marginally significant ($P < 0.1$), with specific effects on the concentrations of limonene, α-caryophyllene, and β-caryophyllene ($P < 0.05$). The

atmospheric concentration of isoprenoids was significantly lower in the drought and N fertilization treatments compared to the control during the winter (2019) and autumn (2020) sampling periods. Among the detected isoprenoids, α-pinene, β-pinene, and limonene dominated the atmospheric BVOCs, accounting for 70.2% of the detected isoprenoid concentrations during summer.

4.3.4. BVOC exchange

The overall exchange rate of isoprenoids indicated that this soil acts as a net sink of BVOCs annually, ranging from −1.30 to −10.33 μg m^{-2} h^{-1} (Table 1). The rate of isoprenoid uptake was highest in the drought treatment (-10.33 ± 8.47 μg m^{-2} h^{-1}, Table 1), and it reached its peak during summer (-25.90 ± 9.36 μg m^{-2} h^{-1}, Table 2). Although not statistically significant, the emission of isoprenoids was highest in the N fertilization treatment, primarily driven by the increased emission of α-pinene (6.22 ± 3.25 μg m^{-2} h^{-1}), which may be derived from litter as its removal led to a significantly lower emission (0.25 ± 1.35 μg m^{-2} h^{-1}, Table 1).

Table 1. Rates of exchange of BVOCs (μg m−2 h−1) from the different treatments (control, drought and N fertilization) during the years of 2019-2020. Rates are means ± SE for the entire data set.

BVOCs / Treatment	Intact soil			Litter removal		
	Control	Drought	N fertilization	Control	Drought	N fertilization
Isoprene	0.20 ± 0.28a	-0.06 ± 0.16a	-0.11 ± 0.06a	-0.24 ± 0.08a	-0.16 ± 0.12a	-0.11 ± 0.04a
Difference to control (%)		-130	-155		+33	+54
Tricyclene	0.01 ± 0.04a	-0.30 ± 0.38a	0.11± 0.07b	-0.03 ± 0.02a	0.69 ± 0.86a	0.11 ± 0.06a
α-Pinene	-1.16 ± 0.37a	0.45 ± 1.19a	**6.22 ± 3.25b**	-1.07 ± 0.53a	-0.67 ± 0.52a	**0.25 ± 1.35a**
Camphene	-0.12 ± 0.04a	0.07 ± 0.08a	**0.25 ± 0.24b**	-0.09 ± 0.05a	**0.57 ± 0.50b**	**0 ± 0.09a**
β-Pinene	-0.46 ± 0.18a	-1.11 ± 1.16a	-0.39 ± 0.47a	-0.41 ± 0.27a	-1.54 ± 1.00a	-0.53 ± 0.45a
β-Myrcene	-0.32 ± 0.18a	-0.71 ± 0.39a	-0.23 ± 0.21a	-0.39 ± 0.122a	-0.70 ± 0.36a	-0.38 ± 0.28a
Δ^3-Carene	-0.14 ± 0.04a	0.07 ± 0.14a	0.01 ± 0.15a	0.08 ± 0.14a	0 ± 0.07a	0.40 ± 0.33a
Limonene	-5.54 ± 3.34a	-7.20 ± 7.55a	-6.91 ± 6.79a	-4.64 ± 2.86a	-7.70 ± 6.39a	-9.05 ± 7.97a

Other monoterpenes	-0.27 ± 0.11a	-0.39 ± 0.71a	0.15 ± 0.20a	-0.07 ± 0.23a	-0.68 ± 0.65a	0.16 ± 0.21a
Total monoterpenes	-8.13 ± 3.52a	-10.15 ± 8.37a	-0.74 ± 5.89a	-6.56 ± 3.46a	-9.51 ± 5.93a	-9.07 ± 7.96a
Difference to control (%)		-25	+91		-45	-38
α-Cubebene	-0.22± 0.10a	-0.05 ± 0.07a	-0.24 ± 0.22a	-0.15 ± 0.11a	-0.04 ± 0.03a	-0.25 ± 0.19a
β-Caryophyllene	-0.11 ± 0.04a	-0.05 ± 0.01a	-0.02 ± 0.05a	-0.11 ± 0.11a	-0.01 ± 0.04a	-0.02 ± 0.07a
α-Caryophyllene	-0.11 ± 0.04a	0.01 ± 0.01b	-0.04 ± 0.02a	-0.04 ± 0.07a	0 ± 0.02a	0 ± 0.03a
Total sesquiterpenes	-0.98 ± 0.53a	**-0.12 ± 0.12b**	-0.47 ± 0.36ab	-0.81± 0.59a	**0.01 ± 0.06a**	-0.42 ± 0.29a
Difference to control (%)		+87	+52		+102	+48
Total	-8.92 ± 3.52a	-10.33 ± 8.47a	-1.30 ± 6.02a	-7.59± 3.59a	-9.66 ± 5.96a	-9.60 ± 9.95a
Difference to control (%)		-16	+85		-27	-26

Values were marked in bold if they differed between litter removal treatments. The exchange rates between the treatments are indicated with different letters if it is significant ($P < 0.05$).

Similarly, the exchange of total sesquiterpenes followed an annual uptake pattern, ranging from -0.12 to -0.98 μg m^{-2} h^{-1} in the intact soil (Table 1). Notably, the drought treatment resulted in a reduction in the uptake rate of total sesquiterpenes, which was statistically significant in the intact soil ($P < 0.05$). A similar decreasing trend was observed in the N fertilization treatments, regardless of whether or not litter was removed, although the differences were not statistically significant.

Table 2. Seasonal mean rates of soil BVOC exchange (μg m^{-2} h^{-1}) combined all plots (control, drought and nitrogen fertilization), standard errors of the means (SE, μg m^{-2} h^{-1}) for intact soil and litter removal during the years of 2019-2020.

Treatment	Intact soil				Litter removal			
Season	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Number of samples	23	24	12	24	23	24	12	24
Isoprene	-0.11 ± 0.05	-0.01 ± 0.24	0.57 ± 0.50	-0.09 ± 0.07	-0.09 ± 0.05	-0.24 ± 0.12	-0.3 ± 0.12	-0.09 ± 0.07
Difference to control (%)					+20a	-230a	-153b	0a
Monoterpenes	3.99 ± 2.56	-24.88 ± 9.29	5.48 ± 7.02	-2.64 ± 3.82	1.53 ± 1.21	-27.96 ± 10.78	-2.04 ± 1.24	-1.57 ± 4.00
Difference to control (%)					-62a	-12a	**-137b**	+41a
Sesquiterpenes	-0.02 ± 0.07	-1.00 ± 0.34	-1.52 ± 0.61	-0.03 ± 0.07	0.14 ± 0.07	-0.57 ± 0.29	-1.95 ± 0.55	-0.02 ± 0.07
Difference to control (%)					+800a	+43a	-28a	+33a
Total	3.86 ± 2.59	-25.9 ± 9.36	4.54 ± 7.18	-2.77 ± 3.84	1.58 ± 1.22	-28.78 ± 10.79	-4.29 ± 1.71	-1.68 ± 4.04
Difference to control (%)					-59a	-11a	-194a	+39a

Values were marked in bold if they differed between litter removal treatments. Letters after the percentage indicate statistical differences of litter removal in each season ($P < 0.05$).

The responses of specific BVOCs to the treatments were found to vary depending on the type of BVOC, season, and litter (Tables 2 and 3, Figure 3). For instance, the exchange rate of isoprene ranged from -0.11 to 0.20 μg m^{-2} h^{-1} (Table 1), and it was slightly lower, although not statistically significant, in the drought and N fertilization treatments. Isoprene emission exhibited a pronounced seasonal pattern (F = 2.70, P < 0.1, Table 3), with net emission occurring only in autumn (0.57 ± 0.50 μg m^{-2} h^{-1}, Table 2). Additionally, the interactions of season and litter removal on isoprene in treatments were both significant (P < 0.05, Table 3).

Table 3. *F* values from the for two-way ANOVAs of the responses of the exchange of soil BVOCs to drought (D), nitrogen fertilization (N), litter removal (LR), and sampling season (S). n = 4. Bold type indicates statistically significant values.

BVOCs/ Treatment	Drought					N fertilization				
	D	LR	S	D × S	LR × S	N	LR	S	N × S	LR × S
Isoprene	0.36	**2.83†**	**2.70†**	0.10	**5.29***	0.22	2.57	0.23	2.56	**5.41***
Tricyclene	0.29	1.59	1.73	1.25	0.37	**4.08***	0.04	0.82	0.08	0.55
α-Pinene	**2.76†**	1.02	**11.05****	0.18	0.24	**3.87†**	1.87	1.49	0.26	0.07
Camphene	**3.85***	1.48	2.16	0.51	0.15	2.57	0.58	1.484	0.02	0.27
β-Pinene	1.23	0.07	2.00	0.04	0.05	0.03	0.05	**11.26****	0.09	0
β-Myrcene	1.33	0.02	2.39	0.06	0	0.15	0.42	**6.36***	0.34	0.17
Δ^3-Carene	0.87	0.39	**7.73****	0.01	0.20	2.15	2.74	0.11	3.00†	0.04
Limonene	0.16	0.03	0.47	0.1	0.03	0.27	0.02	0.6	0.20	0.002
Total monoterpenes	0.17	0.02	0.04	0.06	0	0.21	0.47	0	0.10	0.04
α-Cubebene	**4.94***	0.31	**27.44*****	**13.43*****	0.10	0.42	0.04	**23.05*****	0.001	0.01
β-Caryophyllene	**6.00***	0.56	**28.99*****	**31.96*****	0.18	**3.62†**	0.07	**32.87*****	**14.15*****	0.25
α-Caryophyllene	**8.13****	0.15	**3.39†**	**2.64†**	0.18	**3.52†**	0.97	**6.47***	0.60	0.01
Total sesquiterpenes	**13.23*****	0.24	**33.8*****	**47.56*****	0.17	2.7	0.05	**71.80*****	**11.01****	0.09
Total	0.08	0.01	0.05	0.19	0.04	0.28	0.51	0.09	0.16	0.08

†P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001.

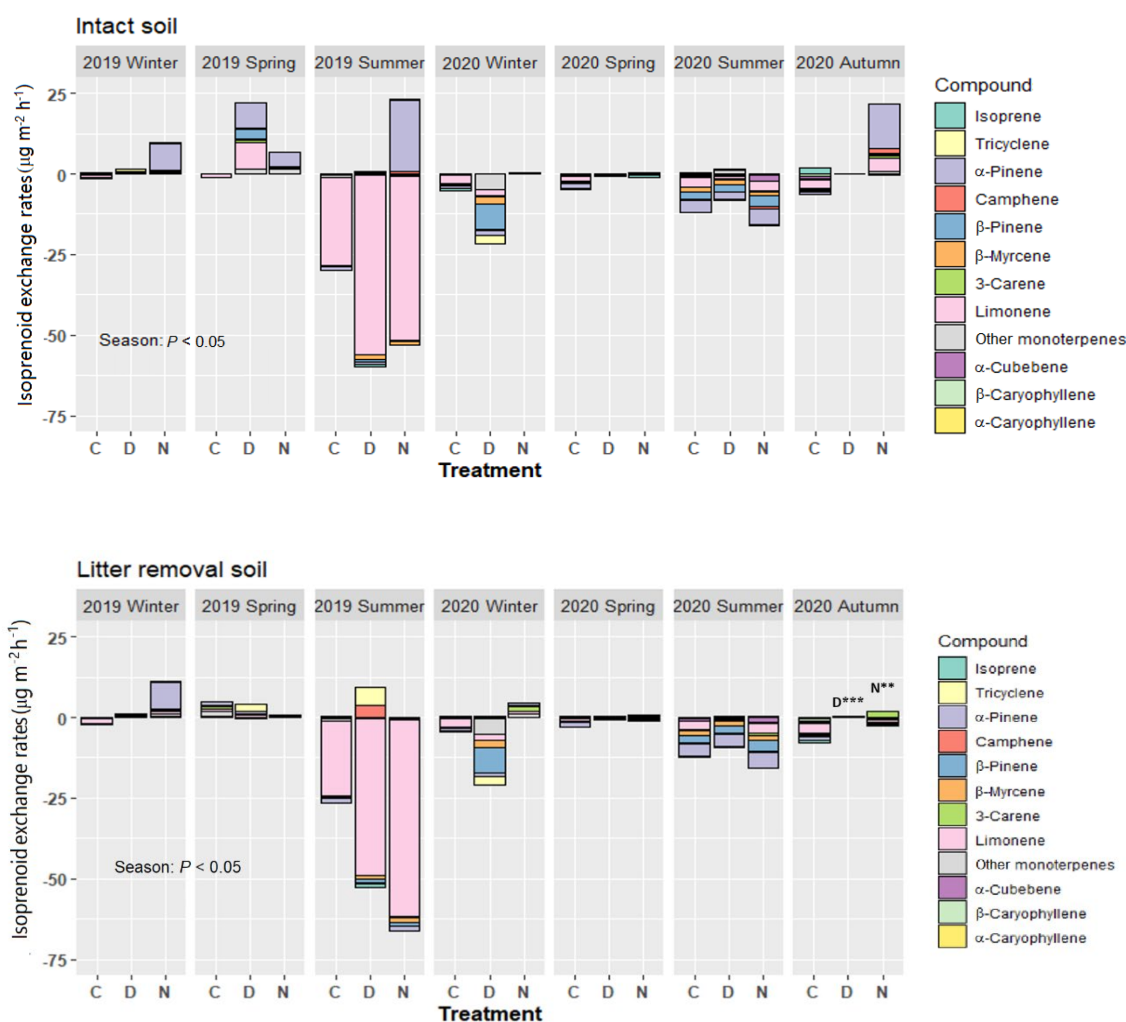

Figure 3. Seasonal course of soil isoprenoid exchange rates under intact soil and litter removal soil. Bars indicate the mean of each compound rate (n = 4). Significant main effects of drought treatment (D) and nitrogen fertilization (N) for linear two-way ANOVAs are indicated by †$P < 0.1$, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ within a date. C refers to control, D refers to plots with drought treatment and N refers to plots with nitrogen fertilization.

Among the seven detected monoterpenes, most of them were taken up by the soil, with limonene being the dominant compound, exhibiting an overall rate ranging from -7.20 ± 7.55 to -5.54 ± 3.34 μg m^{-2} h^{-1} across the treatments (Table 1). These findings have been summarized in the heatmap (Figure 4), which clearly illustrates the effects of treatments and seasons on the exchange rates of soil BVOCs.

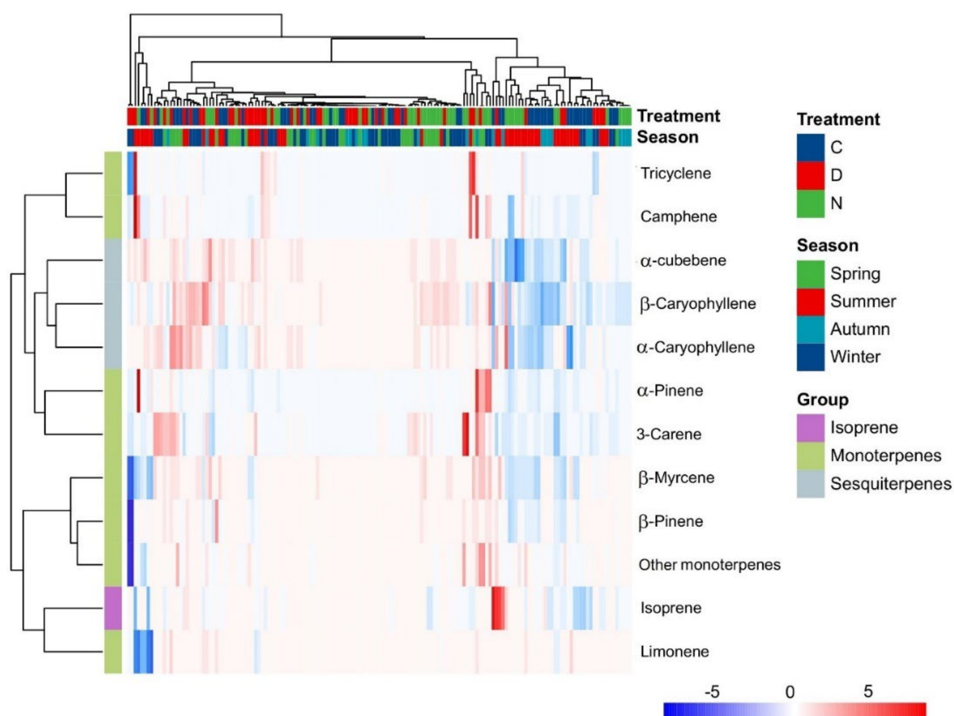

Figure 4. Heatmap of the exchange of soil BVOCs in different treatment within seasons. On the vertical axis, different compounds are represented. On the upper horizontal axis, different replicates of the treatments are represented forming a dendrogram. The blue to red scale represents the fluxes strength of different compounds, i.e., red for higher levels of emission, blue for higher levels of uptake. Data including BVOCs from intact soil and litter removal soil. C refers to control, D refers to plots with drought treatment and N refers to plots with nitrogen fertilization.

4.3.5 Effects of the treatments on the amounts of BVOCs adsorbed to the SPMEs in topsoil

We identified several compounds from the forest soil (Table 4), with α-pinene being the most abundant BVOC, followed by limonene and α-caryophyllene. Some sesquiterpenes, such as α-cubebene and β-caryophyllene, were present in high amounts but occurred only once in a single plot. For example, α-cubebene (3.6 ± 0.36 ng) was detected in one drought plot in spring 2020 and in one control plot in summer 2020 (3.0

± 0.51 ng), while β-caryophyllene (2.99 ± 0.51 ng) was found in one N fertilization plot in winter 2020.

The amounts of total soil isoprenoids ranged from 0.38 to 6.25 ng in the control treatment, 0.72 to 6.95 ng in the drought treatment, and 0.68 to 4.86 ng in the N fertilization treatment (Table 4). Drought had a marginal effect on the amount of limonene ($F = 3.10$, $P < 0.1$), and N fertilization significantly influenced the amounts of α-caryophyllene and total monoterpenes ($P < 0.01$). Season had a significant impact on the amount of isoprene and total monoterpenes (all $P < 0.01$), with the highest values observed in autumn. The response of soil BVOC amounts to N fertilization mainly depended on the season, with total BVOCs increasing in winter and decreasing in summer under the N fertilization treatment (Table 4).

Table 4. Main BVOC amounts (ng) detected in the soil during the 2019-2020 sampling period using SPME that presented significant rates differences among seasons. Significance for season, treatment and interaction is indicated for compounds using two-way ANOVAs (means ± SE, n = 3). ND, not detected. C refers to control, D refers to plots with drought treatment, N refers to plots with nitrogen addition and S refers Season.

Season	Treatment	Isoprene	α-Pinene	Limonene	α-Caryophyllene	Total monoterpenes	Total sesquiterpenes	Total
2020 Winter	C	ND	0.15 ± 0.09	0.54 ± 0.03	0.57 ± 0.18	0.70 ± 0.13	0.69 ± 0.10b	1.39 ± 0.23
	D	ND	0.03 ± 0.02	0.38 ± 0.06	0.30 ± 0.08	0.41 ± 0.08	0.31 ± 0.08a	0.72 ± 0.15
	N	ND	0.09 ± 0.06	0.38 ± 0.07	0.09 ± 0.06	0.61 ± 0.01	4.25 ± 3.00b	4.86 ± 3.01
2020 Spring	C	ND	0.04 ± 0.03	0.22 ± 0.05	0.08 ± 0.05	0.26 ± 0.08	0.12 ± 0.08a	0.38 ± 0.16
	D	ND	1.63 ± 1.29	0.34 ± 0.08	0.54 ± 0.12	1.97 ± 1.36	4.98 ± 3.75b	6.95 ± 5.11
	N	ND	0.08 ± 0.05	0.27 ± 0.03	0.18 ± 0.13	0.48 ± 0.07	0.25 ± 0.18a	0.73 ± 0.11
2020 Summer	C	ND	0.67 ± 0.43	0.15 ± 0.03	0.21 ± 0.11	0.82 ± 0.42	5.43 ± 2.91b	6.25 ± 3.31
	D	ND	0.59 ± 0.03	0.30 ± 0.10	0.11 ± 0.07	0.89 ± 0.08	0.13 ± 0.09a	1.02 ± 0.17
	N	ND	0.13 ± 0.09	0.54 ± 0.20	ND	0.68 ± 0.30	0.01 ± 0.01a	0.68 ± 0.30
2020 Autumn	C	0.16 ± 0.05	0.51 ± 0.15	0.37 ± 0.01	0.11 ± 0.01	1.86 ± 0.26a	1.44 ± 0.95	3.45 ± 1.13
	D	0.18 ± 0.13	0.50 ± 0.10	0.48 ± 0.01	0.16 ± 0.07	2.46 ± 0.09ab	0.81 ± 0.31	3.45 ± 0.10
	N	0.17 ± 0.04	0.40 ± 0.01	0.39 ± 0.05	0.14 ± 0.05	3.58 ± 0.02b	0.84 ± 0.21	4.59 ± 0.19

F-value	Residuals							
D	22	0.11	0.15	**3.10†**	0.11	1.19	0.03	0.03
N	22	0.04	1.37	2.45	**5.31***	**5.32***	0.26	0.01
S	34	**22.19*****	0.65	**2.25†**	**2.30†**	**10.13*****	0.07	0.69
D × S	20	0.11	1.18	**2.85†**	0.39	1.26	2.06	2.07
N × S	20	0.04	0.77	**6.01****	**5.10***	**5.46****	**2.90†**	**2.58†**

Different letters within a season differed significantly from each other ($p < 0.05$). †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

4.3.6 Correlations of soil isoprenoid exchanges with environmental parameters

The exchange of isoprenoids showed a relationship with soil temperature irrespective of treatments, exhibiting a significant decrease when soil temperature exceeded 15 °C (Figure 5a). Increasing soil water content resulted in a reduction in the uptake rate of isoprenoids, although this relationship was only significant in the control plots ($P < 0.001$, Figure 5b). Overall, the exchange of isoprenoids could be explained by soil temperature ($P < 0.001$, Figure 5a), soil water content ($P < 0.01$, Figure 5b), and soil CO_2 flux ($P < 0.05$, Figure 5c).

In the control treatment, soil CO_2 flux accounted for 95% of the variation in the exchange of isoprenoids ($P < 0.05$, Figure 5c). However, the effects of drought and N fertilization decoupled the relationship between isoprenoid emission and soil CO_2 flux. Generally, as soil CO_2 flux increased, there was a concurrent increase in isoprenoid emission at low values of soil CO_2 flux, but the emission of isoprenoids stabilized at around zero at higher soil CO_2 fluxes ($P < 0.05$, Figure 5c). Although litter removal significantly decreased the emission of certain individual BVOCs (Table 1), such as α-pinene and camphene, there was no significant correlation between litter mass and the overall exchange rate of isoprenoids.

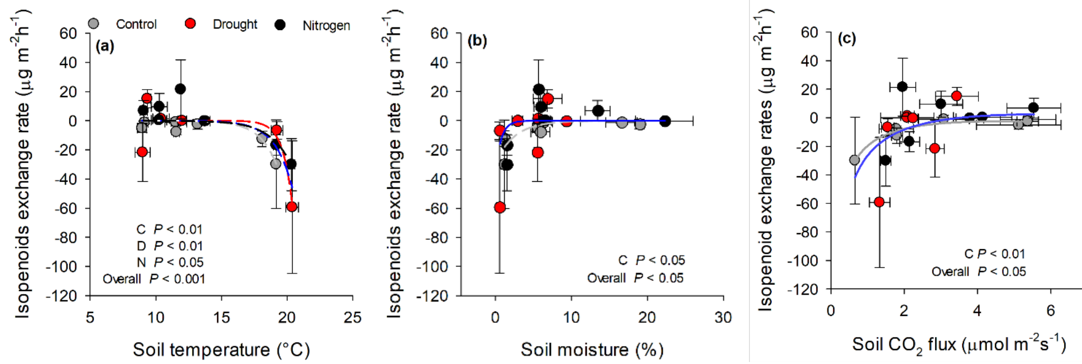

Figure 5. The nonlinear regressions were carried out for the relationship of soil temperature (a), soil-moisture content (b) and soil CO_2 flux (c) with the exchanges of soil BVOCs. Each point represents the mean of different seasonal data (mean ± SE, N = 4). The grey dashed line indicated the regression of control; the red dashed line indicated the regression of drought treatment; the black dashed line indicated the regression of N fertilization; the blue line indicated the regression of overall data.

To analyse the relationships between the amounts of soil isoprenoids and soil environmental factors, a Principal Component Analysis (PCA) was conducted. The PCA results showed that PC1 and PC2 explained 29.9% and 21.5% of the variation, respectively, in soil temperature, soil moisture content, soil CO_2 efflux, litter mass, and the amounts of soil BVOC (Figure 6a and 6b). Total monoterpenes were positively correlated with soil temperature, while total sesquiterpenes were positively correlated with litter mass. Isoprene and monoterpenes exhibited a negative correlation with soil water content and soil CO_2 flux (Figure 6a), and soil water content showed a strong positive correlation with soil CO_2 flux. Seasonality had a significant impact on the amounts of soil BVOCs (Table 5), with higher amounts of isoprene and monoterpenes observed in autumn, and higher amounts of sesquiterpenes observed in spring and winter (Figure 6b).

Table 5. The overall Pearson correlation coefficients between the exchange of soil BVOCs and litter mass and atmospheric isoprenoids concentration.

Soil BVOCs	Litter mass	Atmospheric isoprenoids
Isoprene	0.03	**-0.20***
Tricyclene	0	-0.17
α-Pinene	-0.08	**0.40****
Camphene	-0.05	0.11
β-Pinene	0.1	**-0.26***
β-Myrcene	**0.14†**	**-0.60****
Δ^3-Carene	0.05	-0.14
Limonene	0.06	**-0.95****
Other monoterpenes	0.07	**-0.33****
Total Monoterpenes	0.06	**-0.85****
α-Cubebene	0.12	-0.01
β-Caryophyllene	0.10	-0.09
α-Caryophyllene	0.10	0.03
Total Sesquiterpenes	**0.14†**	-0.01
Total	0.07	**-0.85****

†$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

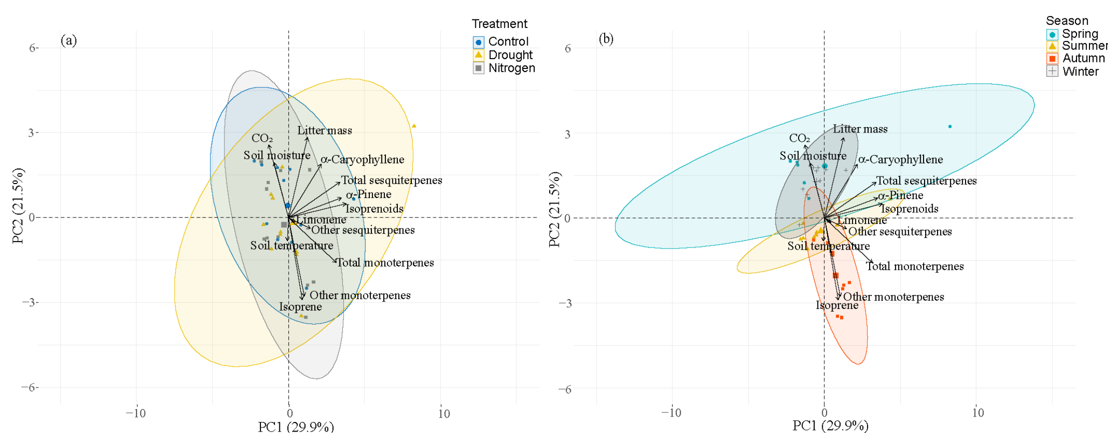

Figure 6. Principal component analysis (PCA) of the amount of soil BVOC, soil CO_2 fluxes, and litter mass affected by treatments (a) and seasons (b). Principal component 1 (PC1) explains 29.9 % of the total variance, while principal component 2 (PC2) explains 21.5 % of the total variance. Ellipses represent 95% confidence levels for each treatment group.

4.4. Discussion

Our findings revealed significant rates of BVOC uptake (net sink) and strong soil CO_2 emissions in the studied soils. Environmental factors, including soil temperature, soil water content, litter mass, and atmospheric BVOC concentrations, were the primary drivers of heterogeneity in soil BVOC exchanges. This led to notable variations in both BVOC uptake and emission rates.

4.4.1 Variations of soil BVOCs under drought and N fertilization

Our findings show that the exchange of soil isoprenoids did not exhibit significant differences among the treatments (Table 1). However, the amounts of BVOCs in the soil were significantly influenced by seasonal variations (Table 4, Figure 6b). Drought affects both the emission of BVOCs from the aboveground tissues of plants (Blanch et al., 2008; Llusià et al., 2006; Mu et al., 2018) and from belowground soil sources (Asensio et al., 2007a; Bourtsoukidis et al., 2018; Kleiber et al., 2017; Lin et al., 2007). Here, drought resulted in an increasing trend in the uptake rate of monoterpenes compared to the control although the difference was not found to be statistically significant, regardless of the presence or absence of litter (Table 1). The observed reduction in soil monoterpene emissions under drought is consistent with the findings of Asensio et al. (2007a) that highlight the importance of soil moisture in regulating soil BVOC fluxes in dry regions.

We demonstrate that the soil functioned as a sink for sesquiterpenes, as evidenced by the net uptake of sesquiterpenes over its emission (Table 1). However, there was a significant decrease in sesquiterpene uptake in response to drought (Tables 1 and 3), as observed in both the chamber data for all seasons and the SPME data specifically in

spring (Table 4). This finding suggests that drought conditions may enhance the emissions of sesquiterpenes from roots, potentially as a response mechanism to counteract the negative effects of drought stress (Quan and Ding, 2017). Roots are a strong source of sesquiterpenes (Tsuruta et al., 2018), and the high variation in the amounts of sesquiterpenes observed among different plots could potentially be attributed to the diversity of root-system development, biomass production and microbes (Bourtsoukidis et al., 2018; Horváth et al., 2011). In addition, physical damage and disturbance to roots can also trigger a high release of sesquiterpenes into the soil, which may have caused an anomalously high emission of sesquiterpenes (Bourtsoukidis et al., 2018; Lin et al., 2007; Tiiva et al., 2019).

N fertilization affects the exchange of soil BVOCs via changes in litter decomposition rates, root traits, and the structure of the microbial community (Gray and Fierer, 2012; Rasheed et al., 2021; Tiiva et al., 2019; Wang et al., 2018a). N addition has been found to be capable of increasing soil BVOC emissions (Raza et al., 2017). Nevertheless, these effects are inconsistent and vary over different time scales (Christodoulou et al., 2021). Our results indicated that N fertilization decreased the overall uptake rate of isoprenoids by the soil even though no statistical significance was found (Table 1). This decrease could be attributed to the marginal stimulation of monoterpene emissions, specifically tricyclene, α-pinene, and camphene, which caused a shift in the soil from being a sink to becoming a source ($P < 0.1$, Tables 1 and 3) (Llusià et al., 2022). The stimulation of monoterpene emissions was likely influenced by the presence of surface litter, as the emission of α-pinene from bare soil was considerably lower after litter removal (Table 1 and Figure 4b). A similar study conducted in a subtropical forest reported an increase in monoterpene emissions (significant for α-pinene) from litter under N addition (Huang et al., 2021). Additionally,

the results of the SPME data showed a significant increase in the total amount of monoterpenes in the soil in response to N fertilization, particularly during autumn compared to other seasons, further supporting the stimulation of litter-derived BVOCs under N fertilization (Table 4). These findings are in agreement with our second hypothesis that the increase in soil BVOC emissions under N fertilization is dependent on compound specificity.

Overall, the effects of N fertilization on soil BVOC emissions are complex and can be influenced by various factors such as litter composition, microbial activity, and decomposition rates. The stimulation of monoterpene emissions under N fertilization observed in this study suggests a potential link between nutrient availability, litter decomposition, and BVOC emissions from the soil.

4.4.2 Contribution of plant litter to soil BVOCs

We removed the litter layer to differentiate between the contributions of BVOCs from litter and those from the soil. *Quercus ilex* (*Q. ilex*) is the dominant species in the region (Peñuelas et al., 2018), and *Q. ilex* litter constitutes the majority of the litter layer, with a dry weight ranging from 436.0 to 1826.7 g per square metre (Figure 3c). It is important to note that *Q. ilex* does not store terpenes, and the emission rate of BVOCs from its litter is negligible, measuring less than 0.10 μg per gram of dry matter per hour at 30 ºC. This emission rate is lower compared to other species that do store terpenes, such as *Thymus vulgaris* (4.7 μg per gram of dry matter per hour at 30 ºC) and pine litter (2.0 μg per gram of dry matter per hour at 20 ºC) (Isidorov et al., 2010).

Considering the low BVOCs emission rates from *Q. ilex* litter and the lack of terpene storage, the removal of *Q. ilex* litter would be expected to have a limited effect on the overall emission of BVOCs from the system (Greenberg et al., 2012). Therefore, we expected the impact of litter removal on the emission of BVOCs in this study to be minor and not significantly alter the conclusions regarding the contributions of BVOCs from the soil and litter sources. However, the removal of litter resulted in a reduction in the emission of monoterpenes, particularly α-pinene ($P < 0.1$, Table 3). Plant litter releases of BVOCs may be minor, but their decomposition process through microbial activity can be a substantial source (Gray et al., 2010). Considering the minor monoterpene release from *Q. ilex* litter alone, the significant reduction of monoterpenes from soil after litter removal may support the notion that, at least when moisture content is sufficient, soil microbes in litter release more monoterpenes than the litter itself. Soil microbes can accelerate litter decomposition and drive the emission of litter-derived BVOCs (Aaltonen et al., 2011; Leff and Fierer, 2008; Staudt et al., 2019). Isoprene emission showed a marginal decrease after litter removal under drought conditions (Table 3), which can be attributed to the fact that isoprene is primarily synthesized in fresh leaves (Viros et al., 2020). Consequently, fresh litter on the soil serves as the primary source of isoprene, and the magnitude of emission is strongly associated with seasonal variations due to plant phenology (Greenberg et al., 2012). Therefore, the influence of litter removal on BVOCs emission may be particularly significant during seasons when leaves are falling (Hayward et al., 2001; Staudt et al., 2019), which is consistent with our results (Table 2).

Although the variation in litter accumulation between seasons (Figure S3) may influence the fluxes of BVOCs, the effect of litter removal on soil BVOCs emission is further modified by biotic and abiotic factors driven by the season (Table 3). This

suggests that the influence of litter removal on soil BVOCs emission is complex and influenced by multiple interacting factors related to both the litter and the surrounding environment.

4.4.3 Relationships of soil BVOCs with the soil environmental conditions

Previous studies have generally observed a positive correlation between soil BVOCs emissions and temperature, either linearly or exponentially (Mäki et al., 2017; Staudt et al., 2019). However, in our study, the exchange of isoprenoids showed a high uptake rate when soil temperature was above 15 ºC (Figure 5a). This difference in results could be attributed to the inclusion of aboveground plant sources in previous studies, as BVOCs emissions from these sources are positively correlated with increasing temperature (Kramshøj et al., 2016; Mäki et al., 2019b). In contrast, our study excluded aboveground plant organs, with our results indicating that the response of BVOCs from bare soil may not be as strong as understorey covered soil (Tang et al., 2018). It should be noted that given that field campaigns were seasonal, measurements of soil temperature were not equally spaced across the full range, with a gap between 13 to 18 ºC, which may affect our estimated relationship between temperature and BVOCs.

Soil water content has strong impacts upon the production and transport of BVOCs in soil, especially in the Mediterranean region (Asensio et al., 2007a; Asensio et al., 2007b; Asensio et al., 2008). Consistent with our first hypothesis, our result demonstrated a significant positive correlation between the exchange of isoprenoids and soil water content, indicating that an increase in soil moisture can promote the production of isoprenoids up to a certain threshold (Figure 5b). Soil acted as a net sink

for isoprenoids when soil water content was below 7%. An earlier study conducted in this site also observed a net rate of uptake when soil water content was lower than 5% in control plots, and also showed a linear increase in total BVOCs along with the increase in soil moisture (Asensio et al., 2007b).

Soil water content was positively correlated with soil CO_2 flux (Figure 3b), and the negative correlation between soil water content and soil CO_2 fluxes with monoterpene emissions (Figure 6a) suggests that these BVOCs may be consumed by soil microbes, which in turn increases microbial activity and leads to higher soil CO_2 emissions (Asensio et al., 2012; de Vries et al., 2018; McBride et al., 2020; Preece et al., 2020). Some soil microorganisms utilize monoterpenes as a carbon source (Albers et al., 2018; Asensio et al., 2012), such as, *Pseudomonas fluorescens* and *Rhodococcus erythropolis*, two soil bacteria, that utilise limonene and a-pinene as energy sources (van der Werf et al., 1999).

The heterogeneity of soil properties within the study area, influenced by the spatial distribution of trees and litter, can contribute to variations in BVOCs emissions, and may explain the large range of exchange rates measured in this study.

4.4.4 Balance of BVOCs between soil and atmosphere

The low emissions and high uptakes of soil monoterpenes observed in this Mediterranean holm oak forest demonstrate that the soil has the capacity to predominantly take up most isoprenoids, given dry conditions (Asensio et al., 2007a; Asensio et al., 2007b), and reveals the importance of soil processes in regulating the overall balance of BVOCs in the ecosystem. Our results indicate an annual net uptake

rate of isoprenoids ranging from 1.34 to 10.33 μg m^{-2} h^{-1} (Table 1). The seasonal pattern of soil BVOCs exchange showed our study site to be a net sink of BVOCs in summer and winter (Table 2), which aligns with previous findings of high uptake rates during the summer 2003 and the winter 2005 at the same site (Asensio et al., 2007a). The interannual variations of soil BVOCs exchange varied under different treatments and could be explained by two potential mechanisms: changes in the emission of BVOCs from aboveground sources (Llusià et al., 2011; Mu et al., 2018) and alterations in the diversity of soil microbial communities (Yuste et al., 2011). The negative correlation between the exchange rate of soil BVOCs and atmospheric BVOC concentrations ($r = -0.85$, $P < 0.01$, Table 5), indicated that the sink capacity may increase with atmospheric concentrations of BVOCs. The average atmospheric concentrations during our study were considerably higher than previous reports from the same site (Asensio et al., 2007a; Asensio et al., 2007b), primarily due to the increased foliar emission of isoprenoids under prolonged drought conditions following the year of 2005 (Llusià et al., 2011, 2013; Mu et al., 2018). The substantial concentration gradient between the soil and atmosphere likely enhanced the soil's capacity to act as a sink for isoprenoid uptake through processes such as adsorption and microbial biodegradation (Pegoraro et al., 2005, 2006; Spielmann et al., 2017).

Microbial processes contribute to the uncertainties associated with the BVOC sink (Albers et al., 2018; Ramirez et al., 2010; Rinnan and Albers, 2020; Trowbridge et al., 2020). The mean annual soil CO_2 flux in our study was twice as high during the period of 2019-2020 (3.26 μmol m^{-2} s^{-1}) compared to 2003-2005 (1.66 μmol m^{-2} s^{-1}) (Asensio et al., 2007a), suggesting that soil microbes may have developed an increased ability to consume BVOCs following long-term acclimation to water scarcity. The higher concentrations of atmospheric BVOCs observed in our study may enhance soil

microbial biomass (Asensio et al., 2012) and influence soil microbial communities (McBride et al., 2020). Soil fungi are generally more resilient to drought than bacteria (de Vries et al., 2018), and drought conditions can lead to an increase in fungal abundance but a decrease in bacterial abundance and diversity (Preece et al., 2019). VOC emissions have been found to have a negative correlation with soil bacterial diversity, and a greater abundance of fungi increases the ability of the soil to remove and absorb BVOCs (Abis et al., 2020). In this Mediterranean forest, soil fungi were found to exhibit better adaptation to drought compared to soil bacteria, and fungal diversity is less sensitive to seasonal changes compared to bacterial diversity (Yuste et al., 2011). Therefore, stable fungal communities may benefit from and enhance their capacity to take up BVOCs, particularly in a drier environment characterized by higher background concentrations of atmospheric BVOCs, which aligns with our findings.

4.5. Conclusions

Through our study, we have gained valuable insights into the specific contribution of soil BVOCs and their interactions with the environment, as we excluded emissions from aboveground organs of green plants. Environmental factors play a crucial role in shaping the source-sink dynamics of soil BVOCs, as demonstrated by our findings. Our first hypothesis was supported, indicating that lower soil moisture resulting from drought or seasonal climatic changes promotes the uptake of soil BVOCs. Notably, the highest BVOC emissions occurred during wet seasons (spring and autumn), while pronounced uptake was observed during the dry season (summer). Furthermore, our second hypothesis was partly supported by the significant increase in emissions of α-pinene and camphene, which were released by litter, suggesting that N fertilization

enhances the emission of soil BVOCs through specific compounds derived from litter. Importantly, the overall sink capacity of the soil was not significantly impacted by the experimental treatments. The soil displayed a prominent net uptake of BVOCs during dry seasons, coinciding with elevated atmospheric BVOC concentrations. These findings highlight the tendency of Mediterranean forest soils to serve as potential sinks for BVOCs. Understanding the variations in atmospheric BVOC concentrations and soil microbial communities provides a foundation for mechanistic analyses and the development of predictive models for soil BVOC fluxes. Our research sheds light on the intricate dynamics of soil BVOCs in Mediterranean forest ecosystems and underscores the pivotal role of environmental factors in driving these fluxes.

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.

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References

Aaltonen, H., Aalto, J., Kolari, P., Pihlatie, M., Pumpanen, J., Kulmala, M., Nikinmaa, E., Vesala, T., Bäck, J., 2013. Continuous VOC flux measurements on boreal forest floor. Plant Soil 369, 24–256.

Aaltonen, H., Pumpanen, J., Pihlatie, M., Hakola, H., Hellén, H., Kulmala, L., Vesala, T., Bäck, J., 2011. Boreal pine forest floor biogenic volatile organic compound emissions peak in early summer and autumn. Agric. For. Meteorol. 151(6), 682–691.

Abis, L., Loubet, B., Ciuraru, R., Lafouge, F., Houot, S., Nowak, V., Tripied, J., Dequiedt, S., Maron, P.A., Sadet-Bourgeteau, S., 2020. Reduced microbial diversity induces larger volatile organic compound emissions from soils. Sci. Rep. 10, 6104.

Albers, C. N., Kramshøj, M., and Rinnan, R., 2018. Rapid mineralization of biogenic volatile organic compounds in temperate and Arctic soils, Biogeosciences, 15, 3591–3601.

Arneth, A., Harrison, S.P., Zaehle, S., Tsigaridis, K., Menon, S., Bartlein, P.J., Feichter, J., Korhola, A., Kulmala, M., O'Donnell, D., Schurgers, G., Sorvari, S., Vesala, T., 2010. Terrestrial biogeochemical feedbacks in the climate system. Nat. Geosci. 3, 525–532.

Asensio, D., Peñuelas, J., Llusià, J., Ogaya, R., Filella, I., 2007a. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. Soil Biol. Biochem. 39(10), 2471–2484.

Asensio, D., Penuelas, J., Ogaya, R., Llusià, J., 2007b. Seasonal soil VOC exchange rates in a Mediterranean holm oak forest and their responses to drought conditions.

Atmos. Environ. 41(11), 2456–2466.

Asensio, D., Peñuelas, J., Prieto, P., Estiarte, M., Filella, I., Llusià, J., 2008. Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland. Eur. J. Soil Sci. 59(5), 878–891.

Asensio, D., Yuste, J.C., Mattana, S., Ribas, À., Llusià, J., Peñuelas, J., 2012. Litter VOCs induce changes in soil microbial biomass C and N and largely increase soil CO_2 efflux. Plant Soil 360(1-2), 163–174.

Bachy, A., Aubinet, M., Amelynck, C., Schoon, N., Bodson, B., Moureaux, C., Delaplace, P., De Ligne, A., Heinesch, B., 2018. Methanol exchange dynamics between a temperate cropland soil and the atmosphere. Atmos. Environ. 176, 229–239.

Barreira, L.M.F., Duporte, G., Parshintsev, J., Hartonen, K., Jussila, M., Aalto, J., Back, J., Kulmala, M., Riekkola, M.L., 2017. Emissions of biogenic volatile organic compounds from the boreal forest floor and understory: a study by solid-phase microextraction and portable gas chromatography-mass spectrometry. Boreal Environ. Res. 22, 393–413.

Blanch, J.S., Peñuelas, J., Sardans, J., Llusià, J., 2008. Drought, warming and soil fertilization effects on leaf volatile terpene concentrations in Pinus halepensis and Quercus ilex. Acta Physiol. Plant. 31(1), 207–218.

Bourtsoukidis, E., Behrendt, T., Yanez-Serrano, A.M., Hellen, H., Diamantopoulos, E., Catao, E., Ashworth, K., Pozzer, A., Quesada, C.A., Martins, D.L., Sa, M., Araujo, A., Brito, J., Artaxo, P., Kesselmeier, J., Lelieveld, J., Williams, J., 2018. Strong sesquiterpene emissions from Amazonian soils. Nat. Commun. 9(1), 2226.

Carrion, O., Gibson, L., Elias, D.M.O., McNamara, N.P., van Alen, T.A., Op den Camp, H.J.M., Supramaniam, C.V., McGenity, T.J., Murrell, J.C., 2020. Diversity of

isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation. Microbiome 8(1), 81.

Christodoulou, E., Agapiou, A., Omirou, M., Ioannides, I.M., 2021. Profiling soil volatile organic compounds after N fertilization in a soil grown with Rosmarinus officinalis. Appl. Soil Ecol. 164.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K.E., Oliver, A., Ostle, N., Prosser, J.I., Thion, C., Thomson, B., Bardgett, R.D., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nat. Commun. 9(1), 3033.

de Vries, F.T., Williams, A., Stringer, F., Willcocks, R., McEwing, R., Langridge, H., Straathof, A.L., 2019. Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. New Phytol. l, 224, 132–145.

Geron, C., Guenther, A., Greenberg, J., Karl, T., Rasmussen, R., 2006. Biogenic volatile organic compound emissions from desert vegetation of the southwestern US. Atmos. Environ. 40(9), 1645–1660.

Gray, C.M., Fierer, N., 2012. Impacts of nitrogen fertilization on volatile organic compound emissions from decomposing plant litter. Glob. Chang. Biol. 18(2), 739–748.

Gray, C.M., Monson, R.K., Fierer, N., 2010. Emissions of volatile organic compounds during the decomposition of plant litter. J. Geophy. Res. 115(G3).

Gray, C.M., Monson, R.K., Fierer, N., 2014. Biotic and abiotic controls on biogenic volatile organic compound fluxes from a subalpine forest floor. J. Geophys. Res.

Biogeosci. 119(4), 547–556.

Greenberg, J.P., Asensio, D., Turnipseed, A., Guenther, A.B., Karl, T., Gochis, D., 2012. Contribution of leaf and needle litter to whole ecosystem BVOC fluxes. Atmos. Environ. 59, 302–311.

Guenther, A., Hewitt, C.N., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., Lerdau, M., McKay, W.A., Pierce, T., Scholes, B., Steinbrecher, R., Tallamraju, R., Taylor, J., Zimmerman, P., 1995. A global model of natural volatile organic compound emissions. J. Geophy. Res. 100(D5), 8873–8892.

Hayward, S., Muncey, R., James, A., Halsall, C.J., Hewitt, C.N., 2001. Monoterpene emissions from soil in a Sitka spruce forest. Atmos. Environ. 35(24), 4081–4087.

Huang, X., Zheng, L., Guo, P., Yi, Z., 2021. Nitrogen addition inhibits total monoterpene emissions in subtropical forest floor of South China. Soil Ecol. Lett. 3(1), 63–72.

Horváth, E., Hoffer, A., Sebők, F., Dobolyi, C., Szoboszlay, S., Kriszt, B., Gelencsér, A., 2011. Microscopic fungi as significant sesquiterpene emission sources. J. Geophys. Res. 116, D16301.

Insam, H., Seewald, M.S.A., 2010. Volatile organic compounds (VOCs) in soils. Biol Fert Soils 46(3), 199–213.

Isidorov, V.A., Smolewska, M., Purzyńska-Pugacewicz, A., Tyszkiewicz, Z., 2010. Chemical composition of volatile and extractive compounds of pine and spruce leaf litter in the initial stages of decomposition. Biogeosciences 7(9), 2785–2794.

Kassambara, A., 2015. Factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0. 3, 2015.

Kesselmeier, J., Staudt, M., 1999. Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. J. Atmo. Chem. 33(1), 23–88.

Kleiber, A., Duan, Q., Jansen, K., Verena Junker, L., Kammerer, B., Rennenberg, H., Ensminger, I., Gessler, A., Kreuzwieser, J., 2017. Drought effects on root and needle terpenoid content of a coastal and an interior Douglas fir provenance. Tree Physiol. 37(12), 1648–1658.

Kramshøj, M., Vedel-Petersen, I., Schollert, M., Rinnan, Å., Nymand, J., Ro-Poulsen, H., Rinnan, R., 2016. Large increases in Arctic biogenic volatile emissions are a direct effect of warming. Nat. Geosci. 9(5), 349–352.

Leff, J.W., Fierer, N., 2008. Volatile organic compound (VOC) emissions from soil and litter samples. Soil Biol. Biochem. 40(7), 1629–1636.

Li, G., Su, H., Li, X., Kuhn, U., Meusel, H., Hoffmann, T., Ammann, M., Pöschl, U., Shao, M., Cheng, Y., 2016. Uptake of gaseous formaldehyde by soil surfaces: a combination of adsorption/desorption equilibrium and chemical reactions. Atmos. Chem. Phys. 16(15), 10299–10311.

Lin, C., Owen, S., Penuelas, J., 2007. Volatile organic compounds in the roots and rhizosphere of Pinus spp. Soil Biol. Biochem. 39(4), 951–960.

Llusià, J., Asensio, D., Sardans, J., Filella, I., Peguero, G., Grau, O., Ogaya, R., Gargallo-Garriga, A., Verryckt, L.T., Van Langenhove, L., Brechet, L.M., Courtois, E., Stahl, C., Janssens, I.A., Peñuelas, J., 2022. Contrasting nitrogen and phosphorus fertilization effects on soil terpene exchanges in a tropical forest. Sci. Total Environ., 802, 149769.

Llusià, J., Peñuelas, J., Alessio, G.A., Estiarte, M., 2006. Seasonal contrasting changes of foliar concentrations of terpenes and other volatile organic compound in four dominant species of a Mediterranean shrubland submitted to a field experimental drought and warming. Physiol. Plant. 127(4), 632–649.

Llusià, J., Peñuelas, J., Alessio, G.A., Ogaya, R., 2011. Species-specific, seasonal, inter-

annual, and historically-accumulated changes in foliar terpene emission rates in Phillyrea latifolia and Quercus ilex submitted to rain exclusion in the Prades Mountains (Catalonia). Russ. J. Plant Physiol. 58(1), 126–132.

Llusià, J., Peñuelas, J., Guenther, A., Rapparini, F., 2013. Seasonal variations in terpene emission factors of dominant species in four ecosystems in NE Spain. Atmos. Environ. 70, 149–158.

Mäki, M., Aalto, J., Hellen, H., Pihlatie, M., Back, J., 2019a. Interannual and Seasonal Dynamics of Volatile Organic Compound Fluxes From the Boreal Forest Floor. Front. Plant Sci. 10, 191.

Mäki, M., Aaltonen, H., Heinonsalo, J., Hellén, H., Pumpanen, J., Bäck, J., 2019b. Boreal forest soil is a significant and diverse source of volatile organic compounds. Plant Soil 441(1-2), 89–110.

Mäki, M., Heinonsalo, J., Hellén, H., Bäck, J., 2017. Contribution of understorey vegetation and soil processes to boreal forest isoprenoid exchange. Biogeosciences 14(5), 1055–1073.

McBride, S.G., Choudoir, M., Fierer, N., Strickland, M.S., 2020. Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics. Ecology 101(10), e03130.

Mu, Z., Llusià, J., Liu, D., Ogaya, R., Asensio, D., Zhang, C., Peñuelas, J., 2018. Seasonal and diurnal variations of plant isoprenoid emissions from two dominant species in Mediterranean shrubland and forest submitted to experimental drought. Atmos. Environ. 191, 105–115.

Mu, Z., Llusià, J., Liu, D., Ogaya, R., Asensio, D., Zhang, C., Peñuelas, J., 2019. Profile of foliar isoprenoid emissions from Mediterranean dominant shrub and tree species under experimental nitrogen deposition. Atmos. Environ. 216.

Ogaya, R., Peñuelas, J., 2007. Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. Plant Ecol. 189(2), 291–299.

Pegoraro, E., Abrell, L., Van Haren, J., Barron-Gafford, G., Grieve, K.A., Malhi, Y., Murthy, R., Lin, G., 2005. The effect of elevated atmospheric CO_2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm. Glob. Chang. Biol. 11(8), 1234–1246.

Pegoraro, E., Rey, A.N.A., Abrell, L., Van Haren, J., Lin, G., 2006. Drought effect on isoprene production and consumption in Biosphere 2 tropical rainforest. Glob. Chang. Biol. 12(3), 456–469.

Peguero, G., Folch, E., Liu, L., Ogaya, R., Peñuelas, J., 2021. Divergent effects of drought and nitrogen deposition on microbial and arthropod soil communities in a Mediterranean forest. Eur. J. Soil Biol. 103.

Peñuelas, J., 2008. An increasingly scented world. New Phytol. 180(4), 735–738.

Peñuelas, J., Asensio, D., Tholl, D., Wenke, K., Rosenkranz, M., Piechulla, B., Schnitzler, J.P., 2014. Biogenic volatile emissions from the soil. Plant Cell Environ. 37(8), 1866–1891.

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., Carnicer, J., Bartrons, M., Rivas-Ubach, A., Grau, O., Peguero, G., Margalef, O., Pla-Rabés, S., Stefanescu, C., Asensio, D., Preece, C., Liu, L., Verger, A., Rico, L., Barbeta, A., Achotegui-Castells, A., Gargallo-Garriga, A., Sperlich, D., Farré-Armengol, G., Fernández-Martínez, M., Liu, D., Zhang, C., Urbina, I., Camino, M., Vives, M., Nadal-Sala, D., Sabaté, S., Gracia, C., Terradas, J., 2018. Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia.

Environ. Exp. Bot. 152, 49-59.

Preece, C., Farre-Armengol, G., Peñuelas, J., 2020. Drought is a stronger driver of soil respiration and microbial communities than nitrogen or phosphorus addition in two Mediterranean tree species. Sci. Total Environ. 735, 139554.

Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol. Biochem. 131, 28–39.

Quan, W., Ding, G., 2017. Root tip structure and volatile organic compound responses to drought stress in Masson pine (*Pinus massoniana* Lamb.). Acta Physiol. Plant. 39(12), 258.

Ramirez, K.S., Lauber, C.L., Fierer, N., 2010. Microbial consumption and production of volatile organic compounds at the soil-litter interface. Biogeochemistry 99(1-3), 97–107.

Rasheed, M.U., Kivimäenpää, M., Kasurinen, A., 2021. Emissions of biogenic volatile organic compounds (BVOCs) from the rhizosphere of Scots pine (*Pinus sylvestris*) seedlings exposed to warming, moderate N addition and bark herbivory by large pine weevil (*Hylobius abietis*). Plant Soil 463(1-2), 379–394.

Raza, W., Mei, X., Wei, Z., Ling, N., Yuan, J., Wang, J., Huang, Q., Shen, Q., 2017. Profiling of soil volatile organic compounds after long-term application of inorganic, organic and organic-inorganic mixed fertilizers and their effect on plant growth. Sci. Total. Environ. 607-608, 326-338.

Rinnan, R., Albers, C.N., 2020. Soil uptake of volatile organic compounds: Ubiquitous and underestimated? J. Geophys. Res. Biogeosci. 125(6), e2020JG005773.

Sindelarova, K., Granier, C., Bouarar, I., Guenther, A., Tilmes, S., Stavrakou, T., Müller, J.F., Kuhn, U., Stefani, P., Knorr, W., 2014. Global data set of biogenic VOC

emissions calculated by the MEGAN model over the last 30 years. Atmos. Chem. Phys. 14(17), 9317–9341.

Spielmann, F.M., Langebner, S., Ghirardo, A., Hansel, A., Schnitzler, J.P., Wohlfahrt, G., 2017. Isoprene and α-pinene deposition to grassland mesocosms. Plant Soil 410, 313–322.

Staudt, M., Byron, J., Piquemal, K., Williams, J., 2019. Compartment specific chiral pinene emissions identified in a Maritime pine forest. Sci. Total Environ. 654, 1158–1166.

Stotzky, G., Schenck, S., Papavizas, G.C., 1976. Volatile organic compounds and microorganisms. CRC Cr. Rev. microbiol. 4(4), 333–382.

Tang, J., Schurgers, G., Rinnan, R., 2019. Process understanding of soil BVOC fluxes in natural ecosystems: A Review. Rev. Geophys. 57(3), 966–986.

Tang, J., Valolahti, H., Kivimäenpää, M., Michelsen, A., Rinnan, R., 2018. Acclimation of biogenic volatile organic compound emission from subarctic heath under long-term moderate warming. J. Geophys. Res: Biogeosci. 123(1), 95–105.

Team, R.C., 2013. R: A language and environment for statistical computing.

Tiiva, P., Julkunen-Tiitto, R., Häikiö, E., Kasurinen, A., 2019. Belowground responses of Scots pine (*Pinus sylvestris*) seedlings to experimental warming, moderate nitrogen addition, and bark herbivory. Can. J. For. Res. 49(6), 647–660.

Trowbridge, A. M., Stoy, P. C., Phillips, R. P. 2020. Soil biogenic volatile organic compound flux in a mixed hardwood forest: Net uptake at warmer temperatures and the importance of mycorrhizal associations. J. Geophys. Res. Biogeosci., 125.

Tsuruta, J., Okumura, M., Makita, N., Kosugi, Y., Miyama, T., Kume, T., Tohno, S., 2018. Comparison of the biogenic volatile organic compound emissions from the fine roots of 15 tree species in Japan and Taiwan. J. For. Res. 23, 242–251.

Vallicrosa, H., Sardans, J., Ogaya, R., Fernández, P.R., Peñuelas, J., 2021. Short-term N-fertilization differently affects the leaf and leaf litter chemistry of the dominant species in a Mediterranean forest under drought conditions. Forests 12， 605.

van der Werf M J, Swarts H J, de Bont J A M. Rhodococcus erythropolis DCL14 contains a novel degradation pathway for limonene. Appl. Environ. Microbiol. 1999, 65(5), 2092–2102.

Viros, J., Fernandez, C., Wortham, H., Gavinet, J., Lecareux, C., Ormeño, E., 2020. Litter of mediterranean species as a source of volatile organic compounds. Atmos. Environ. 242.

Wang, C., Liu, D., Bai, E., 2018a. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol. Biochem. 120, 126–133.

Wang, M., Schurgers, G., Hellen, H., Lagergren, F., Holst, T., 2018b. Biogenic volatile organic compound emissions from a boreal forest floor. Boreal Environ. Res. 23, 249–265.

Wickham, H., 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York.

Yang, W., Cao, J., Wu, Y., Kong, F., Li, L., 2021. Review on plant terpenoid emissions worldwide and in China. Sci. Total Environ. 787: 147454. https://doi.org/10.1016 /j.s citotenv.2021.147454.

Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17(3), 1475–1486.

General conclusions

This thesis aimed to discern the responses of soil respiration and soil BVOCs (Biogenic Volatile Organic Compounds) to climate change in two disparate forest ecosystems. To achieve this objective, I examined two ecosystems: the eastern forests of the Qinghai-Tibet Plateau, known for thier high sensitive to global temperature rise, and the water-stressed Mediterranean forests. As a result of the thesis research I can now conlcude:

1- The reduction in snow cover due to global warming has led to more frequent and severe freeze-thaw cycles within the soil, thereby altering soil aggregate composition and influencing soil microbial and enzymatic activities. The exclusion of snow has resulted in diminished concentrations of PLFAs and enzyme activities across three aggregate fractions

2- Notably, soil microbial attributes exhibited significant responses exclusively within small macroaggregates, indicating that aggregate size may have a stronger impact than snow exclusion on microbial variables. Overall, the reduction in snow is inducing discernible effects on both soil structure and function, which may hold subtantial implications for overall ecosystem health and functioning.

3- Increased frost intensity, stemming from snow exclusion, has led to a 17% decrease in average soil respiration rates during winter in comparison to control plots. Concomitantly, soil basal respiration, microbial phospholipid fatty acid levels, and enzyme activities have also experienced a reduction due to elevated frost exposure during winter periods.

4- Although soil nitrogen availability was augmented in snow-excluded treatments during winter in contrast to control plots, these effects did not extend to the subsequent

growing season. These findings imply that increased frost reduces soil respiration during winter through direct environmental impacts like soil temperature and indirect biological processes such as microbial biomass and activity, while refraining from inducing cross-seasonal effects.

5- The composition and emission rates of VOCs exhibit variability across diverse ecosystem soils, with forests primarily emitting isoprenoids, while non-isoprenoids dominate in tundra and cropland soils. The emission magnitude of soil VOCs across varied ecosystems is influenced by temperature, soil moisture content, litter type and season.

6- The critical mechanisms contributing to the soil VOCs sink function encompass deposition, adsorption, and microbial degradation of VOCs, resulting in the soil absorption of 17-36% of annual VOCs emitted to the atmosphere.

7- Climate change exerts influence over the soil carbon cycle, with potential implications for increased soil VOC emissions if warming and nitrogen enrichment augment soil organic matter decomposition.

8- Environmental factors wield pivotal roles in shaping the source-sink dynamics of soil BVOCs within Mediterranean forests. Reduced soil moisture stemming from drought or seasonal climatic shifts has been found to promote the uptake of soil BVOCs, accompanied by BVOC emissions during wet seasons. N fertilization can amplify the emission of soil BVOCs through the augmentation of specific compounds (α-Pinene) from litter.

9- The overarching sink capacity of the soil has not been significantly altered by experimental treatments, with the net uptake of BVOCs being particularly pronounced

during dry seasons when atmospheric BVOC concentrations are elevated. The outcomes suggest that Mediterranean forest soils hold the potential to serve as net sinks for BVOCs within future drier conditions.

Supplementary material

Chapter 4. Impacts of seasonality, drought, nitrogen fertilization, and litter on soil fluxes of biogenic volatile organic compounds in a Mediterranean forest

Table S1. Background concentrations of atmospheric BVOCs (ppbv) during the year of 2019-2020 in the Prades forest. Significance for season, treatment, and their interactions is indicated for the compounds using a linear mix-effects model, with plot as a random effect (means ± SE, n = 4). ND, not detected. C refers to control, D refers to plots with drought, N refers to plots with nitrogen fertilization and S refers to sampling season.

Season	Isoprene	Tricyclene	α-Pinene	Camphene	β-Pinene	β-Myrcene	Δ^3-Carene	Limonene	α-Cubebene	β-Caryophyllene	α-Caryophyllene	Total
2019 Winter	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.03	ND	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0	0.13 ± 0.09	ND	0.01 ± 0	ND	0.24 ± 0.15
2019 Spring	0.02 ± 0.01	ND	0.04 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.14 ± 0.05	ND	ND	0.01 ± 0	0.24 ± 0.06
2019 Summer	0.14 ± 0.10	0.02 ± 0.01	0.51 ± 0.07	0.05 ± 0.01	0.21 ± 0.02	0.24 ± 0.06	0.06 ± 0.03	9.47 ± 1.56	0.01 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	10.71 ± 1.67
2020 Winter	0.06 ± 0.05	0.18 ± 0.17	0.17 ± 0.08	0.03 ± 0.01	0.49 ± 0.46	0.17 ± 0.15	0.03 ± 0.01	0.29 ± 0.14	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0	1.65 ± 1.23
2020 Spring	0.05 ± 0.02	0.01 ± 0.01	0.18 ± 0.15	0.01 ± 0.01	0.05 ± 0.04	0.02 ± 0.01	0.01 ± 0.01	0.13 ± 0.13	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.45 ± 0.37
2020 Summer	0.09 ± 0.03	0.04 ± 0.01	1.24 ± 0.12	0.13 ± 0.04	0.73 ± 0.10	0.29 ± 0.02	0.08 ± 0.03	0.60 ± 0.03	0.15 ± 0.06	0.06 ± 0.01	0.02 ± 0.01	3.18 ± 0.32
2020 Autumn	0.12 ± 0.07	ND	0.15 ± 0.06	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.23 ± 0.14	0.04 ± 0.02	0.04 ± 0.03	0.01 ± 0.01	0.53 ± 0.29
F-value												
D	**3.25†**	1.67	2.23	**3.30†**	0.76	0.34	1.70	**5.20***	**9.29****	**11.96*****	**6.51***	**3.38†**
N	**2.90†**	2.10	**3.11†**	2.44	**5.90***	2.62	0.14	**3.84***	0.26	**4.40***	**4.31***	**3.09†**
S	**3.45****	**5.18*****	**21.23*****	**15.03*****	**23.63*****	**8.91*****	**4.49*****	**11.26*****	**17.12*****	**9.28*****	1.44	**13.35*****
D × S	**2.75***	**3.15****	1.64	**2.58****	**2.23***	1.17	**3.68****	**3.21****	**4.56*****	**5.69*****	1.42	**3.20****
N × S	0.75	1.54	0.93	1.02	1.43	0.61	**4.52*****	**1.77†**	**2.79***	**2.45***	0.44	0.99

†*P* < 0.1, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

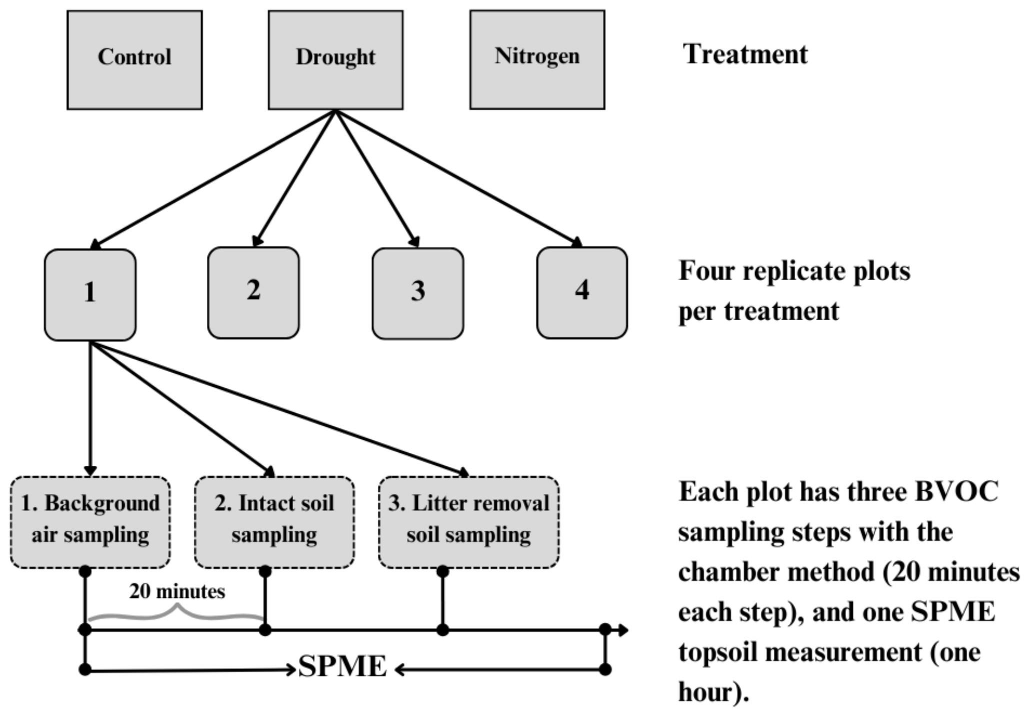

Figure S1. The experiment schematic diagram of BVOCs sampling study in the Prades Forest.

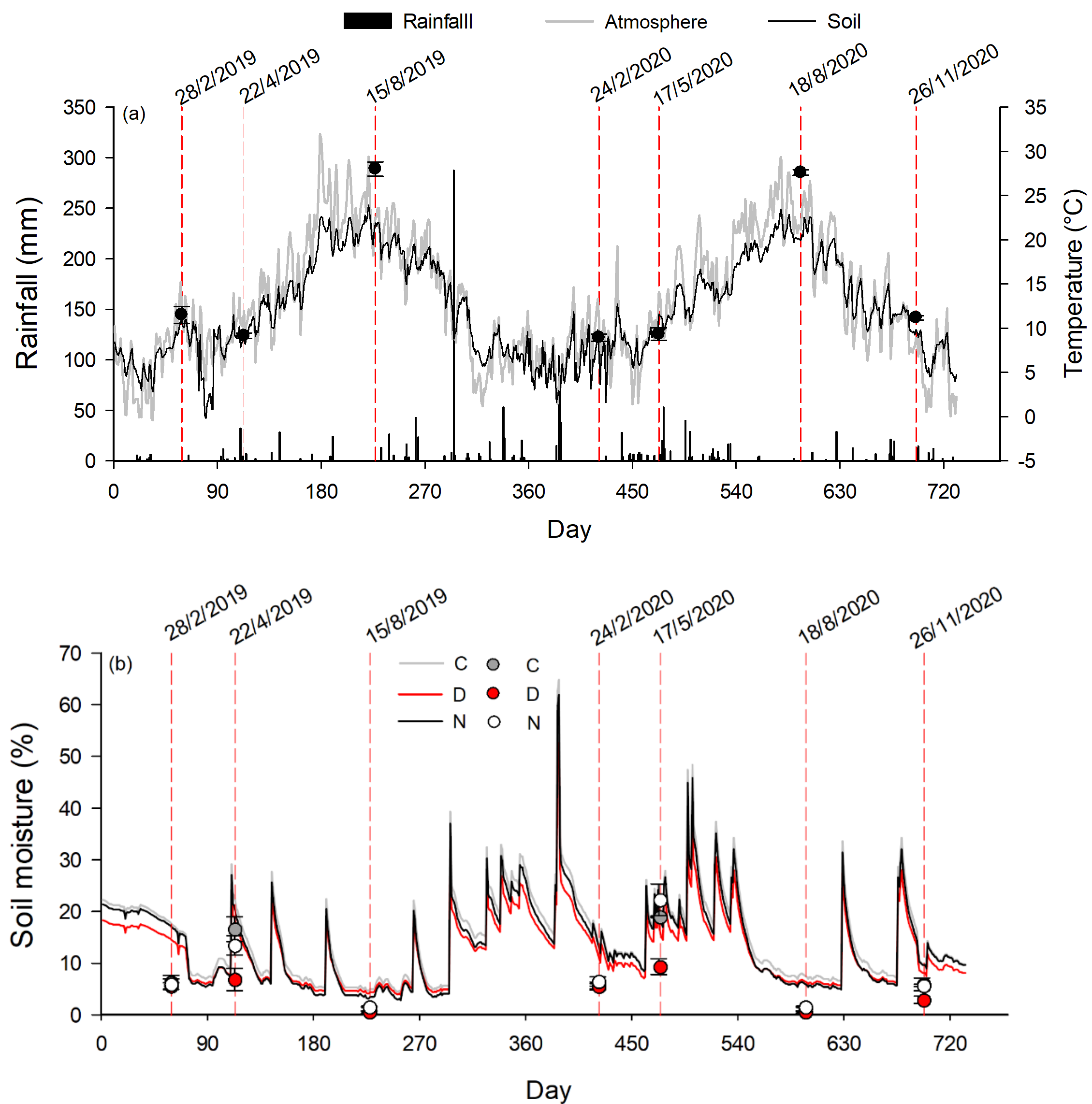

Figure S2. Variation in rainfall, soil temperature (a), and soil moisture content (b) measured during 2019-2020. Dots represent the soil temperature (or moisture content) on the date of sampling. Error bars indicate standard errors for each treatment (n = 4). Different letters within a season indicate significant differences (Kruskal–Wallis test; $p < 0.05$). †$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***, $P < 0.001$. C refers to control, D refers to plots with drought treatment and N refers to plots with nitrogen fertilization.

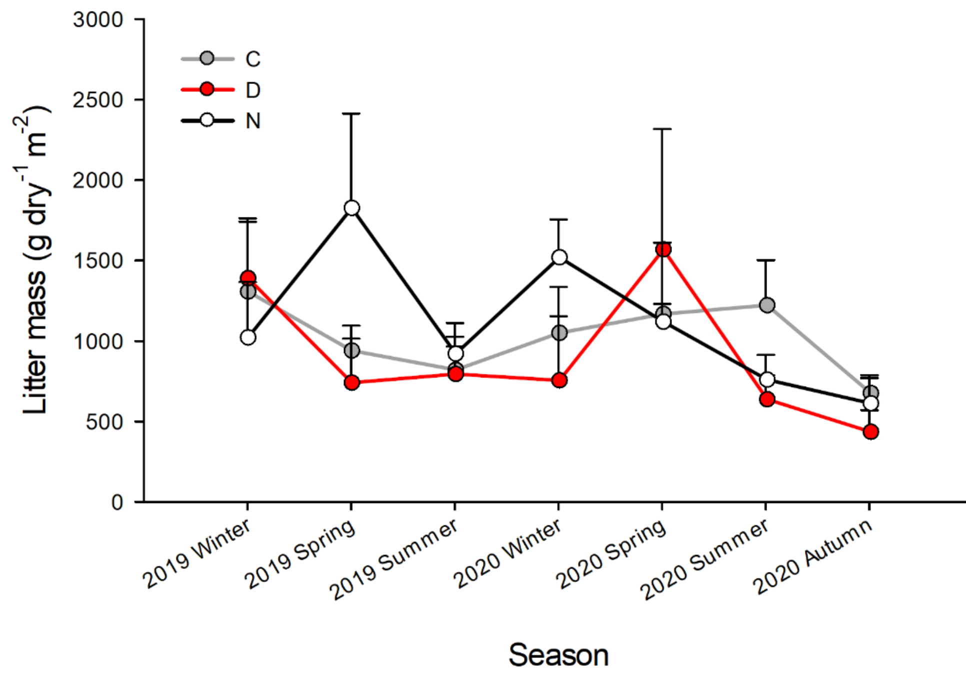

Figure S3. Seasonal variation of collected litterfall during the periods of years 2019-2020. C refers to control, D refers to plots with drought treatment and N refers to plots with nitrogen fertilization.