Land Use Effects on Carbon and Nutrient Fluxes in Soils

Dissertation

zur Erlangung des akademischen Grades doctor rerum naturalium
(Dr. rer. nat.)

vorgelegt dem Rat der Chemisch-Geowissenschaftlichen Fakultät der
Friedrich-Schiller-Universität Jena

von MSc in Environmental Engineering, Antonios Apostolakis
geboren am 1992-06-12 in Rethymnon, Griechenland

Dissertation, Friedrich-Schiller-Universität Jena, 2022

Gutachterinnen:

1.

2.

Tag der Verteidigung:

Acknowledgements

I sincerely thank my supervisors, Susan Trumbore, Beate Michalzik, Marion Schrumpf and Ingo Schöning for sharing with me their scientific enthusiasm and time, and for all the help they provided over the course of my PhD.

I thank Theresa Klötzing, Ines Hilke and Michael Rässler for their contribution in my laboratory work, and Steffen Ferber and Marco Pöhlmann for their contribution in my field work. I thank the members of the *IMPRS-gBGC* office, Steffi Rothhard, John Kula and Stefanie Burkert for their help and support. I thank all members of the *Soil Biogeochemistry* group for their company and feedback on my work.

My PhD was part of the *Biodiversity Exploratories* project. In this regard, I thank Christian Ammer, Daniel Prati, Elisabeth Schurig, Ellen Kandeler, Emily Solly, Falk Hänsel, Margot Neyret, Mark van Kleunen, Markus Fischer, Norbert Hölzel, Peter Manning, Peter Schall, Ralph Bolliger, Runa S. Boeddinghaus, Sven Marhan, Thomas Nauss, Till Kleinebecker, Valentin H. Klaus and Yvonne Oelmann for providing data related to soil, plant and microbial properties and environmental conditions, as well as Andreas Schwarz and Wolf-Anno Bischoff for sharing their expertise on nutrient leaching methods, and for reviewing different Chapters of my Thesis. I also thank the managers of the three *Exploratories*, Kirsten Reichel-Jung, Florian Staub, Juliane Vogt, Miriam Teuscher and all former managers for their work in maintaining the plot and project infrastructure; Christiane Fischer for giving support through the central office, Andreas Ostowski for managing the central database, and Eduard Linsenmair, Dominik Hessenmöller, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the project. I thank the administration of the Hainich national park, the UNESCO Biosphere Reserve Swabian Alb and the UNESCO Biosphere Reserve Schorfheide-Chorin, as well as all stakeholders for granting me access to their fields. I thank the state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg for the field work permits. I thank the DFG Priority Program 1374 "*Biodiversity- Exploratories*" (SCHR 1181/2-3) and the Max Planck Society for funding my research.

Finally, I thank my family and friends.

Table of Contents

List of Tables

List of Supplementary Tables

List of Figures

List of Supplementary Figures

1 Introduction

1.1 Motivation

Based on reports from the United Nations (2017), global population is expected to exceed 9 billion by the middle of the 21st century. This profound population growth, accompanied with the resource-consuming welfare of developed countries, is expected to impose a significant increase in the demand for resources (Godfray et al., 2010). At the same time, the need to mitigate global and climate change imposes additional restrictions to the exploitation and management of natural ecosystems (IPCC, 2014; Vitousek, 1994). Thus, the necessity to ensure ecosystem services and functions, through sustainable management, is more imperative than ever.

Global change in terrestrial ecosystems can be described as a shift in core characteristics of ecosystems at large scales due to human activities. Land-use change and management intensification are main causes of global change, which lead to less natural and more intensively managed ecosystems. Conversion of forested areas to grasslands or croplands, and selection of tree species with high financial value are common examples of land-use change and management intensification in the silvicultural sector. Similarly, increased nutrient inputs via fertilization and increased grazing per land area are common examples of management intensification in grasslands. These changes can provide financial benefits, at least, in the short-term. However, they are also linked to changes in biodiversity and element cycling, which can potentially impose severe impacts on ecosystems in the long-term. To this end, investigating the causes and effects of global change is tightly linked with sustainable development.

Terrestrial ecosystems support important services that can be separated into four categories, namely, the provisioning, regulating, supporting and cultural services (Haines-Young and Potschin, 2018). These services include, for instance, biomass production (provisioning), climate regulation, and energy, water and element cycling (regulating/supporting). Interestingly, all of these services interact directly and/or indirectly with soils and soil functions, rendering soils an integral component of ecosystems, and of pivotal importance for ecosystem services. This study investigates the effects of land-use and management intensity on two major and interrelated soil functions in temperate forests and grasslands: the soil respiration and the nutrient leaching.

1.1.1 Importance of soil respiration

Soil respiration, i.e. the CO_2 efflux from soil surface, is an indication of the metabolic activity of all living organisms in the soil, and it consists of a heterotrophic component that originates from soil fauna and microorganisms, and autotrophic component that originates from roots, mycorrhiza and rhizospheric microorganism (Bond-Lamberty et al., 2004; Hanson et al., 2000; Subke et al., 2006). It is one of the largest carbon fluxes in terrestrial ecosystems, second only to the gross primary production (Luo and Zhou et al., 2006). Thus, soil respiration represents the main pathway of organic carbon losses in soils, and, in some ecosystems, it might determine whether soils will act as a C sink or source (ecosystem service: *C cycling*). Apart from C cycling, the heterotrophic component of soil respiration is an indicator of microbial activity and of plant litter and soil organic matter decomposition, through which nutrients become available for acquisition by plant and microbes, or for leaching. Especially in unfertilized ecosystems, heterotrophic soil respiration controls nutrient dynamics and availability in soils and thus plant growth (ecosystem service: *nutrient cycling* and *biomass production*).

Temperate forests and grasslands store large amounts of C as soil organic matter (SOM), the fate of which is however uncertain due to global and climate change (Achat et al., 2015; Jobbagy and Jackson, 2000; Melillo et al., 2017). For instance, silvicultural and grassland management can influence organic C losses from soils via soil respiration through their effects on soil, plant and soil organism properties, as well as via their effects on climatic conditions (Rodeghiero et al., 2005; Conant et al., 2017). In addition, climate change might result in more frequent and/or severe droughts with important implications for C cycle and soil respiration (Allen et al., 2010; Bastos et al., 2020; Trenberth et al., 2014). As a result, understanding the interactions between ecosystem management, biotic drivers and soil respiration, while accounting for environmental (i.e. climate and soil) conditions, can provide important insights for sustainable ecosystem management and climate change mitigation (ecosystem service: *climate regulation*, FAO, 2010).

1.1.2 Importance of nutrient leaching

Leaching is defined as the downward flux of nutrients in soils with the percolating water. In unfertilized ecosystems, nutrient leaching represents the net balance between nutrient inputs to the soil solution (e.g. by organic matter mineralization or dissolution) and outputs from the soil solution (e.g. due to uptake by plants, or immobilization in microbial biomass), as well as nutrient exchange with the soil matrix (i.e. via (de)sorption to minerals). Nutrients leached below the rooting zone are lost from the ecosystem, and represent losses of important agricultural resources with considerable monetary value (ESF, 2011). At the same time, nutrient leaching, especially in the case of nitrate and phosphate, is a potential threat to public health and aquatic ecosystems (ecosystem services: *water quality control* and *biomass production*, EEA, 2018; EU-Nitrate Directive, 2018; WHO, 2011).

Management intensification places temperate ecosystems at considerable risk of nutrient losses via leaching (Christiansen et al., 2010; Gundersen et al., 2006; Klaus et al., 2018). While several studies investigate the effects of management on (mostly inorganic) nitrogen (N) leaching, management effects on the leaching of other elements, like phosphorus (P) and sulphur (S), are less studied, despite their agricultural and environmental importance (Eriksen, 2009; Withers et al., 2014; Gallejones et al., 2012). In addition, studies that focus simultaneously on multiple nutrients are, in general, scarce (Klaus et al., 2018). Understanding the relationships between ecosystem management, biotic drivers and nutrient leaching, while accounting for environmental (i.e. climate and edaphic) conditions, can provide important insights for sustainable grassland management, and can help secure associated ecosystem services.

1.2 State of the art

1.2.1 Research gaps in land-use, management intensity and biodiversity research

Land-use and management intensity exert strong effects on soil biogeochemical cycles in temperate regions. In forests, management choices (such as the selection of tree species and the timing and intensity of harvest) shape forest properties, like the stand age, density and canopy openness, as well as the tree species richness and functional diversity of the stand. These forest properties influence the environmental conditions and soil properties of the forest stand, by determining, among others, forest and soil climate, understory plant biomass and diversity, and the production and quality of above and belowground plant litter (Berg and McClaugherty, 2003; Ehbrecht et al., 2019; Felipe-Lucia et al., 2018; Finér et al., 2011; Hobbie et al., 2007; Jewell et al., 2017; Solly et al., 2014; Figure 1.1). Thus, silvicultural management has important implications for biogeochemical cycling in soils.

Grassland management, which is mainly expressed by the intensity of fertilization, mowing and grazing (Blüthgen et al., 2012), influences soils and plants that in turn influence soil biogeochemical cycles. For example, intensively managed grasslands have higher plant productivity, lower plant species richness and more exploitative plant community traits compared to extensively managed grasslands (Allan et al., 2015; Blüthgen et al., 2012; Busch et al., 2018; Socher et al., 2012). This differentiation between intensively and extensively managed grasslands affects carbon and nutrient cycling in grassland soils. This is because a higher plant productivity is associated with higher plant litter inputs in the soil and higher nutrient outputs (e.g. harvest) from the ecosystem, but, at the same time, plant species-poor grasslands are linked to lower C storage, increased C losses and lower soil N retention (Lange et al., 2015; Leimer et al., 2016; Steinbeiss et al., 2008). Thus, similar to silvicultural management, grassland management has also important implications for biogeochemical cycling in soils.

Figure 1.1 A coniferous (left, SEW21) and a deciduous (right, SEW36) forest stand in Schorfheide-Chorin that are part of the German Biodiversity Exploratories project. These two forest stands are located approximately 6 km apart. The two photos were taken on 2019-06-25 and, more specifically, at 08:48 in SEW21 and at 09:07 in SEW36 (by A. Apostolakis). The forest properties of the two stands, as shaped by silvicultural management, promote differences in the light conditions under the canopy (climatic conditions), in the understory biomass and richness (plant properties), and in the accumulation of the organic layer (soil properties).

Different land-use types, like forests and grasslands, have distinct vegetation, environmental conditions and soil properties, which can lead to divergent drivers of soil biogeochemical cycles. For instance, temperate grasslands have often higher soil pH, fine root biomass, and microbial biomass than forests and lower soil C:N ratio than forests, which favour microbial activity and lead to higher decomposition rates of above- and belowground plant litter, and higher mineralization rates of soil organic carbon (SOC) (Gan, 2019; Solly et al., 2014; Zhang et al., 2008). Furthermore, silvicultural management happens at longer time scales compared to grassland management that usually happens annually. Grassland ecosystems experience biomass removal, due to harvest or grazing, multiple times per year, which reduces C inputs in the soil and increases nutrient outputs from the ecosystem. However, temperate grasslands also receive high amounts of fertilization (Blüthgen et al., 2012; Gilhaus et al., 2017), while, typically, silvicultural management dispenses with fertilization in temperate regions such as those of central Europe. Studies that investigate and compare the drivers of soil functions in different land-use types of a landscape under a common sampling protocol can provide interesting insights for landscape management, and identify opportunities for global and climate change mitigation.

Land-use, management intensity and biotic effects on soil biogeochemical cycles depend, to some extent, on environmental (i.e. climatic and edaphic) conditions. To date, several studies have investigated these effects in common garden experiments to minimize environmental effects that might mask other drivers of soil biogeochemical cycles (Christiansen et al., 2010; Vogel et al., 2019; Vesterdal et al., 2012). Yet, to gain knowledge transferable to sustainable ecosystem management, we need to investigate ecosystems along broad environmental gradients (Manning et al., 2019). This can be achieved by simultaneously studying a large number of sites with different conditions at the regional scale of multiple study regions.

Biotic effects, e.g. plant and microbial effects, on biogeochemical cycles are frequently investigated through experimental studies. Such studies are useful to gain mechanistic insights in biogeochemical processes, but they often do not consider realistic management intensity gradients, even though management is a main determinant of biotic communities. For instance, nitrogen fertilization is a dominant driver of plant species loss in grasslands (Blüthgen et al., 2012; Midolo et al., 2018) and, thus, it can contribute to nitrate leaching risk directly (i.e. increased inputs) and indirectly, because plant species-poor grasslands are associated with high nitrate leaching risk (Klaus et al., 2018; Leimer et al., 2016). Studies that do not include management intensity gradients often consider plant community assemblages created randomly based on a given seed bank (Klaus et al., 2020a), and therefore they might fail to capture the interactions developed in the real world. Thus, their results do not provide the necessary information for the stakeholders and policy makers in terms of sustainable ecosystem management. For these reasons, studies that investigate biotic effects on soil functions under broad management intensity gradients are needed.

Silvicultural and grassland management can influence soil functions both directly and indirectly. For instance, in intensively managed grasslands, fertilization directly increases nutrient concentration in soil solution leading to increased leaching risk, but it also increases the decomposability of plant litter which increases available nutrients for leaching compared to extensively managed grasslands (de Vries and Bardgett, 2016; Klaus et al., 2018). Therefore, responses of soil functions to management intensity should not be reported only as direct effects, but, following a holistic approach, also through the changes that management intensification causes in soils, plants and soil organisms (Koncz et al., 2015; Pommier et al., 2017).

To summarize, the complex interactions between land-use, management intensity, biodiversity and environmental conditions, demand for studies that i) investigate the drivers of soil functions in real-world ecosystems, ii) cover both broad management intensities and iii) involve broad environmental (both climatic and soil) gradients. To fulfil these criteria, future research attempts should study a high number of broadly managed ecosystems under different land-use types in multiple study regions. However, obtaining forest and grassland inventory data, while accounting for a high spatial scale, leads to considerable logistical constraints, especially if seasonal monitoring is needed. For this reason, such studies are generally scarce.

1.2.2 Innovation of the Thesis

To address the aforementioned research gaps, this Thesis investigates two major soil functions i) in a large number of real-world ecosystems, ii) including two land-use types and a range of different management intensities, and iii) in multiple study regions. In more detail, this Thesis focuses on in-situ soil respiration and nutrient leaching in forests and grasslands, and involves 300 managed ecosystems that cover broad management intensity gradients in three German regions with distinct environmental conditions. As part of the German *Biodiversity Exploratories* project (Fischer et al., 2010), information related to management intensity, climatic conditions, and soil, plant and soil organisms for all 300 ecosystems were available. Therefore, I had the unique opportunity to investigate the drivers of soil respiration and nutrient leaching, including both management intensity and soil, plant and soil organism effects, in a well-established framework that alleviated the logistical constraints associated with large-scale studies on multiple land-use types and study regions (Figure 1.2).

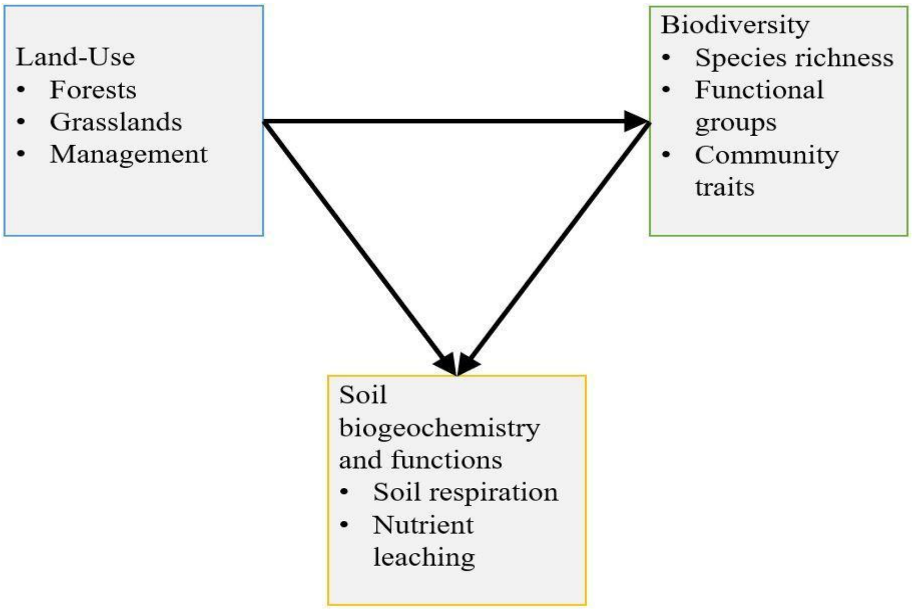


Figure 1.2 Interdisciplinary scope of the Thesis. Investigation of land-use and biodiversity effects on major soil functions. Land-use types include forests and grasslands, each covering broad management intensity gradients representative of each study region. Biodiversity refers mainly to plant diversity and is examined with species richness, functional groups and/or community traits. Soil functions included the early-summer soil respiration (measured as total soil CO_2 efflux) and annual nutrient leaching risk (measured as downward nutrient flux at 10 cm depth).

However, both soil respiration and nutrient leaching, as most biogeochemical processes, depend on the seasonal variation of climate, and, for this reason, they are traditionally monitored with high temporal resolution at few sites. In this Thesis, the conflict between space and time was resolved by employing methodologies and sampling protocols that estimate soil respiration and nutrient leaching as cumulative fluxes over time, and, in the case of soil respiration, by assuming that its spatial patterns remain relatively stable over the growing season (Søe and Buchmann, 2005). For soil respiration, the soda-lime absorption method was used to determine soil CO_2 efflux (Keith and Wong, 2006; Janssens and Ceulemans, 1998). In this application, the soda-lime method integrated soil respiration over time periods ranging from three to six days, depending on the land-use type. In-situ soil respiration measurements were taken in early summer, when soil respiration peaks in temperate ecosystems. For nutrient leaching, a resin method, namely the Self-Integrating Accumulators, was used to estimate the leaching of four nutrients (i.e. nitrate, ammonium, phosphate and sulphate) over the course of a year (Bischoff, 2009). These methods integrate the climatic variability in the measurements over the application periods, and allow the investigation of drivers that emphasize other causes of variability (Grogan, 1998; Klaus et al., 2018; Klaus et al., 2020b).

This Thesis involves 150 forests (*Chapter 2*) and 150 grasslands (*Chapters 3* and *4*) in an attempt to explain soil respiration (single-in-time measurements obtained in 2018 and 2019) and nutrient leaching (annual fluxes from spring 2018 to spring 2019) in the landscape scale. Investigating the drivers of soil functions in a high number of sites imposes considerable logistical constraints. This is especially true when multiple drivers are considered. In this Thesis, data on forest and grassland inventory, soil properties and microbial properties were not always measured on the same year(s) as the soil respiration and nutrient leaching. For instance, forest inventory data in the 150 forests of the *Biodiversity Exploratories* were collected from 2014 to 2018 (Schall et al., 2018). In contrast, grassland inventory data were collected once per year in late spring time. Most soil and microbial properties were determined from soil samples collected in 2017 during an extensive field campaign in all 300 sites of the *Biodiversity Exploratories*. The only exceptions were: i) the soil texture and fine root biomass which were determined in composite samples collected in 2011 (Solly et al., 2014) and ii) the soil bulk density, soil oxalate extractable aluminum and soil inorganic (Olsen) P which were determined in 2014 (Sorkau et al., 2018) always with the same sampling procedure. To this end, similar to previous studies (Felipe-Lucia et al., 2018; Klaus et al., 2018, Le Provost et al., 2021), I assumed that the intra- and inter-annual variability of the aforementioned variables will not obscure their effects on soil respiration and nutrient leaching when focusing at the landscape scale.

1.3 Aims and objectives

To address the aforementioned research gaps, this Thesis aims to identify the main drivers of soil respiration and nutrient leaching in temperate forests and grasslands. Emphasis is given on the effects of management and management intensification on these two soil functions. Apart from direct management effects, the effects of management-driven changes in soils, plants and/or microbes on soil functions are investigated. Silvicultural and grassland management follow distinct practices, and, for this reason, the two ecosystem types are mostly investigated separately. However, a comparison of the drivers of the two soil functions between temperate forests and grasslands is given as a synthesis at the end of the Thesis. The main objectives of this Thesis are:

1. To determine in-situ soil respiration in a high number of forest ecosystems over multiple regions, and to identify the main drivers of soil respiration under different silvicultural

practices (from natural to intensively exploited forests) and under broad climatic and soil conditions.

2. To determine in-situ soil respiration in a high number of managed grassland ecosystems over multiple regions, and to identify the main drivers of soil respiration under a broad management intensity gradient (from unfertilized pastures to intensively fertilized meadows) and under broad climatic and soil conditions.
3. To determine the leaching of nitrate, ammonium, phosphate and sulphate in a high number of managed grassland ecosystems over multiple regions, and to identify the main drivers of leaching risk over a management gradient ranging from extensively managed and plant species-rich grasslands to intensively managed and plant species-poor grasslands.

In this Thesis, an observational approach was followed, which allowed the investigation of a large number of forest and grassland ecosystems, at the landscape scale in three regions. More specifically, this Thesis is part of the German *Biodiversity Exploratories* project (Fischer et al., 2010), a long-term and large-scale project that investigates the effects of land-use and management on biodiversity and ecosystem services and functions. The German *Biodiversity Exploratories* project is globally one of the largest biodiversity projects, and includes 300 sites in Germany that have been intensively and continuously studied since 2008. It involves three study regions in Germany (Figure 1.3) that are the Biosphere Reserve Schwäbische-Alb in the state of Baden-Württemberg, the National Park Hainich and the surrounding of Hainich-Dün in the state of Thüringen, and the Biosphere Reserve Schorfheide-Chorin in the state of Brandenburg. Each of the three study regions has distinct climatic, geological and edaphic conditions. Within each region, 50 forest and 50 grassland sites were established in 2008. These sites are located within larger ecosystems and are managed by stakeholders and land owners. Management practices in these sites are representative of the common practices within each study region.

Figure 1.3 The three study regions of the Biodiversity Exploratories project in Germany.

1.4 Outline of the Thesis

This Thesis includes five main chapters. The current, first Chapter introduces the main concepts of the Thesis, the research gaps that the Thesis addresses, and the objectives of the Thesis. Chapters 2 to 4 address the main objectives of the Thesis. In more detail, Chapter 2 examines the importance of forest properties, as shaped by silvicultural management, for in-situ soil respiration over multiple regions, and identifies the main drivers of soil respiration in temperate forests. Similarly, Chapter 3 examines the effects of grassland management on in-situ soil respiration over multiple regions, and, in addition, investigates how management-driven changes in soils, plants and soil microbial properties alter in-situ soil respiration in temperate grasslands. Chapter 4 examines the importance of grassland management for the leaching risk of multiple nutrients (i.e. nitrate, ammonium, phosphate and sulphate), not only through the effects of management on nutrient inputs and outputs (i.e. fertilization and biomass removal via harvest or grazing) in an ecosystem, but, similar to Chapter 3, also through management-driven changes in soil, plant and soil microbial properties in temperate grasslands. Finally, Chapter 5 provides a synthesis on silvicultural and grassland management effects on soil respiration and nutrient leaching in order to determine common drivers of the two soil functions within and between the two land-use types.

2 Drivers of soil respiration in temperate forests under drought

Abstract

Forest soil respiration is a major pathway for soil carbon losses but rarely evaluated spatially at the regional scale, where forest and soil properties can influence soil CO_2 emissions. The importance of forest and soil properties for soil respiration can only be assessed with high spatial coverage of forest sites at the regional scale and for multiple study regions.

We measured the in-situ soil respiration with the soda-lime method along with an open, static chamber in 150 temperate forest stands in three German regions in early summer 2018 and 2019. Both years were affected by successive naturally occurring droughts. Results from the field were compared to soil respiration from lab incubations under optimal soil moisture (60% water holding capacity) of topsoil samples from the same sites collected in 2011. The impact of silvicultural management on soil respiration was studied with forest structural and compositional properties, as well as with physico-chemical soil properties.

Forest properties explained a large portion (>30%) of the soil respiration, highlighting their importance as soil respiration drivers at the regional scale. While soil organic carbon concentration was the most important predictor of soil respiration in the incubations, organic carbon stocks were not important for explaining variations in the in-situ respiration measurements, which were best explained by fine root biomass. Comparing in-situ soil respiration measurements with and without the organic layer revealed that the organic layer did not contribute significantly to total soil respiration during the studied dry periods. Taken together, our results indicate that autotrophic respiration was possibly the main contributor to total in-situ soil respiration during the dry periods of our study. Of six examined forest properties, mean diameter at breast height and conifer share were the most important drivers of soil respiration. A persistent positive effect of mean diameter at breast height on in-situ soil respiration revealed higher fluxes in older stands with thicker trees and small percentage of conifers under dry conditions. In contrast, under non-limiting water conditions (lab incubation), stands with low mean diameter at breast height and high conifer share had increased heterotrophic respiration.

Forest properties, and especially mean diameter at breast height and conifer share, can influence autotrophic and heterotrophic soil respiration and should be considered when predicting the spatial variation of soil respiration at the regional scale.

2.1 Introduction

Temperate forests store about 14% of the global forest carbon stock with the bigger part being stabilized in the soil (Pan et al., 2011). Forest soils have a high potential for carbon storage but uncertainties arise due to intensive silvicultural management (Achat et al., 2015), that can increase soil carbon losses through increased decomposition, leading to higher respiration losses. Forest properties can influence soil respiration, since they control multiple ecosystem services and functions. For instance, they control above and belowground litter quantity, quality and decomposition and forest microclimate (Berg and McClaugherty, 2003; Ehbrecht et al., 2019; Felipe-Lucia et al., 2018; Finér et al., 2011), which can be especially important under extreme climatic events, like droughts that affect ecosystem productivity and carbon fluxes (Bastos et al., 2020). Understanding the direct and indirect effects of different forest properties on soil respiration is therefore necessary for appropriate management recommendations to reduce greenhouse gas emission in the land-use, land-use change and forestry sectors.

Forest soil respiration depends on climatic conditions and forest and soil properties (Rodeghiero et al., 2005). Several studies have investigated the effects of forest type and tree species on soil respiration, but often came to inconsistent conclusions (Gan et al., 2020; Jewell et al., 2017; Vesterdal et al., 2013). Tree species can influence soil carbon fluxes by determining the amount and chemistry of litter inputs, that further can affect soil properties, like soil pH, cation composition and nutrient content, and, thus, the fate of organic carbon in forest floors and soils (Angst et al., 2019; Berg and McClaugherty, 2003; Hobbie et al., 2007). Moreover, tree species differences in root morphology and exudation (August et al., 2002), microbial community (Purahong et al., 2015), and forest microclimate are also important factors. In addition, Raich and Tufekciogul (2000) suggested that differences in carbon allocation patterns and/or autotrophic soil respiration could explain tree species influences on soil respiration. Many studies point out that broadleaf species lead to higher soil respiration rates based on the higher nutrient status of their leaf litter compared to coniferous species (Borken et al., 2002; Raich and Tufekciogul, 2000). In contrast, other studies did not observe differences between soil respiration fluxes of deciduous and coniferous forests (Borken and Beese, 2005; Subke et al., 2006; Vesterdal et al., 2012). Vesterdal et al. (2012) attributed the discrepancy between different studies to the effect of multiple and interacting factors controlling soil respiration. This is supported by a study of Berger et al. (2010), who measured soil respiration in pure spruce, mixed and pure beech forests in two regions and reported different results depending on the bedrock. Accordingly, larger regions with different parent material and soil types should be considered before generalizing tree species or management effects for soil carbon storage.

At present, most studies relating forest properties to soil respiration mainly focus on single or dual forest properties. The most commonly investigated forest structural properties are stand basal area and diameter at breast height, and many studies have investigated soil respiration across an age gradient (Kukumagi et al., 2017; Zhao et al., 2016). Forest compositional effects on soil respiration have been investigated as single species effects (Borken et al., 2002; Vesterdal et al., 2012) or along a gradient from pure deciduous to pure evergreen forests (Berger et al., 2010). Tree species diversity, however, is not frequently considered in soil respiration studies (Chen and Chen, 2019). Forest inventory data are in general limited due to the large amount of effort required, and the soil respiration literature that includes forest inventory data is even more restricted. A holistic approach, considering multiple forest properties (Schall et al., 2018), can therefore provide useful insights on the importance of different aspects of silvicultural management for soil carbon losses.

Temporal monitoring of soil respiration while maintaining a high spatial coverage at the regional scale requires a large amount of effort. For this reason, most field studies on soil respiration temporal variability are conducted in single regions and include only a small number of replicated plots. Similarly, studies addressing the spatial heterogeneity of soil respiration are often limited in the time of observations. However, forest and physicochemical soil properties, like soil texture, pH and organic carbon stocks, change in slow rates that can be effectively considered constant over short periods of time, especially if no management activities (like tree harvest and soil amendments) take place over the examined time period. In addition, the spatial patterns of soil respiration remain relatively stable over the growing season (Søe and Buchmann, 2005). Thus, measuring in-situ forest soil respiration with single measurements in time can reduce the aforementioned constraints and allow the investigation of the spatial variation of soil respiration across a large number of sites.

In-situ forest soil respiration integrates components derived from the organic layer and the underlying mineral soil. The carbon stored in these two pools is not equally protected and frequently studies compare their carbon losses through respiration. Under field conditions, this can be done by comparing total soil respiration fluxes with fluxes measured after removing the organic layer (Borken and Beese, 2005), while in the laboratory this can be done by incubating separately organic layer and mineral soil samples (Hobbie et al., 2007).

Forest soil respiration has an autotrophic component, i.e. carbon respired by roots, mycorrhiza and rhizospheric microorganism, and a heterotrophic component derived from microbial and soil fauna respiration (Bond-Lamberty et al., 2004; Hanson et al., 2000). Laboratory soil incubations can provide useful insights on the drivers of the heterotrophic component of soil respiration, while controlling for soil temperature and moisture (Gan et al., 2020). However, incubations have well-known shortcomings and fail to capture the complex interactions that exist in the field. Forest properties can influence soil respiration from laboratory incubations only indirectly, through effects on soil and microbial properties. Results obtained from incubation studies are not necessarily transferable to field conditions, but the controlled conditions provide a standardized base for comparison by removing the direct influence of environmental conditions on respiration.

Environmental conditions are subject to extreme events like droughts, which are likely to increase with future climate change (Allen et al., 2010; Trenberth et al., 2014), and that can strongly impact soil respiration. A growing scientific literature deals with the impact of drought on forest soil respiration through field or laboratory experiments by rainfall exclusion or artificial wetting-drying cycles, respectively. Results from such experiments indicate a significant reduction in soil respiration under drought conditions (Borken et al., 2006; Nikolova et al., 2009), while post-drought effects have also been reported (Muhr and Borken, 2009; Selsted et al., 2012; Schindlbacher et al., 2012). Droughts differentially affect autotrophic and heterotrophic respiration in directions that vary among ecosystem types and climatic regions (Casals et al., 2011; Hinko-Najera et al., 2015; Sun et al., 2019). It is still unclear how the different components of soil respiration will respond to drought in temperate forests, where trees grow deep rooting systems (Jackson et al., 1996), and, thus, root activity might be less affected than microbial activity by dry conditions due to access to deep water. At the same time, soil respiration data under naturally occurring droughts are limited (Nikolova et al., 2009), and even fewer studies investigate effects of forest properties on soil respiration under natural drought events. Chang et al. (2016) performed a rainfall exclusion (15% rainfall reduction) experiment in a Mediterranean forest stand dominated by two oak species and reported contrasting responses for soil respiration measured under *Quercus corrioides* and under *Quercus ilex*. Compared to the control conditions, soil respiration declined due to rainfall exclusion under *Q. corrioides*, but marginally increased under *Q. ilex*, indicating that soil respiration responses to drought can be species dependent even within a stand. Long term monitoring experiments provide platforms to observe natural disturbance events without the biases associated with manipulation experiments and allow the study of complex ecosystem interactions between plants and soil organisms that a disturbance triggers in the real world.

To resolve the conflict between spatial and temporal restrictions affecting soil respiration studies, we obtained one-time soil CO_2 efflux measurements in early summer 2018 and 2019 from 150 forest sites in the three study regions of the Biodiversity Exploratories project. Our forest sites covered a broad range of forest structural and compositional properties. In both years, a summer drought occurred in central Europe that also affected our study regions, with 2018 being drier than 2019. The measurements took place from June to July, with four simultaneous measurements at each site. To determine the contribution of the organic layer to the total soil respiration (Borken and Beese, 2005), we measured the CO_2 efflux from the mineral soil after removing the organic layer at a subset of 29 forest sites. To have a time-integrated measure at a large number of plots, we used a soda-lime absorption material in an open, static chamber. In-situ soil respiration fluxes were compared to soil respiration rates from laboratory incubations performed at optimal soil moisture (60% water holding capacity) of (top)soils from the same sites conducted in 2011 (Gan et al., 2020). We hypothesized that:

1. In addition to basic physico-chemical soil properties, forest structure and composition, as shaped by silvicultural management, affect soil respiration at the regional scale.
2. Since soil respiration consists of an autotrophic and a heterotrophic component that are driven by different processes, we hypothesized that i) multiple forest properties, both structural and compositional, drive the spatial variation of soil respiration at the regional scale and ii) the effects of forest properties on soil respiration will differ between the in-situ and soil incubation measurements.
3. In-situ soil respiration mainly originates from autotrophic respiration in early summer conditions under drought.

2.2 Methods

2.2.1 Study regions

This study was conducted in the framework of the Biodiversity Exploratories project that investigates interactions between biodiversity and land-use and their impact on ecosystem functions and services (Fischer et al., 2010). The Biodiversity Exploratories include three study regions in Germany, the Schwäbische-Alb (ALB), the Hainich-Dün (HAI) and the Schorfheide-Chorin (SCH). The study regions differ in their geology, climate and topology (Table 2.1), and thus have different soil types. ALB soils developed mainly on Jurassic limestone and were clay-rich Leptosols or Cambisol (IUSS Working Group WRB, 2014). In HAI, soils had a loamy or clayey texture due to the dominant geological substrate of loess over limestone, and the main soil types were Luvisols and Stagnosols. Soils in SCH had a sandy texture as the geological substrate was glacial till covered by glacio-fluvial or aeolian sand, and were classified as Arenosols or Cambisols.

Table 2.1 Geographical, topological and climatic characteristics of the three study regions included in the Biodiversity Exploratories project after Fischer et al. (2010). Abbreviations: ALB: Schwäbische-Alb, HAI: Hainich-Dün and SCH: Schorfheide-Chorin, AMT: Annual Mean Temperature, AMP: Annual Mean Precipitation, asl: above sea level.

Parameter	ALB	HAI	SCH
Size (km^2)	~422	~1300	~1300
Geology	Calcareous bedrock with karst phenomena	Calcareous bedrock	Young glacial landscape
Altitude (m asl)	460-860	285-550	3-140
AMT (°C)	6.0-7.0	6.5-8.0	8.0-8.5
AMP (mm)	700-1000	500-800	500-600

2.2.2 Forests and forest properties

In each of the three study regions 50 forest sites were established, resulting in a total of 150 forest sites. Each forest site covered an area of 100 m × 100 m within larger forests (Fischer et al., 2010). The forests were dominated by European beech (*Fagus sylvatica*), oak (*Quercus robur* and *Quercus peatrea*), Norway spruce (*Picea abies*), or Scots pine (*Pinus sylvestris*). Basic forest and soil properties for each of the three study regions are listed in Table 2.2. The wide regional range of stand basal area, mean diameter at breast height and conifer share indicates the broad range of forest types, stand densities and developmental phases found in the three study regions.

To explain forest soil respiration at the regional scale, we selected four structural (i.e. stand age, basal area, mean diameter at breast height and stand density) and two compositional (i.e. conifer share and tree species richness) forest properties. These forest properties are associated with multiple functions of temperate forests, like soil organic carbon storage, organic matter and root decomposition and (forest) climate regulation (Ehbrecht et al., 2019; Felipe-Lucia et al., 2018; Herold et al., 2014). The forest inventory of the 150 forest sites of the Biodiversity Exploratories was conducted between 2014-2016 (Schall et al., 2018) and included trees with a diameter at breast height equal or greater than 7 cm. Forest structure was described by the basal area (m^2 ha^{-1}), the mean diameter at breast height (cm) and the stand density (number of trees per ha). Stand age data were obtained from forest administration records or, in the case of HAI unmanaged forests, estimated from the diameter of the largest 30 trees per ha. Forest composition was expressed by the conifer share (%) calculated as the cumulative basal area of conifer trees over the total basal area. Tree species richness is the number of different tree species found in a forest stand. In addition, we used the Silvicultural management intensity (SMI) index that consists of i) a risk component that reflects the probability of stand loss based on the stand age and the tree species identities and ii) a stand density component that reflects the difference between the actual and the potential stand stocking and thus the intensity of harvesting and thinning (Schall and Ammer, 2013). This index quantifies the current managemental status of a forest stand and thus provides useful insights for non-experimental forest studies (Gan et al., 2020).

Table 2.2 Forest and soil properties for the three study regions. Regional mean values and standard deviations are given. Names and units are given in the first column where the '-' represents coefficients or count data. Lower case letters indicate significant differences (p-value <0.05) according to Tukey's Honest Significant Differences test. Abbreviations: ALB: Schwäbische-Alb, HAI: Hainich-Dün and SCH: Schorfheide-Chorin, SMI: Silvicultural management intensity index (Schall and Ammer, 2013), OC: organic carbon.

Region	ALB	HAI	SCH
Forest properties			
Stand age (y)	87±49b	133±48a	107±40b
Basal area (m^2 ha^{-1})	30.0±10.0	30.5±9.1	30.5±7.5
Mean diameter at breast height (cm)	25.4±10.0b	26.6±10.0b	33.3±11.3a
Stand density (-)	701±550a	568±448ab	442±492b
Conifer share (%)	30.1±40.7a	6.6±22.3b	37.4±44.2a
Tree species richness (species ha^{-1})	6.2±3.1a	5.6±2.1a	3.7±1.7b
SMI (-)	0.32±0.15a	0.18±0.13b	0.22±0.09b
Soil properties			
Organic layer OC stock (kg C m^{-2})	0.95±0.45b	0.59±0.18b	1.8±1.3a
Mineral soil OC stock (kg C m^{-2})	3.8±0.06a	3.3±0.07b	2.4±0.05c
pH	5.3±0.8a	4.8±0.9b	3.5±0.1c
Silt content (g kg^{-1} soil)	444.5±107.6b	646.0±94.4a	84.8±49.2c
Clay content (g kg^{-1} soil)	496.1±104.8a	301.0±100.3b	44.8±18.9c
Total soil depth (cm)	36.4±21.2c	54.5±13.1b	88.8±6.3a

2.2.3 Soil properties

Fourteen soil samples of the upper 10 cm of the mineral soil were collected along two intersecting 40 m transects in each of the 150 sites in May 2017. There was no change in silvicultural management within these transects. The upper 10 cm of the mineral soil reflect approximately the thickness of the A horizon (Herold et al., 2014), however, samples could contain a bit of B or E horizon as well. Undisturbed samples were collected with a split-tube with a diameter of 48 mm. They were used to prepare one composite soil sample per forest site. The forest floor at each sampling point was removed beforehand within a 15 cm × 15 cm metal frame. All soil samples were air-dried and sieved to <2 mm, and a portion was ground for elemental analysis. Total carbon (TC) and total nitrogen (TN) were determined by dry combustion at 1,100°C with an elemental analyser VarioMax (Elemental, Hanau, Germany). Soil inorganic carbon concentration was determined with the same analyser after removing organic carbon by exposing 250 mg of soil to 450°C for 16 h. The soil organic carbon concentration was calculated as the difference between total and inorganic carbon concentration. Organic carbon stocks were calculated for i) the organic layer and ii) the upper 10 cm of the mineral soil, by considering the dry weight of the sample and the area sampled. Hereafter, we refer to the sum of the organic stocks from the organic layer and the 10 cm of mineral soil as total organic carbon stocks. Soil texture and fine root (<2 mm) biomass were determined previously for the same sites by Solly et al. (2014). They used a combined sieving and sedimentation method (DIN ISO 11277, 2002) for soil texture determination and weighed the isolated, cleaned and oven-dried roots (at 40°C for two days) for fine root determination.

To explain forest soil respiration at the regional scale, we selected the following physico-chemical soil properties: total organic carbon stock (for in-situ soil respiration) or mineral soil organic carbon concentration (for soil respiration from incubation experiment), carbon to nitrogen ratio, silt content and pH. Fine root biomass from a depth 0-10 cm of the mineral soil was also used (Solly et al., 2014). While clay content is typically assumed to be important for carbon stabilization in soils (Grünberg et al., 2013), it was not an important predictor of soil organic carbon stocks in our study, after accounting for the effects of study regions (Herold et al., 2014). This is possibly because clay content, and, generally, soil texture, variations were greater between the study regions than within them, so that variance explained by study regions could be partly due to differences in particle size distribution. As silt content is more relevant for plant available water content than clay (Salter and Williams, 1965; Figure S2.1), and clay and silt content were strongly correlated in our study (R^2=0.96, with study region effects included), we decided to use the silt content in the models to avoid collinearity and as an indicator for available soil water during the dry soil conditions of our study.

2.2.4 Soil temperature and volumetric water content

Soil temperature and volumetric water content were recorded in 30-minute intervals in each of the 150 forest sites since 2008 using the ADL-MX Data Logger System (Meier-NT GmbH). Soil temperature (°C) was monitored at 5 cm depth below the surface of the mineral soil with the MNT FExtension. The Delta-T ML2X Soil Moisture Probe (Delta-T Ltd) was installed at 10 cm depth below the surface of the mineral soil and the voltage measurement was transformed to volumetric water content (%) using a generalized equation applicable for mineral soils. Soil temperature and water content sensors were installed in a fenced area (20 m × 20 m) located within each forest site (100 m × 100 m), but not falling within the 40 m × 40 m area selected for soil sampling.

2.2.5 In-situ soil respiration

In this study, the soda-lime absorption method was used with an open and static chamber to determine soil CO_2 efflux. Soda-lime, i.e. mainly $Ca(OH)_2$ and NaOH, was used as the absorption material (Keith and Wong, 2006), and the chemical reactions involved in the absorption of CO_2 are (Grogan, 1998):

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

The soda-lime method has been tested against dynamic systems with Infrared Gas Analyzers and is suitable for applications with large numbers of measurements (Keith and Wong, 2006; Janssens and Ceulemans, 1998). Moreover, the soda-lime method provides a measure of the cumulative soil CO_2 efflux over periods of hours (Keith and Wong, 2006) to weeks (McCoy et al., 2015). Long installation periods integrate the diurnal and climatic variability in the measurements (Grogan, 1998) and allow the investigation of variables that emphasize other causes of variability, including forest and soil properties.

The chamber design was a modification of previous work from Bierbaß et al. (2014) and Näthe et al. (2018). The chamber consisted of a PVC ring with an internal diameter of 10.5 cm and a height of 12.0 cm, a PVC lid and an O-ring ensured the air-tightness of the chamber (Figure S2.2). Inside the chamber, soda-lime was contained in a 50 mL syringe with 64 1-mm-holes held on the lid. A plastic tube passing through a hole on the lid allowed pressure equilibrium between the chamber and the ambient air. The outer ending of the tube was connected to a syringe containing 10 g of soda-lime to filter incoming ambient air CO_2. To correct for atmospheric CO_2 absorbed during the laboratory and field work, bottom-sealed chambers following the aforementioned design were used as controls.

Non-hygroscopic soda-lime with a diameter range of 2.4-5.0 mm and a saturation point of about 28% was acquired from the Fisher Scientific GmbH, Germany. Soda-lime is mainly $Ca(OH)_2$ and NaOH and reacts with CO_2 to form $CaCO_3$. The mass of the absorbed CO_2 can be determined by the difference of soda-lime mass before and after the field measurement after drying at 105°C for 48 h. Dry soda-lime mass was weighed with an accuracy of 0.1 mg before and after the exposure to soil CO_2 efflux. A soda-lime mass of about 4 g d^{-1} was used and the exposure time was 5 to 7 days. Each 50 mL syringe containing soda-lime was sealed in a sampling bag, while syringes of each forest site were stored in CO_2-free sampling bags until field installation. The soil efflux is calculated by the equation (Keith and Wong, 2006)

$$R_s[gCO_2 - C\, m^{-2}\, d^{-1}] =$$

$$\frac{WG_{sample}\,[gCO_2] - WG_{blank}[gCO_2]}{CA\,[m^{-2}]} \times \frac{24\left[\frac{h}{d}\right]}{T\,[h]} \times \frac{12\left[\frac{gC}{mol}\right]}{44\left[\frac{gCO_2}{mol}\right]} \times 1.69, (Eq. 2.1)$$

where WG is the weight gain [g], CA is the chamber basal area in [m^{-2}], T is the implementation time in [h] and the factor 1.69 compensates for the H_2O formed during CO_2 sorption and lost during drying (Grogan, 1998).

The soda-lime method can yield misleading results for soil respiration determination if the soda-lime mass is not sufficient, and thus becomes saturated during the exposure to the soil CO_2 efflux. Janssens et al. (2000) proposed that the mass increase should be less than 10% of the initial dry mass given a saturation point of about 28%. In this work, the soda-lime mass gain did not exceed the 9.0% in any of the 1416 measurements taken (Table 2.3). The lowest mass gain observed was 0.4% in a measurement taken in SCH in 2018 and the highest was 8.5% in HAI in 2019 followed by a 6.6% gain in ALB also in 2019. Regarding the control measurements, the lowest observed mass gain was <0.1% and the highest was 1.3% (data not shown). Thus, the increase in soda-lime method was shown to be an appropriate method for soil respiration estimation in our study.

Table 2.3 Minimum, average and maximum values of soda-lime sample mass increase (% of the initial dry mass) for the 2018 and 2019 campaigns in the three study regions; Schwäbische-Alb: ALB, Hainich-Dün: HAI and Schorfheide-Chorin: SCH. Mass gain did not exceed the limit of 9.0% proposed by Janssen et al. (2000).

Region	Year	Average	Min	Max
ALB	2018	2.2	0.4	4.9
	2019	3.1	1.5	6.6
HAI	2018	2.8	0.5	4.9
	2019	3.3	0.4	8.5
SCH	2018	2.0	0.5	5.3
	2019	3.5	1.0	6.6

Soil respiration was measured from late June to July in 2018 and 2019. Measurements started in HAI followed by the SCH and ALB study regions. In each forest site, we installed four chambers in the 2.5 m projections of the soil sampling transects (cardinal orientation forming a 40 m length cross) together with one control chamber. Aboveground vegetation was carefully clipped and removed from the installation area. PVC rings were installed to a depth of 1 to 2 cm into the soil to restrict severing roots. PVC rings and soda-lime were installed simultaneously. Soda-lime was rewetted before the installation to compensate for the initial moisture content of about 18% since CO_2 needs to be hydrated before reacting with the soda-lime. In a subset of 29 out of the 150 forest sites, we measured the mineral soil respiration after removing the organic layer. The installation of the chambers for the mineral soil respiration happened right after the removal of the organic layer to ensure similar environmental conditions and comparability between the two treatments (total and mineral soil respiration). Total and mineral soil respiration fluxes were measured at the same time. Over the two years, we conducted 1,200 total and 216 mineral soil CO_2 efflux measurements and used 300 controls.

2.2.6 Incubation experiment

Soil respiration data from incubation experiments were provided by Gan et al. (2020) for the same forest sites of the Biodiversity Exploratories. In that study, soil sampling of the upper 10 cm of the mineral soil took place in 2011 following the same protocol described for 2017. Soil samples were incubated for 14 days under standard conditions of 60% of the samples’ water holding capacity and a constant temperature of 20°C. The CO_2 release from the soil samples was measured after 14 days with a LI-6262 CO_2/H_2O infrared gas analyser (LI-COR-Environmental, Lincoln, Nebraska, USA). Data were expressed as [$\mu g\ CO_2\text{-}C\ g^{-1}\ d^{-1}$].

2.2.7 Data analysis

Sub-hourly soil climate data were averaged for the period of the soil respiration measurements for each forest site and year. Forest soil respiration, temperature and moisture were tested for differences among the three study regions and the two sampling years using a two-way Analysis of variance model. Tukey’s Honest Significant Difference (HSD) test was employed to identify significant differences among the levels of the independent variables.

Pooling the data from the three study regions, we performed variance partitioning models in each of the three dataset on soil respiration (in-situ measurements in 2018 and 2019 and soil incubation experiment) to identify the relative importance of forests and soils using the properties described above. Then, a backward stepwise analysis was employed to assess the impact of the individual forest and soil properties on model performance based on the Akaike information criterion. We used Analysis of covariance (ANCOVA) to model the effects of study regions and the forest and soil properties (selected from the stepwise analysis) on total and mineral soil respiration. ANCOVA models were performed assuming a type II sum of squares, which is not influenced by the order in which the explanatory variables are introduced in the model (Zuur et al., 2009). Diagnostic plots were investigated for every linear model applied to evaluate the assumptions of linearity, normality and homoscedasticity in the residuals, and to check for influential values, i.e. outliers (Figure S2.3). Heteroscedasticity issues were assessed with logarithmic or square-root transformation of the response variable. To reject the null hypothesis, we selected a p-value threshold of 0.05 and in the following we refer to some relationships as marginally significant if the p-value was in the range 0.05-0.10.

To understand the causal structure that governs the effect of forest properties on soil respiration, the structural equation modeling technique was employed (Shipley, 2016). We selected mean diameter at breast height, conifer share and silt content as exogenous variables. As mediation variables we selected fine root biomass and either total organic carbon stock (for field soil respiration measurements) or mineral soil organic carbon concentration (for incubation experiment soil respiration measurements) or soil volumetric water content. We started from a full model including all possible pathways among the variables and reduced the model removing non-significant paths one at a time. Causal structures with p-values higher than 0.05 were considered acceptable. Models with a root mean square error of approximation (RMSEA) and a standardized root mean square residual (SRMR) lower than 0.05 were also considered acceptable. The high number of observations compared to the number of estimated parameters provided safety against multivariate non-normality issues (Shipley, 2016). As an additional measure, we used a bootstrapping method and observed how the p-value of the causal structure changed between the non-bootstrapped and the bootstrapped models. Since there were no important differences, we only reported the p-values of the non-bootstrapped models.

Structural Equation Modeling is among the few statistical methods claiming to prove, or disprove, causal relationships in a proposed structure. Natural ecosystems are characterized by complex interactions between processes involved in element cycling. This makes the development of a unique causal structure challenging. Therefore, we recognize that the results of our Structural Equation Models represent associations between variables. However, we interpret these associations as evidence of management effects and for simplicity we often use terms such as ‘effects’ and ‘drivers’ hereafter (in Chapters 2, 3 and 4).

Statistical analyses were performed with the R statistical software (R Core Team, 2019). Analysis of variance and covariance models were performed with the *lm* function and information was extracted with the *summary* function. Tukey's HSD test was performed with the *TukeyHSD* function of the *stats* package. For the backward elimination stepwise process, the *step* function from the *stats* package was used. Redundancy analysis for the variance partitioning was performed with the *rda* function and the permutation analysis of variance with the *anova* function of the *vegan* package. The structural equation modeling was performed with the *sem* function from the *lavaan* package (Rosseel, 2012).

2.3 Results

2.3.1 Forest soil temperature and water content

Soil temperature during respiration measurements ranged from 13.9 to 16.2°C across the three study regions and two sampling years and increased in the order SCH<ALB<HAI both in 2018 and 2019 (Figure 2.1a). Soil temperature did not differ significantly between the years in any of the study regions. Soil volumetric water content ranged from 10.4 to 25.2% in 2018 and from 10.0 to 31.9% in 2019 and also increased in the order SCH<ALB<HAI (Figure 2.1b). Differences between years were observed only in HAI, where soil water content in 2018 was lower than in 2019. Low soil water content in SCH is probably explained by the sandy soils found in the region (Table 2.2) and their low capacity to hold water. In ALB, despite the high mean annual precipitation (Table 2.1) and the high silt and clay content (Table 2.2), the soil water content was between that of SCH and HAI, showing that sampling in ALB took place during a very dry period of the year. Table 2.4 gives the monthly regional soil temperature and volumetric water content for the 10-year-average 2008-17 and the sampling years 2018 and 2019 from March to July. Higher soil temperature and reduced water content during the summer months of 2018 and 2019 revealed two successive naturally occurring droughts in central Europe (Figure S2.4). Only in HAI 2019, soil water content was not below the long-term average in June.

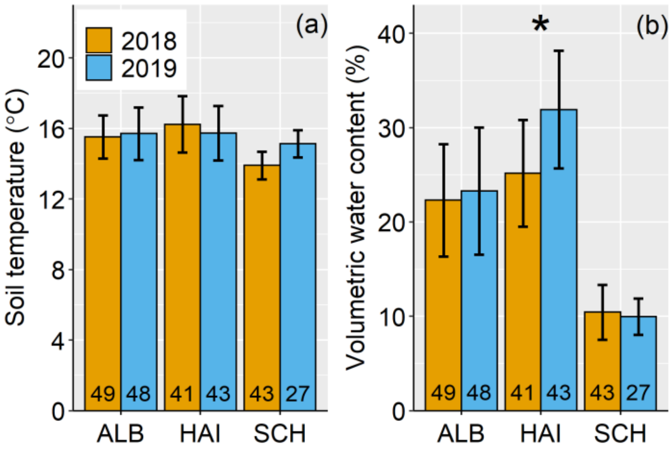


Figure 2.1 (a) Soil temperature and (b) volumetric water content during the campaigns in 2018 and 2019 for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Bars represent mean values and the error bars the standard deviation. Numbers at the base of each bar indicate the sample size. Asterisks indicate significant differences between the years for a given study region.

Table 2.4 Soil temperature and volumetric water content per month from March to July for the 10-year-average (2008-17) and the two sampling years 2018 and 2019 in the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Values represent mean values and standard deviations. Soil temperature and volumetric water content for the 10-year-average (2008-17) and the two sampling years (2018 and 2019) for every month and study region can be found in Figure S2.4.

		Soil temperature (°C)			Volumetric water content (%)		
Region	Month	2008-17	2018	2019	2008-17	2018	2019
ALB	March	3.8±1.4	2.5±1.5	4.7±1.1	39.2±5.4	39.3±7.2	33.5±7.1
	April	6.9±1.4	8.0±2.2	7.2±1.5	38.1±6.3	35.2±8.6	31.9±7.8
	May	9.7±1.1	10.9±1.5	8.4±1.5	37.2±5.9	31.1±7.4	33.3±7.8
	June	12.5±1.1	13.6±1.1	13.5±1.8	33.6±6.0	29.7±7.9	28.9±7.3
	July	14.5±0.9	14.7±1.2	15.0±1.6	30.7±6.1	22.4±6.4	23.1±6.8
HAI	March	3.7±1.2	1.7±1.5	5.2±1.1	36.8±5.0	37.2±8.4	33.8±6.2
	April	7.5±1.3	8.3±2.4	7.1±1.6	35.1±5.5	37.0±7.4	32.2±6.1
	May	10.3±1.1	11.0±1.9	8.8±1.8	32.5±6.0	28.4±7.1	33.1±6.2
	June	12.7±0.9	13.9±1.7	14.0±1.7	28.7±6.4	21.5±6.5	29.6±7.1
	July	14.7±1.0	18.4±5.3	14.3±2.1	26.1±6.4	18.4±5.3	21.1±5.4
SCH	March	4.2±1.2	1.4±1.3	6.0±0.9	15.8±3.2	17.0±4.8	14.4±3.5
	April	7.5±1.1	8.3±2.6	8.3±1.5	15.2±3.4	17.4±4.0	12.8±3.1
	May	10.8±1.3	12.3±1.6	10.4±1.6	13.8±3.5	12.7±3.4	12.8±3.4
	June	13.7±1.0	15.1±1.0	16.2±1.2	12.6±3.5	11.6±3.6	12.0±3.7
	July	15.9±0.9	16.2±1.4	16.1±1.5	11.9±3.0	11.1±4.0	8.2±1.9

2.3.2 Forest soil respiration

In-situ soil respiration rates ranged from 0.9 to 3.3 gC-CO_2 m^{-2} d^{-1} in 2018 and from 1.0 to 3.9 gC-CO_2 m^{-2} d^{-1} in 2019. Highest fluxes were at HAI in both years (Figure 2.2a), while SCH and ALB had the lowest fluxes in 2018 and 2019, respectively. Interestingly, HAI was also the region with the highest soil water availability in both years (Figure S2.5), which possibly influenced in-situ soil respiration. Comparing the two years, in-situ soil respiration differed only in SCH and it was higher in 2019. In-situ soil respiration was not related to soil temperature, but was significantly correlated with soil volumetric water content across study regions both in 2018 and 2019 ($p<0.010$, Figure S2.6). In contrast to in-situ soil respiration, the soil respiration from the incubation experiments significantly increased in the order SCH<HAI<ALB (Figure 2.2c). The incubation experiment was conducted with sieved samples of mineral topsoil under standardized moisture and temperature conditions, and thus represents the heterotrophic potential of the mineral topsoil. Across the study regions, a significant positive relationship between the soil organic carbon concentration and the incubated soil respiration was found (Figure S2.7).

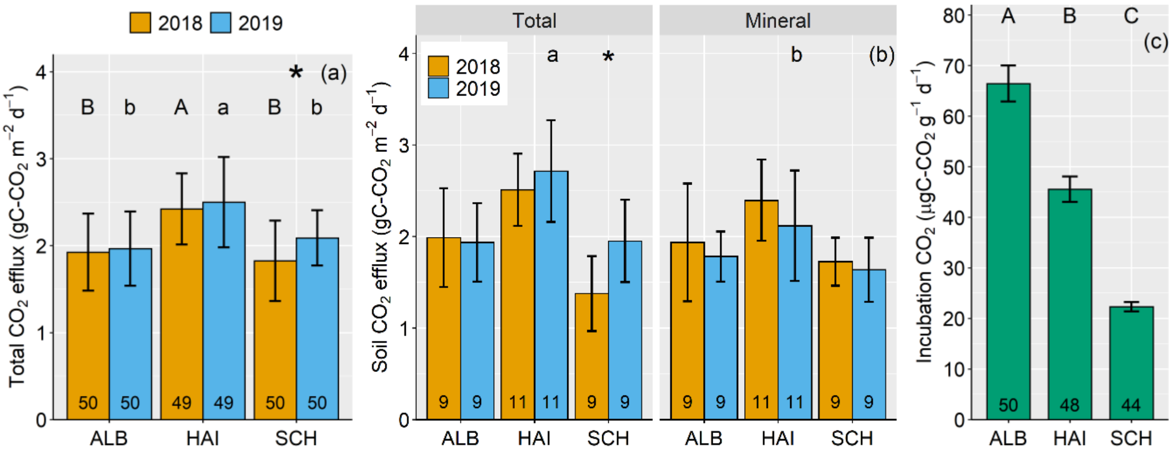


Figure 2.2 (a) Soil respiration fluxes for 2018 and 2019 in the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Error bars represent the standard deviation of the sample. Numbers at the base of each bar indicate the sample size and asterisks indicate significant differences between the years for a given study region. Upper- and lower-case letters indicate differences among the study regions in 2018 and 2019, respectively. (b) Total and mineral soil respiration for a subset of 29 forest sites. Asterisks indicate significant differences between the years for a given study region. Lower case letters indicate differences among respiration source (total or mineral soil) for a given study region in 2019. (c) Laboratory incubation soil respiration from soils samples in 2011. Upper case letters indicate differences among the three study regions.

In-situ mineral soil respiration from a subset of 29 forest sites in all study regions ranged from 1.4 to 3.5 in 2018 and from 1.1 to 3.8 $gC\text{-}CO_2\ m^{-2}\ d^{-1}$ in 2019 and no differences between the two years were observed in any of the study regions (Figure 2.2b). In contrast, for the same subset of 29 forest sites, the in-situ total soil respiration in SCH was higher in 2019 than 2018

(Figure 2.2b) verifying the differences observed in the full dataset (150 forest sites, Figure 2.2a). Comparing total and mineral soil respiration of the same subset of sites (Figure 2.2b), the total soil respiration in HAI in 2019 was higher than the respective mineral soil respiration, while no differences were observed for the other regions and in 2018.

2.3.3 Forest and soil properties effects on soil respiration

We partitioned the explained variance of soil respiration between forest (including fine root biomass) and soil related properties for three soil respiration datasets; i) the in-situ soil respiration in 2018 and ii) 2019 and iii) the soil respiration from the lab incubation experiment. Forest properties explained 30 and 31% of the variance under the 2018 and 2019 in-situ conditions and 33% of the variance under incubation conditions (Figure 2.3), while soil properties explained 13, 4 and 37%, respectively. Forest properties explanatory power was more consistent among the three datasets compared to that of soil and the magnitude underlined the importance of forest properties as soil respiration predictors at the regional scale. Forest properties explained a big portion of the (heterotrophic) soil respiration variance measured under the lab incubation conditions, suggesting the existence of legacy effects from forest to soil properties, and thus to respiration. Incubation data showed the largest explained variance (70%) possibly due to the standardized conditions and the consideration of heterotrophic respiration only.

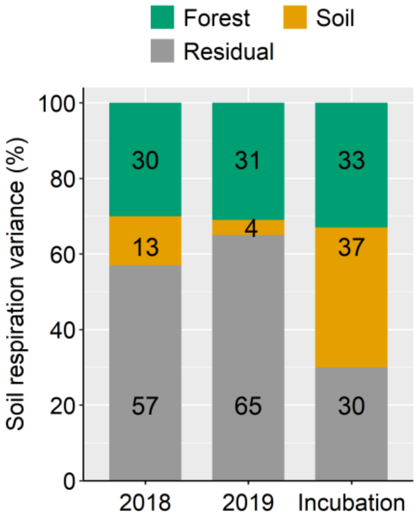


Figure 2.3 Soil respiration variance partitioning among two explanatory groups; the forest (stand age, basal area, mean breast height diameter, stand density, conifer share, tree species diversity and fine root biomass) and soil (soil organic carbon stock for in-situ soil respiration and soil organic carbon concentration for soil respiration from incubation experiment, C:N ratio, silt content and pH) properties for the in-situ soil respiration datasets in 2018 and 2019 and the laboratory incubation experiment dataset. Soil temperature and volumetric water content were not included in the variance partitioning.

We selected a subset of forest and soil properties with stepwise backwards elimination and used ANCOVA models to investigate their effects on the aforementioned soil respiration datasets. Study regions were always important predictors of soil respiration. In 2018, the mean breast height diameter, fine root biomass and silt content increased the in-situ soil respiration (Table 2.5), while the carbon to nitrogen ratio (marginally) decreased it. In 2019, the mean diameter at breast height and fine root biomass had again a positive effect on in-situ soil respiration (Table 2.5). The two in-situ soil respiration models explained 37% and 40% of variance in 2018 and 2019, respectively, while applying a similar model for soil respiration from the incubation experiment explained 65% variance (Table 2.5). Under the incubation conditions, the mineral soil organic carbon concentration significantly increased the soil respiration, while the conifer share had a marginal negative effect. After accounting for mean breast height diameter and conifer share, the Silvicultural management intensity index had no significant effect on the in-situ soil respiration and the incubation experiments.

Table 2.5 ANCOVA (SS type II) models of soil respiration data from 2018, 2019 and incubation experiments with forest and soil properties. Full models were stepwise reduced. Soil temperature, soil water content and soil pH were included in the full models, but were never selected in any final model. Incubation data were log transformed. Study region effects are given relative to the Schwäbische-Alb region. Standardized β coefficients and p-values are given for each explanatory variable. Abbreviation: HAI =Hainich-Dün and SCH =Schorfheide-Chorin, DBH =mean diameter at breast height, SMI =Silvicultural management intensity index, SOC stock/conc. =soil organic carbon stock for the in-situ soil respiration models and soil organic carbon concentration for the incubation experiment and CN ratio =carbon to nitrogen ratio.

Soil respiration	**2018**	Model	**2019**	Model p	**Incubation**	Model p
	df=135	p-value	df=136	p-value	df=136	p-value
	R^2=0.37	<0.001	R^2=0.40	<0.001	R^2=0.65	<0.001
Parameter	Stand. β	p-value	Stand. β	p-value	Stand. β	p-value
Intercept	-0.382	<0.001	-0.359	<0.001	3.389	<0.001
Region HAI	0.241	0.338	0.915	<0.001	-0.113	0.196
Region SCH	0.877	<0.050	0.193	0.245	-0.434	<0.010
DBH	0.229	<0.010	0.245	<0.010		-
Conifer share		-		-	-0.066	0.091
SMI		-	0.149	0.060	0.074	0.061
Fine root biom.	0.182	<0.050	0.393	<0.001		-
SOC stock/conc.		-		-	0.268	<0.001
CN ratio	-0.203	0.097		-		-
Silt content	0.523	<0.010		-		-

A direct comparison of soil respiration measured in-situ and under incubation conditions is always a difficult task. Soil respiration is a complex process that is controlled by physical, chemical and biological processes and soil properties. Under field conditions physical processes that relate to gas emissions (e.g. gas diffusivity, soil structure, porosity, pore size distribution and connectivity, soil water content) are very important. In contrast, under incubation conditions soil structure is disrupted and soil water content is controlled, so that soil respiration is mostly governed by biochemical processes, and not by soil physical properties. The lower R^2 of the in-situ soil respiration models compared to the models under incubation conditions might be due to unaccounted soil physical properties, like porosity, pore size distribution and connectivity that control gas emissions.

Soil organic carbon was an important predictor for the soil respiration under the incubation conditions, but not for the in-situ soil respiration suggesting a shift in the respiration source. To understand how forest and soil properties influence this shift and test the existence of indirect paths from soil organic carbon and fine root biomass to soil respiration, we applied structural equation modeling. Both soil organic carbon stocks (or concentration) and fine root biomass were influenced by mean diameter at breast height and silt content (Figure 2.4), while effects of conifer share on soil organic carbon mainly represented differences among the three study regions. Similar to the ANCOVA results, the fine root biomass drove the in-situ soil respiration, but the total soil organic carbon stock did not, even though there was remaining explanatory power in the exogenous variables (direct paths from mean diameter at breast height and conifer share to soil respiration in Figure 2.4a and c). Direct effect of silt content on soil respiration mainly represented differences among the three study regions. Under the incubation conditions, only the mineral soil organic carbon concentration directly drove the soil respiration, while all forest and soil effects were completely mediated by the mineral soil organic carbon (Figure 2.4e). Additional structural equation models for the in-situ soil respiration were developed to test if the forest and soil effects on soil respiration could be explained by soil volumetric water content. Only silt content caused differences on soil water content (Figure 2.5a and c) and in 2019 the direct effect of silt content on soil respiration was completely mediated by the soil water content (Figure 2.5c).

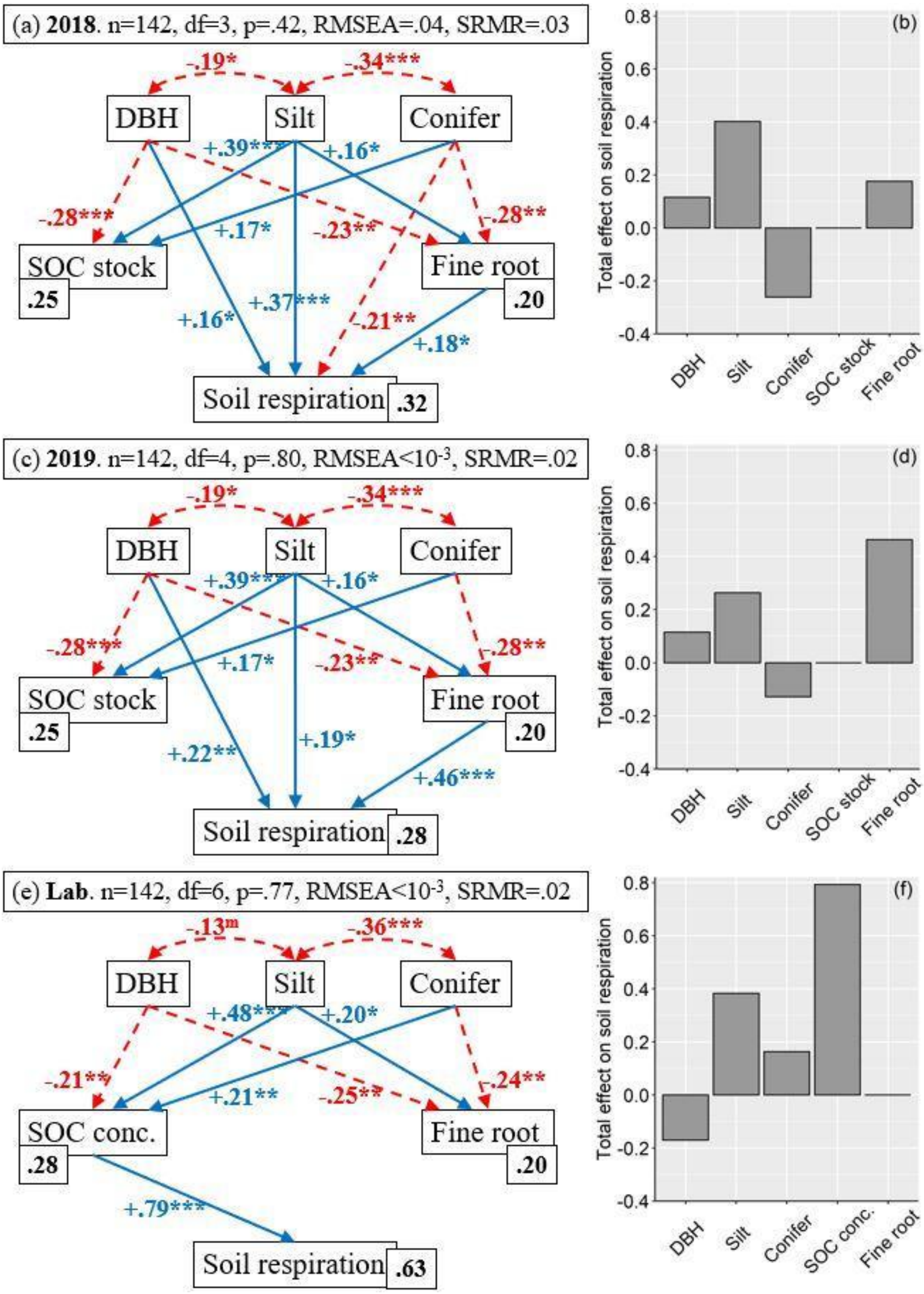


Figure 2.4 Structural equation models (left) and total effects (right) for the in-situ soil respiration measurements in 2018 (a and b) and 2019 (c and d) and the soil respiration from the incubation experiment (e and f). Silt content (Silt), mean diameter at breast height (DBH) and conifer share (Conifer) were selected as exogenous variables and fine root biomass (Fine root) and total soil organic carbon stock or mineral soil organic carbon concentration (SOC stock or conc.) as mediation variables. Solid, single-headed arrows represent direct causal paths and dashed double-headed arrows represent covariances. Standardized path coefficients are given together with their significance level and coefficients of determination for the endogenous variables. Significance levels: ^{m}p<0.010; *p<0.050; **p<0.010; ***p<0.001.

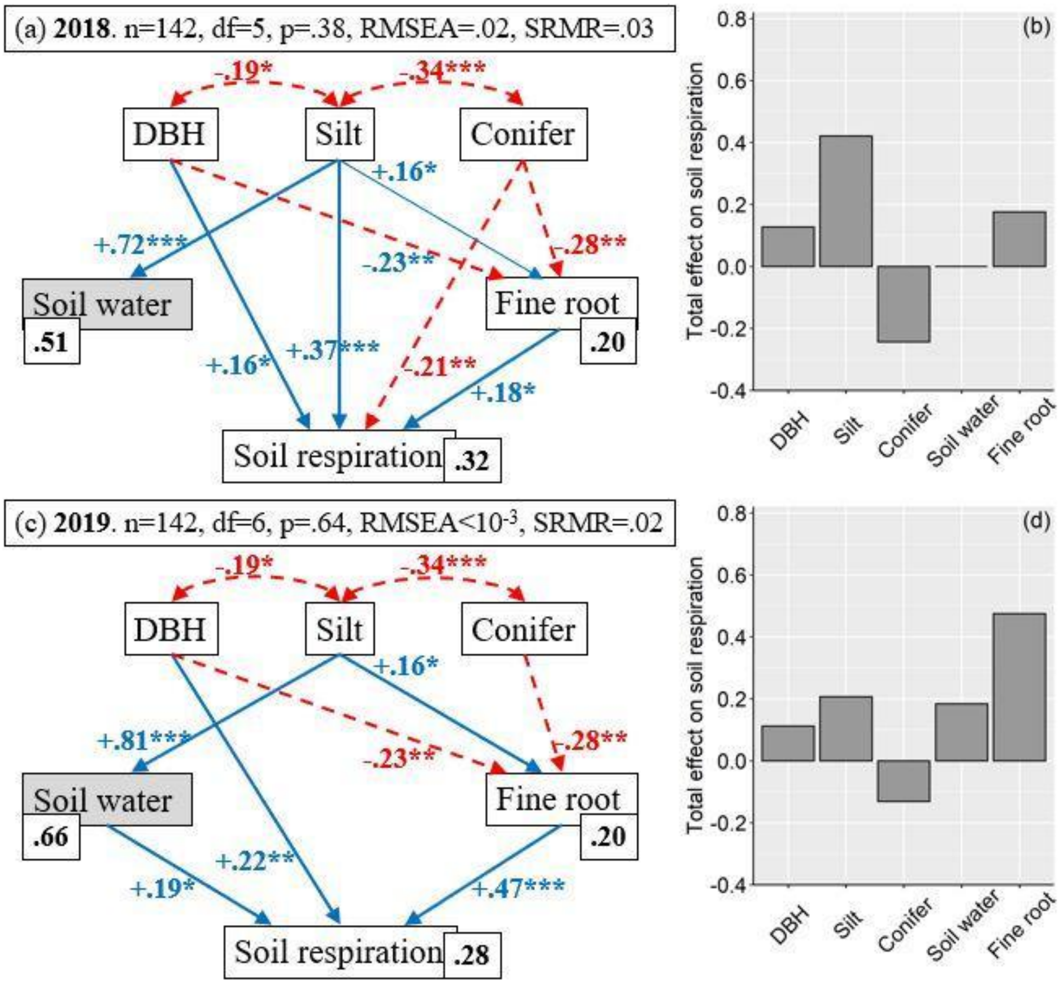


Figure 2.5 Structural equation models (left) and total effects (right) for the in-situ soil respiration measurements in 2018 (a and b) and 2019 (c and d). Silt content (Silt), mean diameter at breast height (DBH) and conifer share (Conifer) were selected as exogenous variables and fine root biomass (Fine root) and soil volumetric water content (Soil water) as mediation variables. Solid, single-headed arrows represent direct causal paths and dashed double-headed arrows represent covariances. Standardized path coefficients are given together with their significance level and coefficients of determination for the endogenous variables. Significance levels: *p<0.050; **p<0.010; ***p<0.001.

In-situ mineral soil respiration was not explained by the study regions (Table 2.6). In 2018, the mean diameter at breast height, fine root biomass and silt content had a positive effect on mineral soil respiration similar to total soil respiration (Table 2.6). Additional positive effects from conifer share and negative effects from basal area and the Silvicultural management intensity index were found. In 2019, only the positive effect of fine root biomass was detected and silt content had a positive, but marginal, effect.

Table 2.6 ANCOVA (SS type II) models of in-situ soil respiration from the mineral soil in 2018 and 2019 with forest and soil properties. Full models were stepwise reduced. Study regions, stand age and density, soil temperature and water content and soil pH, organic carbon and C:N ratio were included in the full models, but were never selected in any final model. Standardized β coefficients and p-values are given for each explanatory variable. Abbreviation: DBH =mean diameter at breast height, SMI =Silvicultural management intensity index.

Soil respiration	**2018**	Model	**2019**	Model
	df=18	p-value	df=23	p-value
	R^2=0.78	<0.001	R^2=0.35	<0.001
Parameter	Stand. β	p-value	Stand. β	p-value
Intercept	-0.002	<0.001	~0	<0.001
Basal area	-1.218	<0.001	-	-
DBH	0.300	<0.050	-	-
Conifer share	1.356	<0.010	-	-
SMI	-1.105	<0.010	-	-
Fine root biomass	0.254	<0.050	0.400	<0.050
Silt content	0.817	<0.001	0.313	0.061

2.4 Discussion

To investigate the effects of forest properties on soil respiration, we measured soil respiration in 150 forest sites. Measurements were performed at a large number of forest sites nearly simultaneously. Forest soil respiration is expected to increase during the growing season in temperate regions, and many studies report highest fluxes in early summer under wet (Zhao et al., 2016) and dry conditions (Borken et al., 2006; Nikolova et al., 2009). We performed the field campaigns in early summer to capture the peak of soil respiration fluxes in temperate forests. Even though forest structure effects on soil respiration may not be constant between years (Powers et al., 2018), the spatial patterns of soil respiration should remain relatively stable over the growing season (Søe and Buchmann, 2005). Thus, our early summer measurements provide a good measure to capture differences and controls of forest soil respiration at the regional scale.

2.4.1 Dry conditions led to low in-situ soil respiration

In this study, in-situ soil respiration measurements averaged 2.1±0.5 and 2.2±0.5 gC - CO_2 m^{-2} d^{-1} in 2018 and 2019, respectively. Reported summer soil respiration fluxes in similar temperate European forests range from 0.9 to 4.9 gC-CO_2 m^{-2} d^{-1} (Borken and Beese, 2005; Borken et al., 2002; Berger et al., 2010; Kukumägi et al., 2017; Nikolova et al., 2009; Schindlbacher et al., 2012; Vesterdal et al., 2012; Søe and Buchmann, 2005). Compared to literature values, our in-situ soil respiration fluxes fell into the lower part of the range.

Summer months in 2018 and 2019 were on average drier and warmer than the 10-year mean (Table 2.4), which probably led to reduced soil respiration.

In temperate forests, soil respiration from the organic layer contributes 38-46% to the total soil respiration over the year (Borken and Beese, 2005). In this study, however, differences between total and mineral soil respiration were only observed in HAI in 2019 (Figure 2.2b). Two processes might explain the lack of differences between total and mineral soil respiration in the other study regions and years: i) the organic layer dried out faster than the underlying mineral soil, and so the soil respiration from the organic layer made a negligible contribution to the total soil respiration and ii) autotrophic respiration was the main contributor to total soil respiration. Regarding the first process, we empirically observed during the fieldwork that the organic layers were drier than the underlying mineral soil, which supports the idea of reduced microbial activity in the organic layer due to water limitation. Low soil moisture in the organic layer, and subsequently reduced CO_2 emissions, have been observed in several throughfall exclusion experiments (Borken et al., 2006; Muhr and Borken, 2009; Schindlbacher et al., 2012). Here, total soil respiration was higher than that from the mineral soil only in the case of HAI in 2019 (Figure 2.2b), and this was also the only region with significantly different soil volumetric water contents between the two years (Figure 2.1b; with 2019 wetter than 2018). However, neither total nor mineral in-situ soil respiration was related to soil organic carbon stocks (Tables 2.5 and 2.6) indicating that organic layer and mineral soil were both affected by the dry conditions.

For the second process, several studies suggest that the autotrophic contribution to total soil respiration peaks in summer (Shabaga et al., 2015), especially given the sensitivity of heterotrophic respiration to water limitations (Moyano et al., 2013). Many studies have reported reductions in both the heterotrophic and autotrophic soil respiration under dry conditions (Nikolova et al., 2009; Schindlbacher et al., 2012), while others determined mainly reductions in the heterotrophic respiration (Borken et al., 2006; Muhr and Borken, 2009). This agrees with the lack of significant correlation between in-situ soil respiration and soil organic carbon, and the significance of fine root biomass (Table 2.5 and 2.6). We suggest that water limitations, caused by two successive summer droughts in Europe in 2018 and 2019, were responsible for the lack of differences between total and mineral soil CO_2 fluxes. In line with our third hypothesis, we assume that autotrophic respiration, i.e. C respired by roots, mycorrhiza and rhizospheric microorganism (Bond-Lamberty., 2004; Hanson et al., 2000), was probably the main contributor to summer soil respiration.

Fine root biomass has been proposed as an important predictor of soil respiration, especially at larger spatial scales and a positive relationship with soil respiration has been shown (Luan et al., 2011; Søe and Buchmann, 2005). Similarly, we identified fine root biomass as the strongest and most persistent predictor of in-situ soil respiration at the regional scale (Table 2.5 and 2.6). Fine root biomass had a positive effect on in-situ soil respiration both in 2018 and 2019 (Figure 2.4a and c), but the effect was stronger in 2019 compared to 2018. However, we observed no significant relationship between fine root biomass and soil respiration under incubation conditions (Table 2.5), because laboratory conditions (i.e. incubation with sieved, root-free soil) minimize vegetation effects on soil respiration. Yet, this could be an indication that fine root biomass does not promote detectable legacy effects on soil properties, i.e. on soil organic carbon (Figure 2.4).

2.4.2 Forest, rather than soil properties drive spatial variation of in-situ soil respiration in early summer

Soil respiration is a major ecosystem function influenced simultaneously by many abiotic and biotic factors (Rodeghiero et al., 2005). Soil temperature and moisture effects on soil respiration are well documented in field and lab studies, and have been the focus of many modeling studies. The purpose of this study was to identify effects of forest properties on soil respiration across regions. At least all sites within a study region were measured as far as possible in parallel, and integrated over several days to obtain values for comparable environmental and weather conditions. Here, early summer soil temperature was not related to in-situ soil respiration in any of the two sampling years. This agrees with previous studies on spatial soil respiration dynamics (Søe and Buchmann, 2005), where the spatial variation of soil temperature was small, and thus did not explain soil respiration variation. Similarly, in our study, soil water content variations in each of the three regions were smaller than the variation between them, and, thus, did not drive differences in the in-situ soil respiration. Accordingly, observed differences in soil respiration rates are not expected to be due to differences in soil moisture or temperature, but rather due to forest or soil properties.

The magnitude of the total soil organic carbon stocks had no effect on soil respiration under field conditions, while the mineral soil organic carbon concentration significantly affected the soil respiration of the incubation experiment (Table 2.5 and Figure 2.4). Both strong (Rodeghiero et al., 2005) and weak (Reichstein et al., 2003) positive and negative (Borken and Beese, 2002) relationships have been reported for soil organic carbon and soil respiration under non-limiting water conditions. Our fieldwork in 2018 and 2019 was conducted under drier and warmer conditions in comparison to the 10-year mean (Table 2.4), which, we hypothesize, reduced the heterotrophic soil respiration, and weakened its dependency on soil organic carbon. Similarly, drought might weaken soil respiration dependency on soil pH and texture, which are important determinants of soil organic carbon stocks and stability in temperate forests (Gan et al., 2020; Grüneberg et al., 2013; Grüneberg et al., 2019). In our structural equation analysis, total soil organic carbon stocks and the mineral soil organic carbon concentration were influenced by mean diameter at breast height, conifer share and silt content (Figure 2.4), but soil organic carbon acted as a mediation variable only for the soil respiration from the incubation experiment. The positive effect of mineral soil organic carbon concentration on incubation respiration highlights the importance of the substrate under non-water limiting conditions.

Silt content had a positive effect on total and mineral soil respiration in 2018 (Table 2.5) and 2019 (Table 2.6 and Figure 2.4). Under the incubation conditions the effect of silt content on soil respiration was completely mediated by the mineral soil organic carbon concentration (Figure 2.4e). However, under field conditions, silt content had an additional indirect effect on soil respiration via the fine root biomass (Figure 2.4a and c), and a direct effect which was explained by soil water content in 2019 (Figure 2.5c), but not in 2018 (Figure 2.5a). Soil moisture in silty soils is more available to plants and microbes compared to clayey soils (Salter and Williams, 1965), and thus it has a greater ecological meaning. However, to understand these processes, soil water tension measurements, rather than soil volumetric water content, are needed. The remaining effect of silt content could represent water availability and release characteristics among the three study regions since HAI had the highest i) silt content and ii) soil depth (Table 2.2), iii) available soil moisture (Figure S2.5) and iv) soil respiration (Figure 2.2).

Despite the different climatic conditions and methods associated with our three soil respiration datasets, forest properties explained about a third of the early summer soil respiration variance at the regional scale (Figure 2.4), which was in line with our first hypothesis. Both forest and soil properties explained higher proportions of soil respiration variance under the lab incubation than field conditions. We suggest that under water limiting conditions forest properties affect soil respiration mainly through autotrophic respiration (Figure 2.4a and b), while under non-limiting water conditions they exert additional legacy effects through soil properties, and especially soil organic carbon content (Figure 2.4c).

Stand age is one of the most frequently used forest properties to explain soil respiration, and positive (Lucas-Borja et al., 2016), negative and nonlinear (Kukumägi et al., 2017; Powers et al., 2018) relationships have been reported. Stand age is strongly related to forest structural properties. For example, basal area (Lucas-Borja et al., 2016) and mean diameter at breast height (Zhao et al., 2016; Kukumägi et al., 2017) increase with stand age, while stand density is reduced (Kukumägi et al., 2017). In this work, mean diameter at breast height was the only forest structural property identified as an important predictor of soil respiration (Table 2.5). Mean diameter at breast height indirectly decreased the soil respiration measured both in-situ via a negative effect on fine root biomass (Figure 2.4a and c), and in the soil incubation experiment via negative effects on soil organic carbon (Figure 2.4e). In addition, mean diameter at breast height had also a positive and direct effect on in-situ soil respiration (Figure 2.4a, c) leading to a positive total effect (Figure 2.4b and d). This direct effect was not explained by soil water content (Figure 2.5), but might be related to root activity or belowground carbon allocation.

The negative effect of mean diameter at breast height on fine root biomass (Figure 2.4) is not in agreement with the literature. Studies investigating deeper soil profiles (Jagodzinski et al., 2016) or the whole rooting depth (Finér et al, 2011) reported positive responses of fine root biomass to stand age, basal area and diameter at breast height (Ammer and Wagner, 2005). Finér et al. (2007) proposed that the relationship between fine root biomass and forest properties differs among tree species. This discrepancy can be related to the negative relationship found between mean diameter at breast height and tree density (R^2=0.59, p<0.001, data not shown) or, most probably, to our methodology since the shallow sampling depth (0-10 cm in the mineral soil) might have resulted in an underestimation of the fine root biomass in some age classes or tree species. In addition, our fine root biomass data originated from 2011, and they do not correspond to the soil respiration sampling years (i.e. 2018 and 2019), under which the fine root biomass might have also been impacted by the two successive droughts. Even so, our topsoil fine root biomass data explained well the soil respiration fluxes, suggesting that i) the upper soil fine root biomass might be more relevant for the soil respiration and that ii) the spatial patterns of fine root biomass remain relatively stable interannually.

Conifer share had, in general, a negative effect on in-situ soil respiration (Table 2.5, Figure 2.4) that could be direct (Figure 2.4a) or indirect through lower fine root biomass (Figure 2.4a, c). Direct negative effects from conifer share to soil respiration could indicate reduced fine root activity, and thus lower autotrophic respiration in conifer compared to broadleaf forests as reported by Nikolova et al. (2009) under a natural drought. Finér et al. (2007) found increasing fine root biomass in the order Scots pine<Norway spruce<European beech, which explains the negative effects of conifer share to fine root biomass (and indirectly to soil respiration).

Comparing the total effects of mean diameter at breast height and conifer share for the in-situ soil respiration, we found that silvicultural management actions that decrease conifer share and increase mean diameter at breast height could increase in-situ autotrophic-dominated soil respiration under water limiting conditions (Figure 2.4b and d). However, these two measures could also increase heterotrophic respiration under non-limiting water conditions, as shown by the laboratory incubation experiment (Figure 2.4f). In line with our hypothesis 2ii, we found some evidence that potential silvicultural management effects on soil respiration depend on the climatic conditions.

From the six forest properties examined, we identified only mean diameter at breast height and conifer share as important drivers of soil respiration, rejecting our hypothesis 2i that multiple properties would provide additional benefits in explaining soil respiration at the regional scale. Even though recent evidence suggests that stand diversity influences soil respiration (Chen and Chen, 2016; Jewell et al., 2017), tree species richness, the only forest diversity property examined in this study, was not selected in any model. However, our measurements were affected by two successive natural droughts, which could have masked additional effects of other forest properties. Further research under wetter years is needed to provide additional information for the importance of forest properties to soil respiration.

2.5 Conclusions

The drier than regular conditions observed in central Europe in 2018 and 2019 reduced the summer soil respiration fluxes. Structural equation modeling indicated that fine root biomass was the most important predictor of forest soil respiration. We found that fine root biomass partially mediated the effects of forest properties on in-situ soil respiration. Reductions in soil moisture associated with drought events might not only reduce soil respiration, but also influence the relative importance of autotrophic and heterotrophic respiration for total soil CO_2 fluxes. Our results indicated that autotrophic respiration was the main contributor to total in-situ soil respiration, though this was possibly emphasized by the drought conditions in 2018 and 2019.

The ability of forest properties to explain soil respiration rates remained stable over the two years and even under the disturbed, vegetation-free, standardized conditions of an incubation experiment. After examining multiple forest properties, we identified mean diameter at breast height and conifer share as the most important properties for the variation of soil respiration at the regional scale. The importance of other forest properties, such as tree species richness, might have been masked by the droughts that occurred during our field campaigns. In drought conditions mean diameter at breast height and conifer share had a positive and a negative effect on in-situ, autotrophic-dominated, soil respiration, respectively. This suggests that older forests with, on average, thicker trees and less conifers have higher respiration fluxes under dry conditions compared to younger and thinner stands with more coniferous trees. In contrast, the opposite was found for heterotrophic respiration in our soil incubation data under non-limiting water conditions. This suggests an environmental dependency on the effects of forest properties on soil respiration. Future studies should investigate the effects of multiple forest properties under wetter conditions and focus on disentangling potential differential effects on heterotrophic and autotrophic respiration under wet and dry conditions.

2.6 Supplementary material

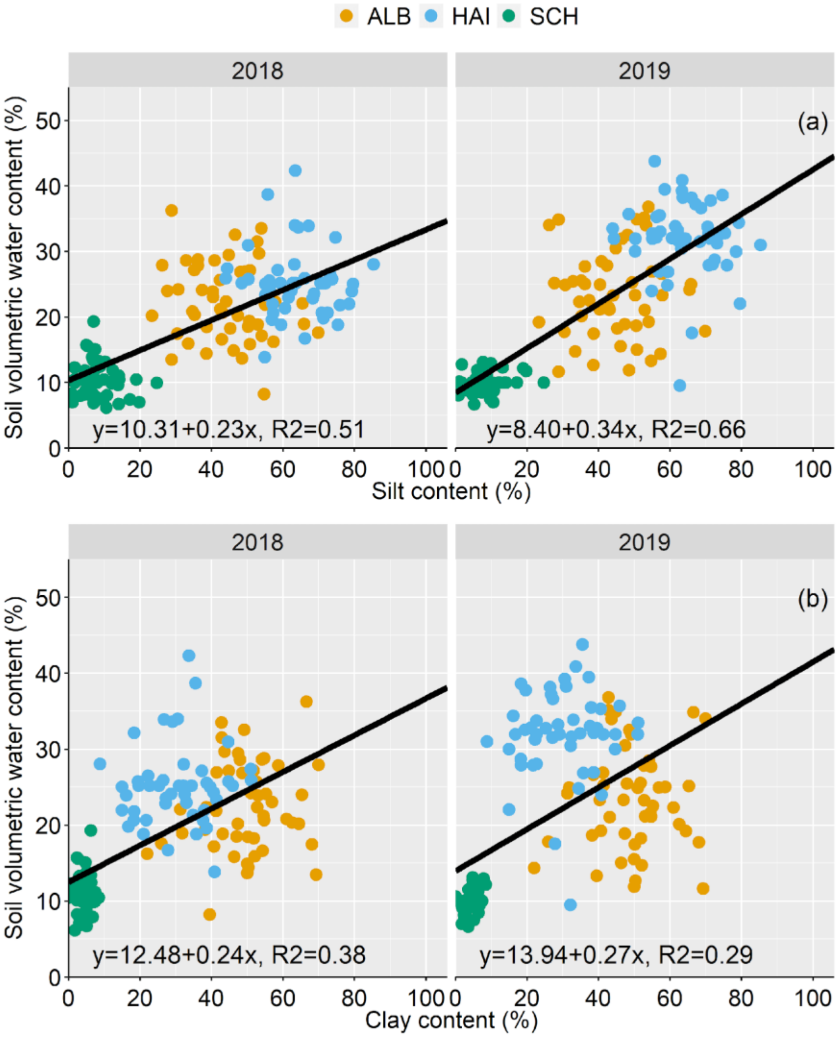


Figure S 2.1 Correlation between soil volumetric water content and (a) silt and (b) clay content for the two sampling years 2018 and 2019 and the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Equation and R^2 describe the linear relationship of soil volumetric water content and silt and clay content over all study regions.

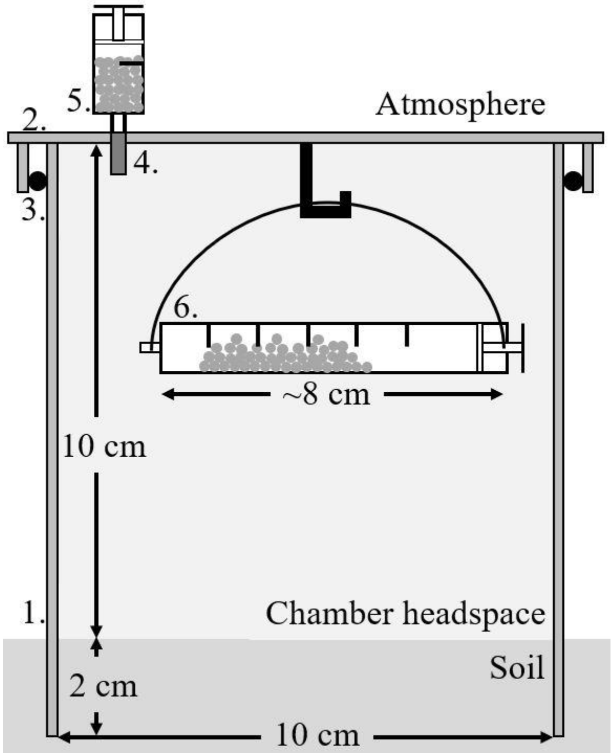
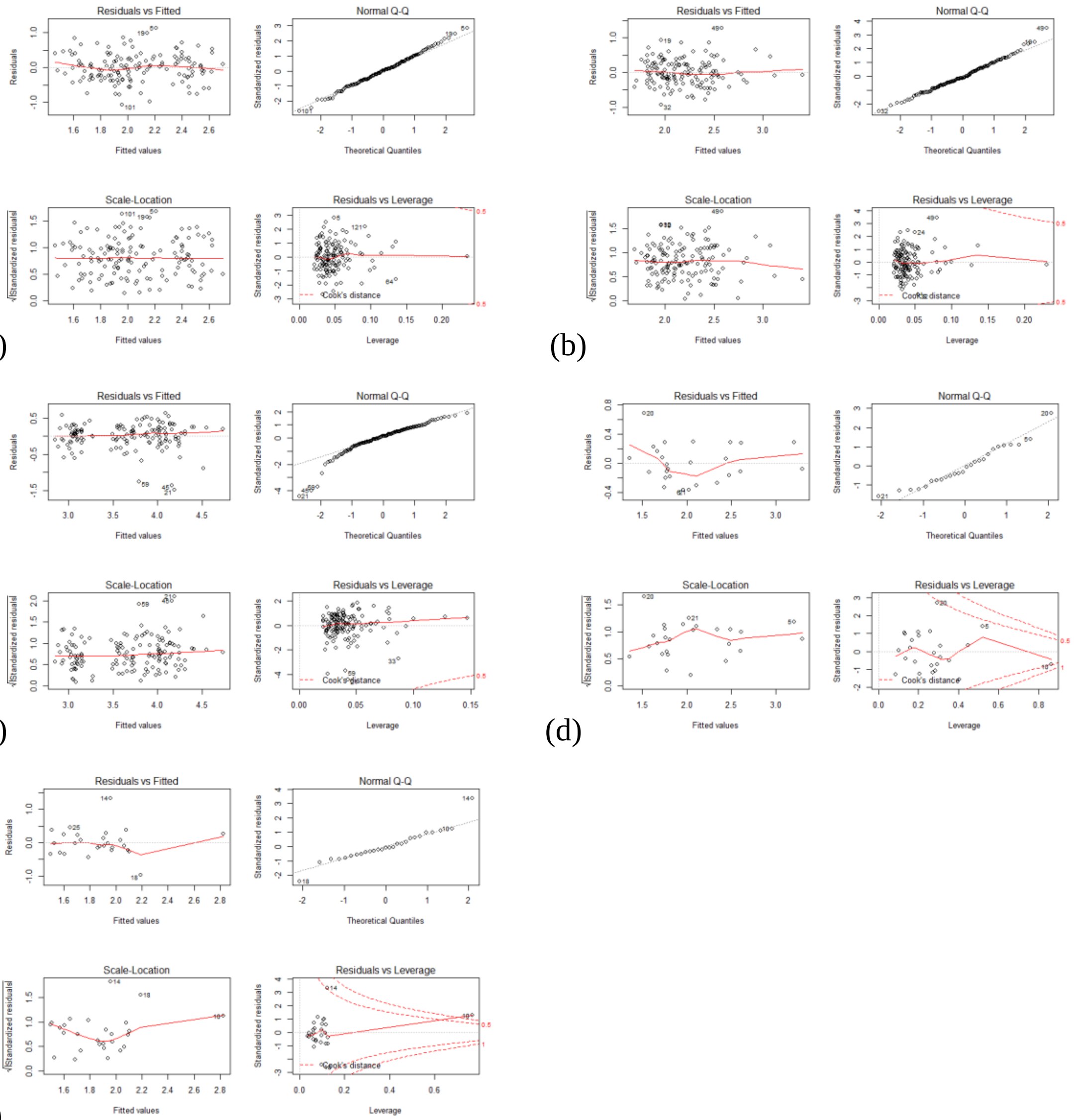


Figure S 2.2 Sketch of the soda-lime method with an open and static chamber. A PVC ring (1.) of 12 cm is inserted to the soil down to 2 cm. A PVC lid (2.) is placed over the PVC ring and a plastic O-ring (3.) ensures the airtightness of the chamber. A plastic tube (4.), which is glued on the PC lid with CO_2-impermeable silicon, passes through the PVC lid. This tube provides a flow-channel between the headspace of the chamber and ambient atmosphere and, thus, pressure equilibrium between the two. In line with the plastic tube, a syringe (5.) containing soda-lime granules is placed out of the chamber to prevent atmospheric CO_2 from entering the headspace of the chamber. Inside the chamber a syringe (6.) with 64 holes (1 mm diameter) is held from a hook and contains soda-lime granules for the determination of soil CO_2 efflux.



(a)

(b)

(c)

(d)

(e)

Figure S 2.3 Diagnostic plots for the assumptions of linearity, normality, homoscedasticity of variance and for testing the existence of influential values (i.e. outliers) for the ANCOVA (SS type II) models of the in-situ soil respiration in 2018 (a) and in 2019 (b), the soil respiration from the incubation experiment (c) and the in-situ soil respiration of the mineral soil in 2018 (d) and in 2019 (e). The diagnostic plots were exported from the function lm() with the function plot() in R. More information on the ANCOVA models is given in Tables 5 and 6.

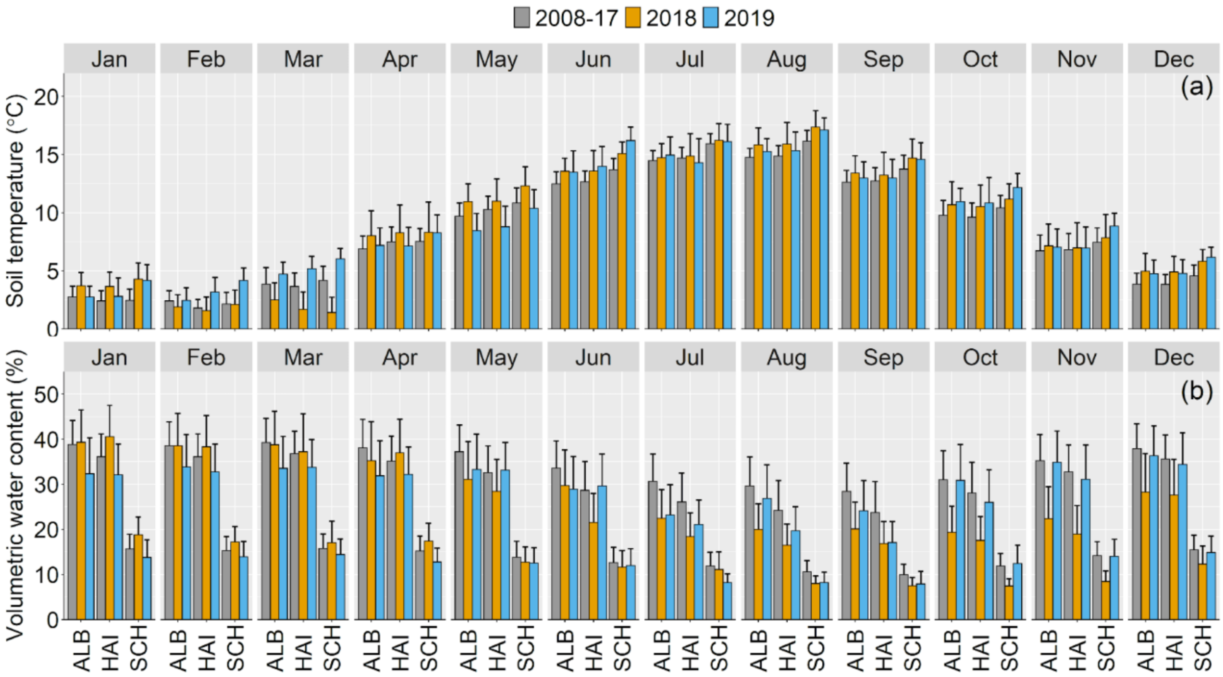


Figure S 2.4 Mean (a) soil temperature (°C) and (b) volumetric water content (%) per month for the 10-year-mean (2008-17) and the sampling years 2018 and 2019 for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Error bars represent standard deviations around the mean.

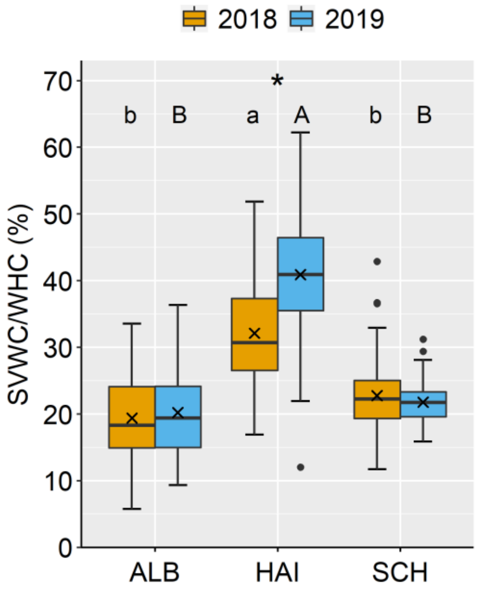


Figure S 2.5 Soil volumetric water content (SVWC) over the Water holding capacity (WHC) for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH and the two years; 2018 and 2019. Asterisks indicate differences between the two years for a given study site. Lower- and upper-case letters indicate differences among study sites in 2018 and 2019, respectively.

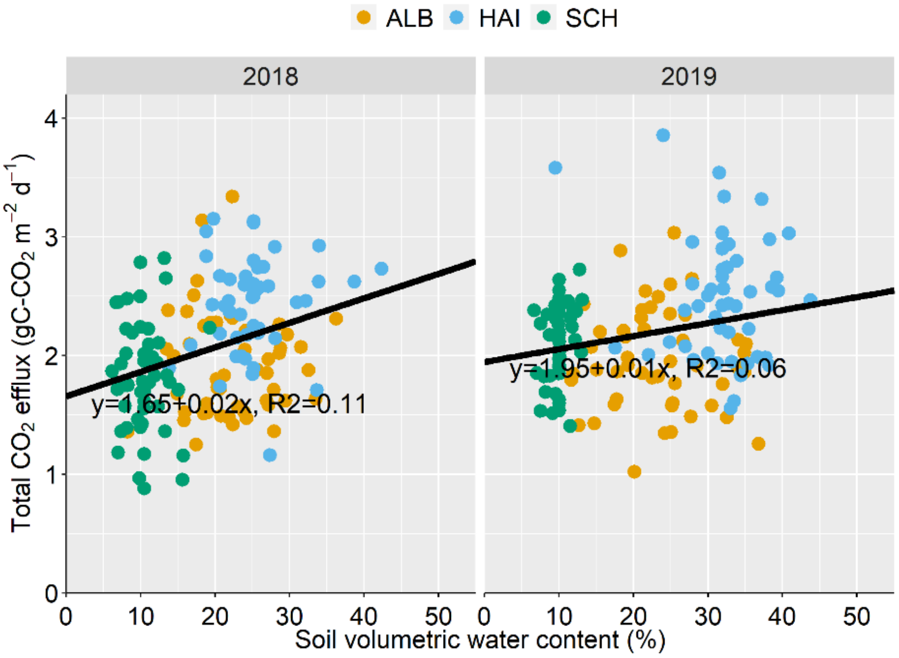


Figure S 2.6 Correlation between total in-situ soil respiration and soil volumetric water content for the two sampling years 2018 and 2019 and the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Equation and R^2 describe the linear relationship of total in-situ soil respiration and soil volumetric water content over all study regions.

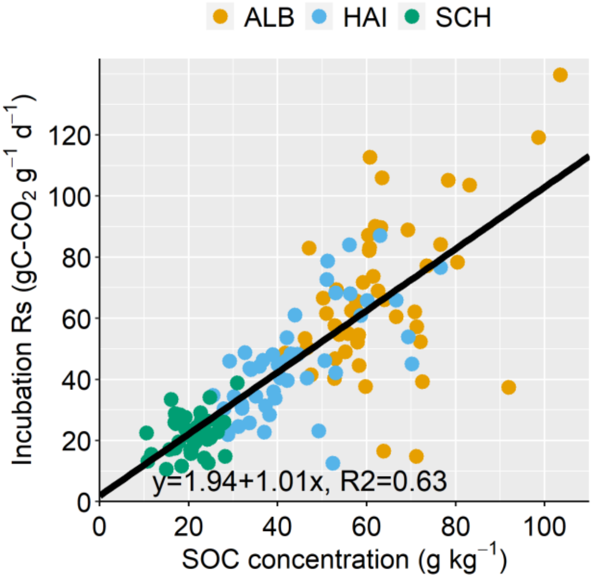


Figure S 2.7 Correlation between soil respiration from laboratory incubation experiments and soil organic carbon (SOC) concentration for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün = HAI and Schorfheide-Chorin =SCH. Equation and R^2 describe the linear relationship of total soil respiration from the incubation experiment and soil organic carbon concentration over all study regions.

3 Drivers of soil respiration across a management intensity gradient in temperate grasslands under drought

Abstract

Management intensification alters the functioning of temperate grasslands, such as carbon cycling, and threatens their biodiversity. Soil respiration is an important path of soil carbon losses however, it is rarely studied across broad management gradients in temperate grasslands.

Using the soda-lime method, we measured in-situ soil respiration with single-in-time measurements in 150 grasslands in three German regions in summer 2018 and 2019. The grasslands ranged from unfertilized and grazed grasslands to intensively fertilized and frequently harvested ones. To assess effects of management intensity and plant diversity on soil respiration, we used Structural Equation Modeling and accounted for both direct and indirect effects through soil and plant properties.

In-situ soil respiration was suppressed by limited water availability caused by naturally occurring droughts in both study years. Under the prevailing environmental conditions, grazing intensity did not affect in-situ soil respiration, while fertilization intensity indirectly increased soil respiration. Fertilization-induced plant species losses reduced the carbon to nitrogen ratio of soils and plants, leading to increased soil respiration, presumably due to substrate of higher quality and decomposability.

We conclude that intensively managed grasslands have higher soil respiration than extensive, plant species-rich grasslands even under the extreme conditions of a natural drought.

3.1 Introduction

Temperate grasslands store large amounts of carbon (C) in soils (Jobbagy and Jackson, 2000), and the fate of this carbon is determined by the balance between carbon inputs and losses due to decomposition. Grassland management intensification threatens soil organic carbon stocks (Conant et al., 2017), since it can alter carbon fluxes, and especially soil respiration. Management intensification influences soil respiration by causing changes related to soil, plant and microbial properties and communities. Understanding the interactions among ecosystem management, biotic drivers and soil respiration, while accounting for environmental drivers (i.e. soil properties and climate), can provide important insights for sustainable grassland management and climate change mitigation (FAO, 2010).

Grassland management intensity, mainly expressed by the intensity of fertilization, mowing and grazing (Blüthgen et al., 2012), increases soil respiration rates (Raich and Tufekcioglu, 2000; Wang and Fang, 2009). Fertilization, in general, increases soil respiration, but its effects also depend on the amounts of nitrogen (N) applied (Luo et al., 2016). Moreover, fertilization can enhance the effects of other practices (e.g. mowing) when combined with them (Jia et al., 2012). Both mowing and grazing reduce soil respiration possibly by removing plant biomass (Bahn et al., 2008), but (mowed) meadows are associated with higher respiration fluxes than (grazed) pastures (Koncz et al., 2015). However, most of the studies investigating management effects on soil respiration do not consider broad management gradients, and they include only a small number of replicated plots. Since the temporal patterns of soil respiration remain constant during the growing season (Johnson et al., 2008), single-in-time measurement campaigns can be used to sample a high number of replicated plots (Dias et al., 2010), and cover broad management gradients.

Many studies suggest that grassland management intensity can influence soil, plant and microbial properties to various degrees (Conant et al., 2017; Herold et al., 2014a; Herold et al., 2014b; Kleinebecker et al., 2014), which might further affect soil respiration. For example, fertilization decreases soil C:N ratio (Herold et al., 2014a) leading to increased carbon losses via decomposition, but it also increases plant productivity (Socher et al., 2012) and carbon inputs into the soil. Moreover, fertilization is a main driver of plant diversity loss in grasslands (Blüthgen et al., 2012; Midolo et al., 2018), which can have an adverse effect on soil organic carbon since recent evidence suggests that plant diversity increases carbon storage and decreases losses (Lange et al., 2015; Steinbeiss et al., 2008). In fertilized grasslands, soil microbial composition shifts towards bacteria-dominated communities (de Vries et al., 2006), but other studies identified soil properties as more important drivers of microbial properties than management (Herold et al., 2014a). Thus, responses of soil functions, like soil respiration, to management intensity should not be reported only as direct effects, but also through changes that management intensification causes in the soil, plant and microbial system (Koncz et al., 2015; Chapter 4).

Many studies investigate the effects of plant diversity on soil respiration, but often to contrasting results, since both positive and neutral effects have been reported (Chen et al., 2019; Chen et al., 2014; Dias et al., 2010; Dietrich et al., 2017). For instance, plant diversity increases plant biomass productivity, and exerts a positive effect on soil respiration (Craine et al., 2001; Dias et al., 2010). In contrast, a higher nitrogen use efficiency found in species-rich grasslands (Kleinebecker et al., 2014) can lead to lower nitrogen concentrations in plant biomass and litter (Fargione et al., 2007), and, thus, to reduced decomposition. Dias et al. (2010), using Structural Equation Modeling, found that plant diversity increased soil respiration in experimental grasslands i) by increasing aboveground plant biomass, and ii) by alleviating the negative effect of plant nitrogen on plant biomass. However, grassland management, and especially fertilization, increases plant productivity and decreases plant diversity (Klaus et al., 2018; Tilman et al., 2014), leading possibly to a decoupling of the aforementioned processes in managed grasslands. Most studies examining grassland diversity effects on ecosystem functions simulate plant species losses by random assemblages of plant communities at different diversity levels, and frequently do not consider management practices (Klaus et al., 2020a), which are, however, a main driving force of plant diversity variations in grasslands at the regional scale.

Together with management intensification, climate change is a main driver of ecosystem degradation and biodiversity loss (Gitay et al., 2001). Drought events are more likely to occur in the future due to climate change (Allen et al., 2010; Trenberth et al., 2014), affecting ecosystem services, like productivity and element cycling (Bastos et al., 2020; Klaus et al., 2016; Klaus et al., 2020b). The dry, water-limited conditions of a drought lead to reduced soil respiration (Burri et al., 2014), but studies investigating management effects on soil respiration under natural droughts are limited.

We obtained single-point-in-time soil CO_2 efflux measurements from the 150 grasslands in the *Biodiversity Exploratories* project, which cover a broad management intensity gradient. Measurements were taken in summer 2018 and 2019 with the soda-lime method that is particularly suitable to study a high number of sites. Two successive droughts occurred in central Europe in 2018 and 2019, and these affected our study regions. Our basic hypotheses were:

1. Grassland soil respiration rates differ among the three study regions due to differences in climatic conditions and soil properties.
2. Grassland fertilization and grazing intensities indirectly increases soil respiration by altering the stoichiometry of soils and plants.
3. Extensive, plant species-rich grasslands have lower soil respiration fluxes than intensively managed grasslands due to higher soil, plant and microbial C:N stoichiometry.

3.2 Methods

3.2.1 Study regions

This study was conducted in the *Biodiversity Exploratories* project that investigates land-use effects on biodiversity and ecosystem services and functions in real-world ecosystems (Fischer et al., 2010) in three study regions in Germany; the Schwäbische-Alb (ALB), the Hainich-Dün (HAI) and the Schorfheide-Chorin (SCH). The study regions differed in their geology, climate and topology (Table 3.1). ALB soils developed mainly on Jurassic limestone and were clay-rich Leptosols or Cambisols (IUSS Working Group WRB, 2014). In HAI, soils had a loamy or clayey texture due to the dominant geological substrate of loess over limestone and the main soil types were Cambisols, Stagnosols and Vertisols. Soils in SCH were drained Histosols with a loamy texture, but also Gleysols, Cambisols, Luvisols and Albeluvisols.

In each study regions, 50 plots with area 50 m×50 m were established within agriculturally managed grasslands (Fischer et al., 2010). Information regarding the regional management practices as well as the basic plant, microbial and soil properties are given in Table 3.2. Grassland management ranged from extensively to intensively grazed and/or fertilized grasslands, covering a wide and realistic management gradient. Fertilization, expressed as nitrogen additions, was applied as organic or mineral (chemical) fertilizers. Grazing, expressed as equivalent-livestock unit per area multiplied with grazing days (i.e. the days the animals are on the plot; Blüthgen et al., 2012), included mainly cattle or sheep grazing.

Table 3.1 Geographical, geological and climatic characteristics of the three study regions included in the Biodiversity Exploratories project after Fischer et al. (2010). Abbreviations: ALB =Schwäbische-Alb; HAI =Hainich-Dün; SCH =Schorfheide-Chorin; a.s.l. =above sea level. Soil types are given according to IUSS Working group WRB (2014).

Parameter	Study region		
	ALB	HAI	SCH
Location in Germany	Southwest	Central	Northeast
Geology	Calcareous bedrock with loam layer and karstic phenomenon	Calcareous bedrock with loess layer	Post glacial landscape with sandy ridges and fens
Elevation (m a.s.l.)	460-860	285-550	3-140
Dominant soil types	Leptosols and Cambisols	Cambisols, Stagnosols and Vertisols	Histosols, Gleysols, Cambisols, Luvisols and Albeluvisols
Annual temperature (°C)	6.0-7.0	6.5-8.0	8.0-8.5
Annual precipitation (mm)	700-1,000	500-800	500-600

3.2.2 Soil properties

Fourteen soil samples of the upper 10 cm of the soil were collected along two intersecting 20 m transects in each grassland in May 2017. They were used to prepare one composite soil sample per grassland. All soil samples were air-dried and sieved to <2 mm, while a portion was ground for the elemental analysis. Soil pH was measured in the extraction solution of 10 g of soil with 25 mL of $CaCl_2$ (0.01 M) with a pH-meter and a glass-electrode (WTW pH meter 538, Gießen, Germany). Total carbon and total nitrogen were determined by dry combustion at 1100°C with an elemental analyser VarioMax (Elemental, Hanau, Germany). Soil inorganic carbon concentration was determined with the same analyser after removing organic carbon by exposing 250 mg of soil to 450°C for 16 h. The soil organic carbon concentration was calculated as the difference between total and inorganic carbon. Soil texture and fine root (<2 mm) biomass were determined in composite samples collected in May 2011 with the same sampling procedure (Solly et al., 2014). They used a combined sieving and sedimentation method (DIN ISO 11277, 2002) for soil texture determination and weighed the isolated, cleaned and oven-dried roots (at 40°C for two days) for fine root determination.

Table 3.2 Mean values and standard deviation of management practices and soil, plant and microbial properties of the three study regions: Schwäbische-Alb =ALB; Hainich-Dün =HAI; Schorfheide-Chorin =SCH. Fertilization, grazing and mowing intensity data were obtained from Ostrowski et al. (2020) and averaged for the period 2015-18 and 2016-19. Lower-case letters indicate differences among the three study regions in a given year. Asterisks indicate significant differences between 2018 and 2019 in a given study region.

Parameter	Study region		
	ALB	HAI	SCH
Management practices 2015-18			
Grazing (livestock unit days ha^{-1})	77.8±117.4b	101.5±138.9 ab	186.6±197.5 a
Fertilization (kg N ha^{-1})	58.2±87.8a	39.1±52.0 a	3.0±13.0 b
Mowing (cuts y^{-1})	1.3±1.1a	1.0±0.7 ab	0.8±0.6 b
Management practices 2016-19			
Grazing (livestock unit days ha^{-1})	77.3±112.7	103.2±169.6	166.9±188.2
Fertilization (kg N ha^{-1})	57.2±77.3a	44.0±52.5a	3.6±12.7b
Mowing (cuts y^{-1})	1.4±1.1a	0.9±0.7ab	0.8±0.6b
Plant properties			
Abovegr. biomass (g m^{-2}, 2018)	178.5±96.5b	145.4±72.0b	364.6±156.2a*
Abovegr. biomass (g m^{-2}, 2019)	128.6±84.3b	158.4±114.3b	223.4±114.1b
Species richness (per 16 m^{2}, 2018)	30.4±8.8b	37.3±13.4a	25.8±7.2b
Species richness (per 16 m^{2}, 2019)	32.0±8.1b	42.2±12.9a	24.1±7.0c
C:N ratio (2018)	24.4±3.8ab*	23.7±2.6b	26.4±5.4a*
C:N ratio (2019)	21.7±4.9	21.7±2.2	22.0±5.6
Fine root biomass (g cm^{-3})	1.3±1.1b	6.0±2.5a	6.9±4.2a
Microbial properties			
Biomass carbon (μg C g^{-1})	815.7±152a	729.1±159.3a	582.7±352.5b
Biomass nitrogen (μg N g^{-1})	137.4±38.7	140.4±41.4	136.3±97.1
Soil properties			
Clay content (g kg^{-1} soil)	536.1±133.3a	422.6±126.6b	173.8±86.5c
Silt content (g kg^{-1} soil)	406.6±114.0b	520.122.0a	372.1±190.7b
Sand content (g kg^{-1} soil)	57.4±45.0b	57.6±23.1b	454.2±221.0a
pH	6.3±0.6b	6.9±0.5a	6.5±0.9b
Organic carbon conc. (g C kg^{-1})	69.8±14.3b	49.9±12.1b	95.5±90.7a
Nitrogen conc. (g N kg^{-1})	6.8±1.5b	4.9±1.2b	10.4±9.0a
C:N ratio	10.4±0.7a	10.2±0.5a	9.4±1.4b

3.2.3 Microbial properties

Aliquots of sieved soil (from the composite samples collected in May 2017) were used for microbial analysis. We determined the microbial C and N concentration with the chloroform-fumigation-extraction method (CFE), according to Vance et al. (1987) and Keil et al. (2011). Fumigated sample replicates were incubated with $CHCl_3$ for 24 hours. C and N were extracted from each fumigated and non-fumigated replicate (5 g) with 40 ml 0.5 M K_2SO_4. The suspension was horizontally shaken (30 min, 150 rpm) and centrifuged (30 min, 4400 x g). C and N concentrations in diluted extracts (1:4, extract: deionized H_2O) were measured with a

TOC/TN analyser (Multi N/C 2100S, Analytik Jena AG, Jena, Germany). No correction factors accounting for the extractable fraction of microbial C and N were used to calculate microbial biomass.

3.2.4 Plant properties

Vegetation information was collected in May 2018 and 2019. Aboveground plant biomass was measured in eight 0.25 m^2 subplots in each grassland. The subplots were fenced to prevent livestock grazing or mowing prior harvest. Vegetation was clipped at 2 cm height, dried at 80°C for 48 hours, weighed and grounded for chemical analysis. In a different 4 m×4 m subplot we recorded all occurring species and determined the plant species richness. We determined the carbon and nitrogen concentration of plant biomass with a Near Infrared Reflectance spectrometer (SpectraStar 2400, Unity Scientific, Columbia, MD, USA) (Busch et al., 2018; Kleinebecker et al., 2011). The reflectance spectrum of each sample, averaged over 24 scans, was recorded from 1250 to 2350 nm with 1 nm intervals.

3.2.5 Soil temperature and volumetric water content

Soil temperature and volumetric water content were recorded in 30-minute intervals in each grassland since 2008 using the ADL-MX Data Logger System (Meier-NT GmbH, Zwönitz, Germany). Instruments were installed in (2 m×2 m) fenced areas within the managed grasslands. Soil temperature (°C) was monitored at 5°cm depth below the surface of the mineral soil with the MNT-FExtension and water content (%) at 10 cm with the Delta-T ML2X Soil Humidity Probe (Delta-T Ltd, Cambridge, United Kingdom). Sub-hourly soil temperature and volumetric water content data were averaged with respect to soil respiration measurement dates for each grassland site and year.

3.2.6 Soil respiration

Soil respiration was measured with an absorption method along with an open and static chamber. The chamber design was based on previous work of Bierbaß et al. (2014), Näthe et al. (2018) (described in *Chapter 2.2.5*). The chamber consisted of a PVC ring (internal diameter =0.5 cm and height =12.0 cm), a PVC lid and an O-ring to ensure air-tightness (Figure S2.2). A plastic tube passing through a hole on the PVC lid allowed pressure equilibrium between the chamber and the ambient air. The outer ending of the tube was connected to a syringe containing 10 g of soda-lime to filter incoming ambient air CO_2. Inside the chamber, soda-lime was contained in a 50 mL syringe with 64 1-mm-holes held on the PVC lid.

Non-hygroscopic soda-lime with a diameter range of 2.4-5.0 mm and a saturation point of about 28% was acquired from the Fisher Scientific GmbH, Germany. Soda-lime is mainly $Ca(OH)_2$ and NaOH and reacts with CO_2 to form $CaCO_3$. Dried soda-lime mass (105°C for

48 h) was weighed with an accuracy of 0.1 mg before and after the exposure to soil CO_2 efflux. Each 50 mL syringe containing soda-lime was sealed in a sampling bag, while syringes of each grassland site were stored in CO_2-free sampling bags until field installation. To correct for atmospheric CO_2 adsorbed during the laboratory and field work, bottom-sealed chambers following the same design were used as controls. The exposure time was <3 days (65-70 hours) and the mass of dried soda-lime for each measurement was 12 g d^{-1}. The mass of the absorbed CO_2 can be determined by the difference of dried soda-lime mass before and after the field measurement. The soil efflux is calculated by the Equation 2.1 (Keith and Wong, 2006):

Soil respiration measurements were taken from June to July in 2018 and 2019 following the order HAI, SCH and ALB. Four chambers, forming a square of 10 m side length, were installed for the measurement of soil respiration in each grassland site and one bottom-sealed trap served as control. Aboveground vegetation was clipped and removed from the installation area and PVC rings were plugged down to 1-2 cm soil depth. Measurements (soda-lime and PVC lids installation) started five to six days after vegetation clipping. Just before installation, we applied deionized water to the dried soda-lime to compensate for the initial moisture content (about 18%) that was lost during drying, since CO_2 needs to be hydrated before reacting with the soda-lime. Over the two years, we installed 1,200 traps and 300 controls for the determination of soil CO_2 effluxes.

The soda-lime method for the soil respiration determination can yield misleading results if the soda-lime mass is not sufficient, and thus becomes saturated during the exposure to soil CO_2 efflux. Janssens et al. (2000) proposed that the mass gain should be lower than 10% per dry mass given a saturation point of about 28% per dry mass. In our study, the soda-lime mass gain did not exceed 6% in any of the measurements (Table S3.1). In addition, the soda-lime method can overestimate soil CO_2 efflux due to outgassing of abiotic CO_2 from the soil solution in soil with high pH and carbonate content. We tested the performance of the soda-lime method against an infra-red gas analyser (Li8100A, LI-COR Biosciences) in a grassland with soil pH =7.5 over a period of three days. Based on a one-way Analysis of Variance (ANOVA) model, there were no significant differences between measurements taken with the two methods (Figure S3.1).

3.2.7 Data analysis

Using ANOVA models and the Tukey's Honest Significant Difference (HSD) test, we tested for differences in in-situ soil respiration, soil, plant and microbial properties and environmental conditions among the three study regions, and, when possible, between the two sampling years. Then, we ran a backward stepwise analysis for soil respiration including i) climate (i.e. soil temperature and moisture), ii) management (i.e. fertilization and grazing intensities), iii) plant properties (i.e. plant species richness, aboveground biomass, fine root biomass and plant C:N ratio), iv) microbial properties (microbial C and C:N ratio) and v) soil properties (SOC, soil C:N ratio, pH and silt content), to evaluate the importance of these variables on model performance based on the Akaike information criterion. Based on the selected variables from the stepwise analysis, we examined the significance of these variables on soil respiration using Analysis of Covariance (ANCOVA) models. ANCOVA models were performed assuming a type II sum of squares, which is not influenced by the order in which the explanatory variables are introduced in the model (Zuur et al., 2009). Diagnostic plots were investigated for every linear model applied to evaluate the assumptions of linearity, normality and homoscedasticity in the residuals, and to check for influential values (Zuur et al., 2009). We applied logarithmic transformations on soil respiration and plant species richness to tackle heteroscedastic issues. After selecting the independent variables for the final model, we used the variance inflation ratio to test for collinearities, and values below 3.0 were considered acceptable.

To separate direct from indirect effects of grassland management on soil respiration, we used Structural Equation Modeling (Shipley, 2016). Fertilization and grazing intensities and soil water content were always exogenous variables. As endogenous variables, we used plant species richness and soil and plant C:N ratio to gain a mechanistic understanding of management effects on soil respiration. Using one-way ANOVA models, we removed the effects of study regions from each variable, and we used the residuals in the Structural Equation models. Similar to models with soil and plant C:N stoichiometry, we ran models with soil OC and aboveground plant biomass. Proposed causal structures with p-values >0.05 were considered acceptable. In addition, we calculated the root mean square errors of approximation (RMSEA) and the standardized root mean square residuals (SRMR), which should be <0.05. In addition to the high number of observations (≥144 observations from 150 grasslands due to missing values) compared to estimated parameters that provide stability against multivariate non-normality issues, we used bootstrapping and monitored changes in the p-value of the causal structure for additional safety. These changes in the p-values of the causal structure were negligible, and they are not shown.

Statistical analysis was performed with the R statistical software (R Core Team, 2019). ANOVA and ANCOVA models were performed with the *lm* function and information was extracted with the *summary* function. Tukey’s HSD test was performed with the *TukeyHSD* of the *stats* package. For the backward elimination stepwise process, the *step* function from the *stats* package was used. The variance inflation ratio was calculated with the *vif* function of the *faraway* package. The structural equation modeling was performed with the *sem* function from the *lavaan* package (Rosseel, 2012).

3.3 Results

3.3.1 Soil temperature and water content

In the three study regions, soil temperature ranged from 14.5 to 22.8°C in 2018 and from 14.6 to 24.2°C in 2019. Soil temperature increased in the order SCH<HAI=ALB in 2018 and in the order HAI<SCH<ALB in 2019 (Figure 3.1a). Moreover, soil temperature differed between the two study years in every study region, and was higher in 2019 than 2018 in ALB and SCH, but lower in HAI. Soil volumetric water content ranged from 7.4 to 51.5% in 2018 and from 7.0 to 43.6% in 2019. In 2018, soil water content did not differ among the three study regions, while in 2019 it increased in the order SCH<ALB<HAI (Figure 3.1b). Between the two study years, soil water content differed only in HAI, and it was wetter in 2019 than 2018. The ratio of soil water content over the water holding capacity increased in the order ALB=SCH<HAI both in 2018 and 2019 (Figure S3.2). Compared to the 10-year average, soil temperature was exceptionally high, and soil water content low, during and before our sampling months suggesting exceptionally dry conditions both in 2018 and 2019 (Figure S3.3).

3.3.2 In-situ soil respiration rates

In-situ soil respiration ranged from 0.5 to 6.9 in 2018 and 1.1 to 9.2 gCO_2-C m^{-2} d^{-1} in 2019 over the three study regions. In 2018, soil respiration did not differ among the study regions, while in 2019 it increased in the order ALB=SCH<HAI (Figure 3.1c). Between the two study years, soil respiration differed only in HAI, and it was higher in 2019 than 2018 following the increase observed in soil volumetric water content (Figure 3.1b).

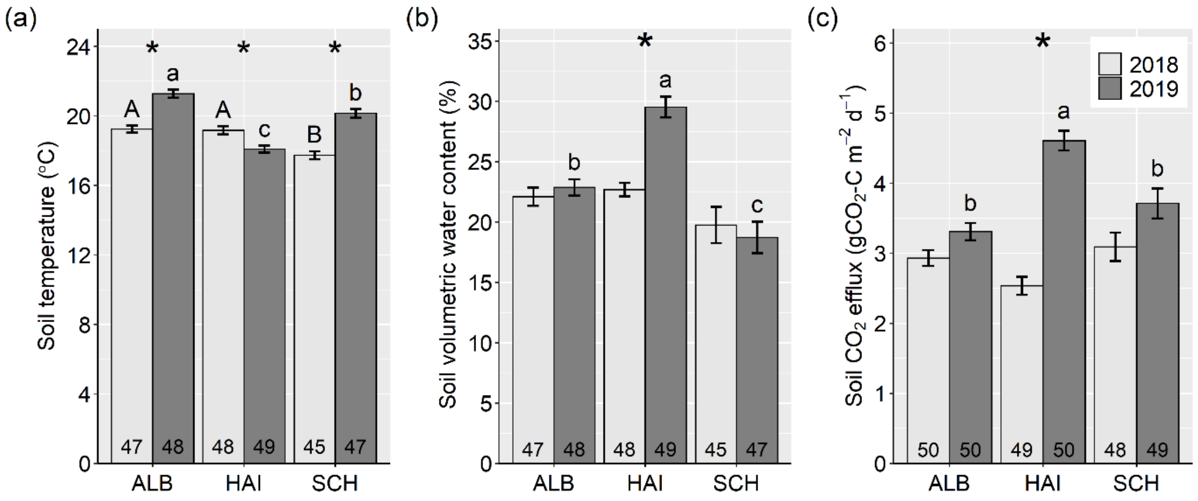


Figure 3.1 (a) Soil temperature at 5 cm, (b) soil volumetric water content at 10 cm soil depth and (c) soil respiration in 2018 (light grey) and 2019 (dark grey) for the three study regions; Schwäbische-Alb =ALB; Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Bars show mean values and error bars show the standard error of the samples. Numbers at the base of the bars indicate the number of observations available in each study region and year. Asterisks indicate significant differences between the years for a given study region according to post-hoc tests. Upper (for 2018) and lower-case letters (for 2019) indicate differences among the three study regions in the two years.

3.3.3 Drivers of in-situ soil respiration

ANCOVA models explained 18% and 46% of the variation in in-situ soil respiration in 2018 and 2019, respectively (Table 3.3). Study regions were important predictors of soil respiration in both study years. Fertilization and grazing intensities were rarely selected in the final models (only fertilization in 2019), and their effects were not significant. This indicates that management affected soil respiration only indirectly. Soil volumetric water content had a strong positive effect on soil respiration, indicating water-limited conditions in both study years. In 2018, plant species richness and soil C:N ratio were selected in our final models, and had a significant and a marginal negative effect on soil respiration, respectively. In 2019, microbial C and silt content had significant positive effects on soil respiration. Soil texture is important for water, nutrient and gas fluxes in soils, and the effect of silt on respiration is possibly related to this. As in 2018, soil C:N ratio had only a marginal effect on soil respiration in 2019. Finally, grazing intensity, soil temperature, plant biomass and C:N ratio, fine root biomass, soil organic carbon concentration and microbial C:N ratio were not selected as predictors of soil respiration in neither of the study years.

Table 3.3 ANCOVA models (SS type II) of in-situ soil respiration in 2018 and 2019 with management intensities, soil water content, plant, microbial and soil properties. Full models were stepwise reduced. Study regions effects are given relative to the Schwäbische-Alb region. Fertilization and grazing intensities refer to average management practices in the periods 2015-18 and 2016-19. Significant predictors are given in bold. The variance inflation factor was 1.4 and 1.6 for the explanatory variables in 2018 and 2019. Grazing intensity, soil temperature, fine root biomass, plant and microbial C:N ratios and soil organic carbon were never selected in the final models. Abbreviation: HAI =Hainich-Dün; SCH =Schorfheide-Chorin; df =degrees of freedom.

In-situ soil respiration	**in 2018**		**in 2019**	
	df=133	Model p	df=138	Model p
	R^2=0.18	<0.001	R^2=0.46	<0.001
Parameter	t-values	p-values	t-values	p-values
Intercept	**4.84**	**<0.001**	**2.08**	**0.040**
Region HAI	-1.12	0.263	**4.38**	**<0.001**
Region SCH	-0.21	0.831	**3.26**	**0.001**
Fertilization (hist.)	-	-	1.86	0.066
Soil water content	**2.22**	**0.028**	**3.51**	**<0.001**
Plant richness	**-2.08**	**0.040**	-	-
Microbial C	-	-	**2.45**	**0.015**
Soil C:N ratio	-1.63	0.100	-1.71	0.090
Soil pH	-	-	-1.60	0.112
Silt content	-	-	**3.02**	**0.003**

3.3.4 Management intensity effects on in-situ soil respiration

After removing the effects of study regions, we used Structural Equation Modeling to investigate the indirect effects of fertilization and grazing intensities on in-situ soil respiration through the stoichiometry of soils and plants (Figure 3.2). Soil water content had a positive effect on soil respiration in both years. In both years, soil C:N ratio was directly and negatively associated with soil respiration (Figure 3.2a), while plant C:N ratio was negatively associated with soil respiration only in 2019 (Figure 3.2c). Plant species richness was indirectly and negatively associated with soil respiration in both year due to its positive correlation with soil and plant C:N ratios. Finally, fertilization intensity was indirectly and positively linked to soil respiration by decreasing plant species richness, while grazing intensity remained unrelated to soil respiration fluxes in both study years.

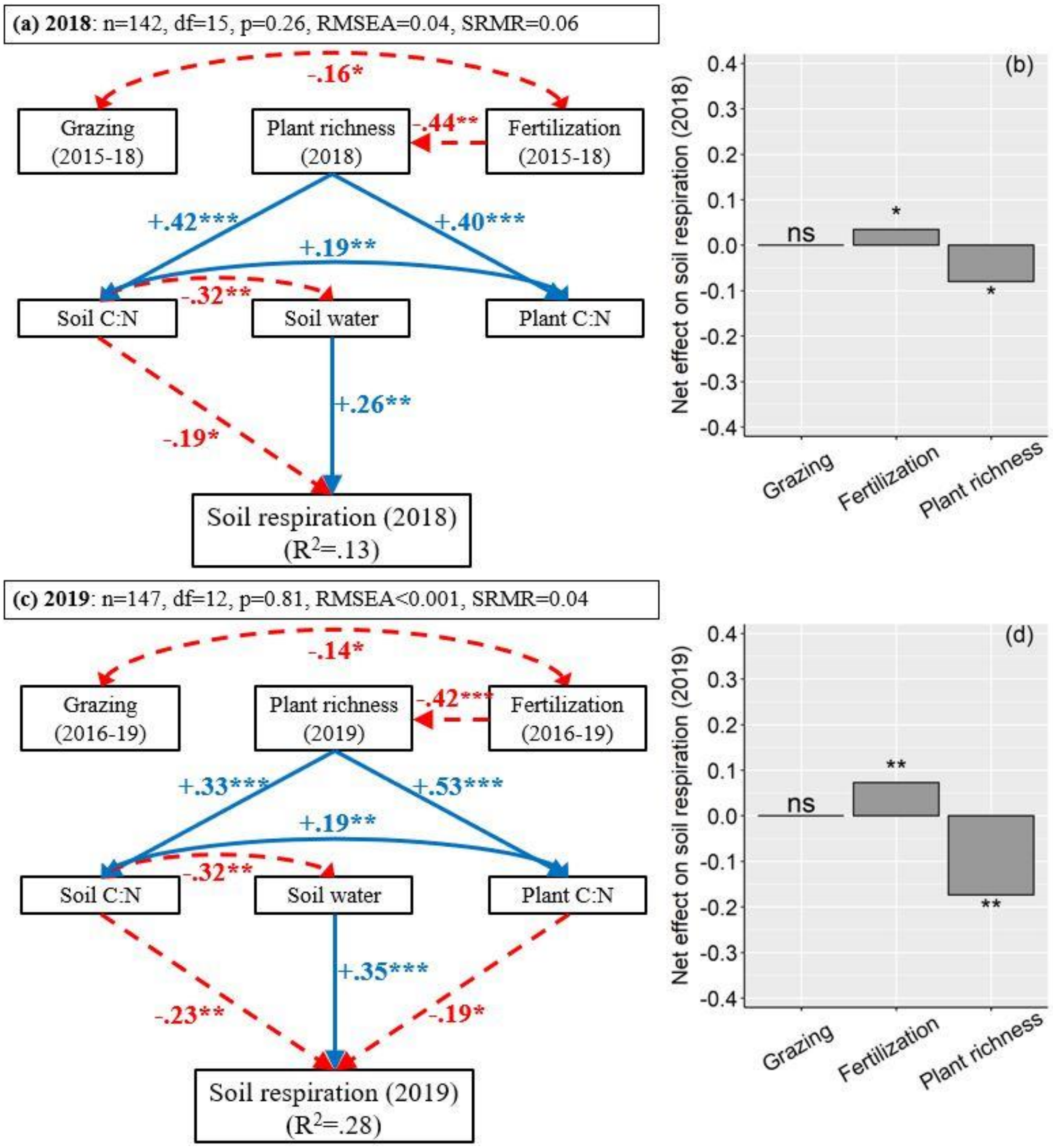


Figure 3.2 Structural Equation Models and net effects on soil respiration (a, b) in 2018 and (c, d) in 2019, explained by fertilization and grazing intensities, plant species richness and C:N ratios of soils and plants. Residuals of each variable are used after removing the effect of study regions. Single-headed arrows indicate direct causal paths, and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Number of observations (n), degrees of freedom (df) and fitness statistics (p-values, RMSEA and SRMR) are given for each model. Standardized path coefficients are given together with their significance level (ns =not significant; *p<0.050; **p<0.010; ***p<0.001). Coefficients of determination are given for soil respiration.

Apart from soil and plant C:N stoichiometry, we also investigated the effects of soil OC and aboveground plant biomass on in-situ soil respiration in alternative SEMs. Soil water content had again a positive effect on soil respiration in both years (Figure S3.5). In 2018, soil OC and aboveground plant biomass were not associated with soil respiration, while plant species richness was directly and negatively link with soil respiration (Figure S3.5a). In contrast, both soil OC and aboveground plant biomass were positively associated with soil respiration in 2019 (Figure S3.5b). In this year, plant species richness was indirectly and negatively linked with soil respiration due to a negative relationship with soil OC. Fertilization intensity was indirectly and positively linked to soil respiration by decreasing plant species richness. In addition, both fertilization and grazing intensities were positively linked with aboveground biomass and thus with soil respiration. Finally, we also tested soil and plant C:P ratios effects on soil respiration (Figure S3.5), but we did not find any significant effects.

3.4 Discussion

Grassland soil respiration is rarely studied over broad management gradients, partially due to logistical constraints associated with covering a high temporal and spatial scale. Since the temporal patterns of soil respiration remain relatively stable in the growing season (Johnson et al., 2008), single-in-time measurements can be used to measure in-situ soil respiration in a large number of grasslands (Dias et al., 2010), and, thus, to identify drivers of soil respiration at the regional scale. Here, we determined in-situ soil respiration with single-in-time measurements in 150 temperate managed grasslands in three regions in Germany to assess fertilization and grazing intensity effects on soil respiration through changes in soil and plant properties.

3.4.1 Grassland soil respiration was severely impacted by dry conditions

In this study, in-situ soil respiration in summer averaged at 2.8±1.1 gCO_2-C m^{-2} d^{-1} in 2018 and 3.9±1.3 gCO_2-C m^{-2} d^{-1} in 2019, respectively. Soil respiration in temperate grasslands peaks in early summer time, and frequently reaches values higher than 5 gCO_2-C m^{-2} d^{-1} (Bahn et al., 2008; Burri et al., 2014; Koncz et al., 2015; Moinet et al., 2019, after unit conversion). Compared to values for similar temperate grasslands from the literature, our summer soil respiration fluxes were relatively low. The strong correlation between soil respiration and soil water content (Table 3.3) indicates suppressed soil respiration because of the (limiting) water conditions which prevailed in central Europe in summer 2018 and 2019 (Figure S3.3; Chapter 2). In our first hypothesis, we expected that soil respiration would differ among the three study regions due to their differences in edaphic, environmental, and ecological conditions (Table 3.1 and 3.2). Indeed, we observed differences among the three study regions in 2019, when soil respiration in HAI was higher than in ALB and SCH, but not in 2018 (Figure 3.1c). This discrepancy between the two years was possibly caused by a slightly wetter summer in 2019 compared to 2018 (Figure S3.3), a condition that was more profound in HAI (Figure 3.1b). Thus, the particularly dry conditions in 2018 most likely suppressed soil respiration, and masked differences among the three study regions.

Apart from masking study region effects on soil respiration, drought also altered the dependency of in-situ soil respiration on soil properties, like the soil organic carbon. In more detail, soil respiration did not correlate with soil organic carbon in 2018 or 2019, and soil C:N ratio was only marginally significant in both years (Table 3.3). Similarly, Dietrich et al. (2017) found that microbial basal respiration did not correlate with soil organic carbon under a naturally occurring summer drought, while they correlated in the summer of the next year that was wetter. Several studies have suggested that droughts suppress heterotrophic respiration more than autotrophic respiration both in forests and grasslands (Moinet et al., 2019; Chapter 2), but such observations are frequently reported together with strong correlations between soil respiration rates and root biomass, which was not observed here.

3.4.2 Fertilization intensity effects on in-situ soil respiration depend on soil water conditions

Grazing and fertilization intensities were not important predictors of in-situ soil respiration (Table 3.3), but, when we accounted for indirect effects (with Structural Equation Modeling), we found that fertilization caused several positive effects (Figure 3.2a, c). Fertilization-induced effects on soil respiration were stronger in wetter (here 2019) than in drier (here 2018) years (Figure 3.2b, d), which is in accordance with previous findings for fertilization effects on microbial basal respiration in temperate grasslands (Dietrich et al., 2017). We suggest that climatic and soil water conditions modify the effects of fertilization on biological processes (e.g. plant and microbial activity) that control soil respiration, and potentially on other ecosystem functions as well. Understanding the interaction among climate, management and biodiversity and their effects on carbon cycling, both during and after drought events, is very important in a future of more frequent and/or intense droughts.

Studies investigating fertilization effects on soil respiration often report contrasting results including positive, negative or neutral responses in grasslands (Jia et al., 2012; Jones et al., 2005; Ward et al., 2017). Fertilization can influence soil respiration through many mechanisms, i.e. by causing changes in plant and microbial communities and properties. For instance, fertilization effects on plant properties involve i) increases in aboveground productivity (Socher et al., 2012), ii) reductions in belowground productivity (Bardgett et al., 1999a), and iii) plant species loss (Isbel et al., 2013; Midolo et al., 2018). In addition, fertilization alters the nutrient status of soils, plants and microbes (Blüthgen et al., 2012; Herold et al., 2014a; Chaper 4), which can further alter soil respiration, and its autotrophic and heterotrophic components (Zechmeister-Boltenstern et al., 2015; Zhang et al., 2014). Environmental conditions, fertilization type and time-length of the experiments (short- vs long-term) possibly determine the relevance of the different mechanisms involved and might obscure comparison between studies. In line with our second hypothesis, we found that fertilization intensity indirectly increased soil respiration by altering the stoichiometry of soils and plants. More specifically, fertilization-induced plant species loss decreased the C:N ratios of soils and plants, and soil (both in 2018 and ’19) and plant (only in 2019) C:N ratios were negatively associated with soil respiration (Figure 3.2). Thus, in our grasslands, soil respiration depended on the environmental conditions (e.g. soil water content) and the quality of the substrate.

Grazing intensity was not associated with in-situ soil respiration neither directly nor indirectly via C:N stoichiometry of soils and plants (Figure 3.2) but, similar to fertilization, it was positively linked to soil respiration in 2019 due to aboveground plant biomass (Figure S3.5b). The (limiting) water conditions in our study years (compared to previous years, Figure S3.3) might, however, have masked additional grazing effects on soil respiration, for instance.

3.4.3 Fertilization-induced plant species losses increase soil respiration under dry conditions

Plant diversity effects on soil respiration were found to be positive or neutral (Chen et al., 2019; Dias et al., 2010; Dietrich et al., 2017), and they are frequently reported as direct effects (Spehn et al., 2000). In contrast to most studies, we found that plant species richness indirectly reduced in-situ soil respiration due to increased soil and plant C:N ratios (Figure 3.2). However, plant diversity effects on soil respiration were ultimately controlled by fertilization since fertilization intensity is a main driver of plant species richness in managed grasslands.

Extensively managed grasslands host richer plant communities compared to intensively managed ones (Blüthgen et al., 2012). Species-rich grasslands are characterized by conservative, slow growing plant communities with a high N use efficiency (Busch et al., 2018; Kleinebecker et al., 2014). This leads to lower N concentrations in plant biomass and litter and, as a consequence, slower litter decomposition (Chen et al., 2017; Fargione et al., 2007). Similarly, in this work, fertilization intensity reduced plant species richness, which was positively associated with plant C:N ratio and thus negatively associated with soil respiration even though indirectly (Figure 3.2c). Apart from plant chemistry, plant species richness was also negatively associated with (aboveground) plant productivity in our grasslands (Socher et al., 2012). Plant productivity increased soil respiration (Figure S3.5b), possibly because of increased C inputs into the soil (Craine et al., 2001; Dias et al., 2010). We suggest that the aforementioned characteristics of extensive, plant species-rich grasslands lead to slower C cycling compared to intensive, species-poor grasslands, and, thus, to lower soil respiration fluxes.

3.5 Conclusions

Management intensification is a main driver of ecosystem degradation in temperate grasslands, driving plant species loss and alterations in C cycling. Under the prevailing dry conditions, fertilization intensity indirectly increased soil respiration, and its effects were mediated by a plant species richness loss and by a higher quality (lower C:N ratios) in soil organic matter and aboveground plant biomass. This suggests that intensively managed, plant species-poor grasslands have a faster C cycling than extensive, species-rich grasslands even under the extreme conditions of a natural drought. Since C cycling is tightly coupled with nutrient cycling, grassland management and plant diversity effects on litter and soil organic matter decomposition will, at least partially, determine the availability of other nutrients for plant uptake or leaching. Grassland management guidelines that consider the protection of biodiversity are needed in order to cease, if not to reverse, plant species loss and to ensure ecosystem functioning.

3.6 Supplementary material

Table S 3.1 Minimum, average and maximum values of soda-lime sample mass increase (%, per dry weight) for the 2018 and 2019 campaigns in the three study regions; Schwäbische-Alb: ALB, Hainich-Dün: HAI and Schorfheide-Chorin: SCH. Mass gain did not exceed the limit of 9.0% proposed by Janssen et al. (2000)*, and the highest value was observed in SCH in 2019. Control samples had mass gains consistently lower than 1.0% (data not shown).

Region	Year	Mean	Min	Max
ALB	2018	2.3	0.2	3.8
	2019	1.6	0.6	2.7
HAI	2018	1.9	0.2	3.4
	2019	2.2	1.3	4.3
SCH	2018	2.2	0.2	5.3
	2019	1.6	0.5	3.7

*Janssens, Ivan a., Kowalski, A. S., Longdoz, B., & Ceulemans, R. (2000). Assessing forest soil CO2 efflux: an in situ comparison of four techniques. Tree Physiology, 20(5), 23–32. https://doi.org/10.1093/treephys/20.1.23

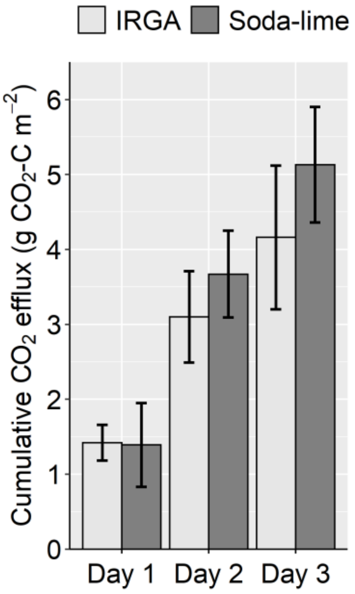


Figure S 3.1 Comparison of cumulative soil CO_2 efflux with the soda-lime method and an infra-red gas analyser (IRGA) (Li8100A, LI-COR Biosciences) in a grassland soil (pH =7.5) over a period of three days. No significant differences were observed between the two methods based on a one-way ANOVA.

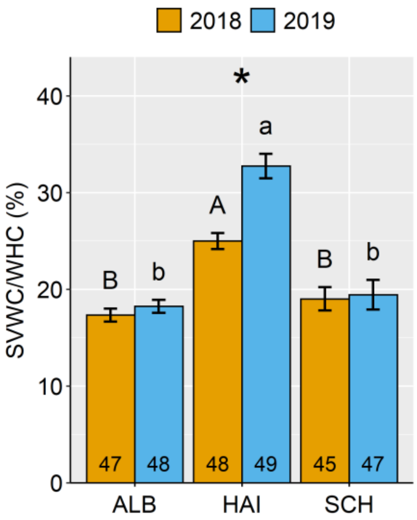


Figure S 3.2 Soil Volumetric Water Content (SVWC) over Water Holding Capacity (WHC) for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH and the two years; 2018 and 2019. Asterisks indicate differences between the two years for a given study region. Lower- and upper-case letters indicate differences among study regions in 2018 and 2019, respectively.

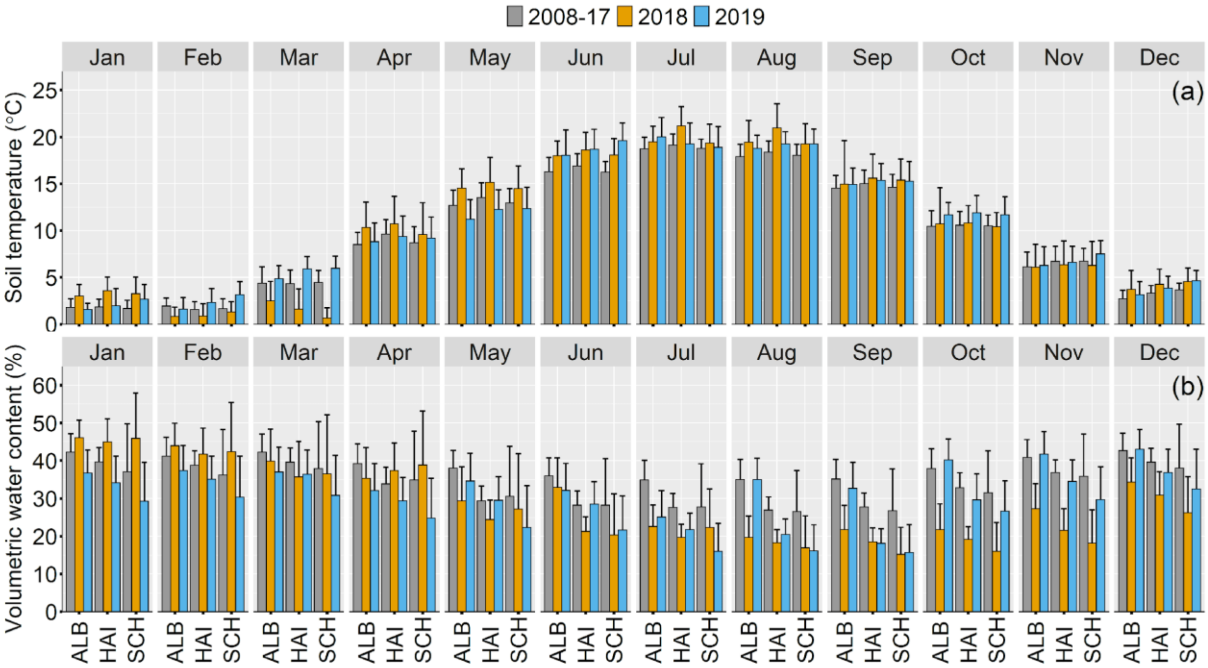


Figure S 3.3 Mean (a) soil temperature (°C) and (b) soil volumetric water content (%) per month for the 10-year-mean (2008-17) and the sampling years 2018 and 2019 for the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Error bars represent standard deviations around the mean.

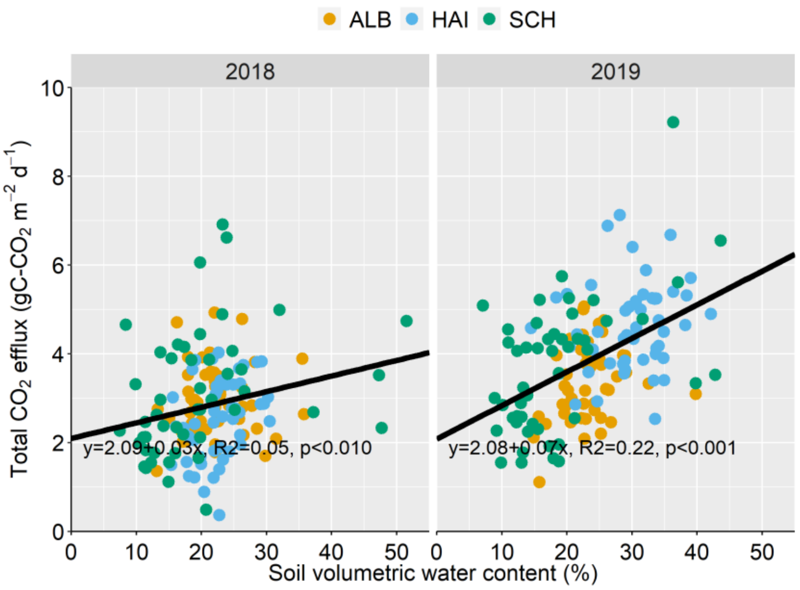


Figure S 3.4 Correlation between in-situ soil respiration and soil volumetric water content for the two sampling years 2018 and 2019 and the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Equation, R^2 and p-value describe the linear regression of in-situ soil respiration and soil volumetric water content over all study regions.

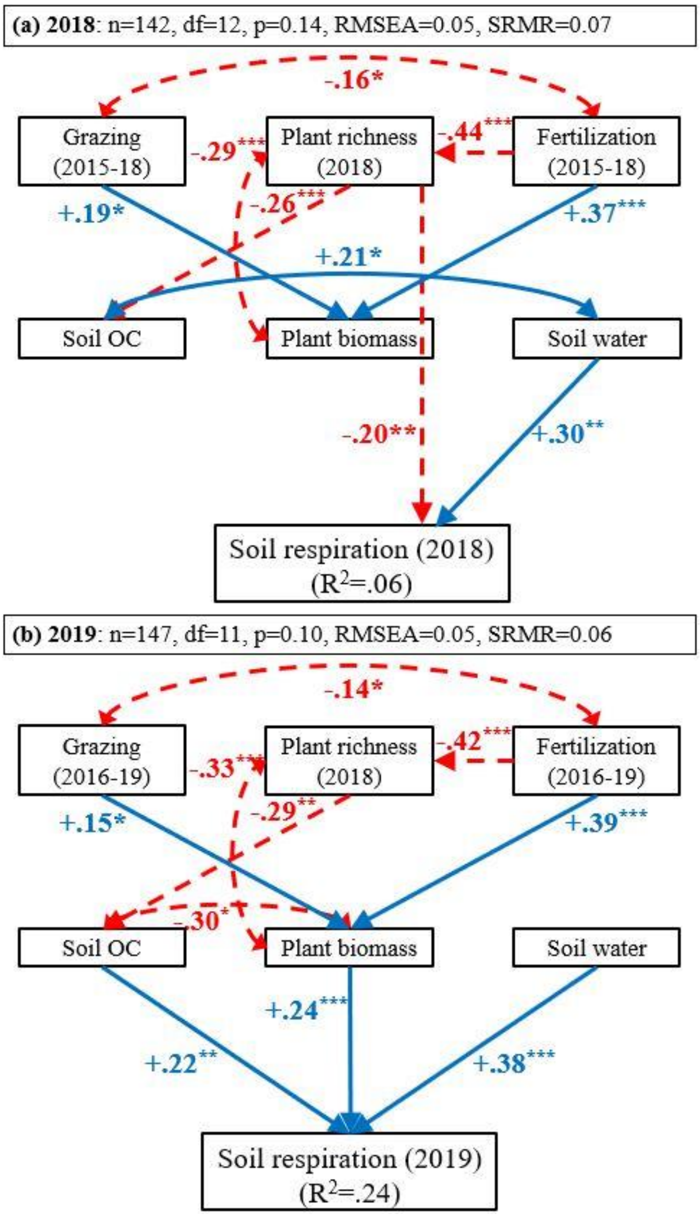


Figure S 3.5 Structural Equation Models for soil respiration explained by fertilization and grazing intensities, soil water content, plant species richness, soil organic carbon and aboveground plant biomass (a) in 2018 and (b) in 2019. Residuals of each variable are used after removing the effect of study regions. Single-headed arrows indicate direct causal paths, and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Number of observations (n), degrees of freedom (df) and fitness statistics (p-values, RMSEA and SRMR) are given for each model. Standardized path coefficients are given together with their significance level (ns =not significant; *p<0.050; **p<0.010; ***p<0.001). Coefficients of determination are given for soil respiration.

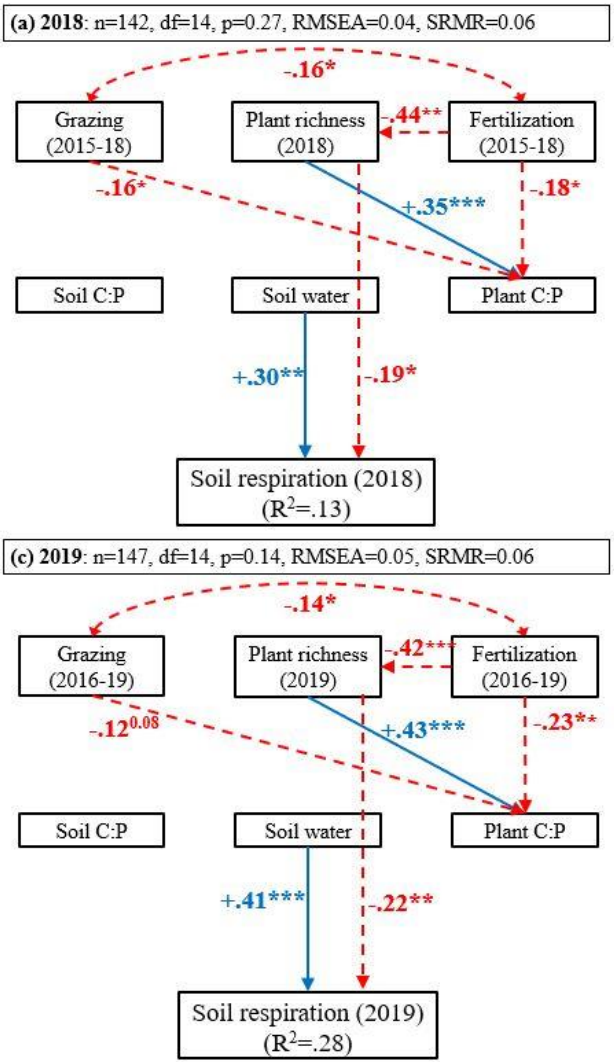


Figure S 3.6 Structural Equation Models for soil respiration explained by fertilization and grazing intensities, soil water content, plant species richness and soil and plant C:P ratios (a) in 2018 and (b) in 2019. Residuals of each variable are used after removing the effect of study regions. Single-headed arrows indicate direct causal paths, and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Number of observations (n), degrees of freedom (df) and fitness statistics (p-values, RMSEA and SRMR) are given for each model. Standardized path coefficients are given together with their significance level (ns =not significant; *p<0.050; **p<0.010; ***p<0.001). Coefficients of determination are given for soil respiration.

4 Drivers of annual nutrient leaching across a management intensity gradient in temperate grasslands

Abstract

Grassland management intensity influences nutrient cycling both directly, by changing nutrient inputs and outputs from the ecosystem, and indirectly, by altering the nutrient content, diversity and functional composition of plant and microbial communities. However, the relative importance of these direct and indirect processes for the leaching of multiple nutrients is poorly studied.

We measured the annual leaching of nitrate, ammonium, phosphate and sulphate at a depth of 10 cm in 150 temperate and managed grasslands using a resin method. Using Structural Equation Modeling, we distinguished between various direct and indirect effects of management intensity (i.e. grazing and fertilization) on nutrient leaching.

We found that management intensity increased nitrate, ammonium and phosphate leaching risk both directly (i.e. via increased nutrient inputs), and indirectly, by changing the stoichiometry of soils, plants and microbes. In contrast, sulphate leaching risk was reduced by management, presumably due to increased outputs from mowing and grazing. In addition, management intensity shifted plant communities towards an exploitative functional composition (characterized by high tissue turnover rates), and, thus, further promoted the leaching risk of inorganic N. Plant species richness was associated with lower inorganic N leaching risk, even after accounting for correlated effects of plant functional groups or community traits.

Maintaining and restoring diverse plant communities may therefore mitigate the increased leaching risk that management intensity imposes upon grasslands.

4.1 Introduction

Grasslands are widespread and highly diverse ecosystems providing a multitude of ecosystem functions and services, including nutrient cycling and food production (Bengtsson et al., 2019). Management intensification of grassland ecosystems places them at considerable risk of nutrient losses via leaching (Klaus et al., 2018, and references therein), especially that of nitrate, which represents the loss of an important resource, and, at the same time, a potential threat to human health and aquatic ecosystems (EEA, 2018; EU-Nitrate Directive, 2018; WHO, 2011). Compared to nitrogen (N), management effects on leaching of other elements, like phosphorus (P) and sulphur (S), are less studied in grasslands, despite their agricultural and environmental importance (Eriksen, 2009; Withers et al., 2014; Gallejones et al., 2012). Unravelling the relationships between ecosystem management, biotic drivers and nutrient leaching will provide important insights for sustainable grassland management, and could help secure associated ecosystem services.

In unfertilized grasslands, inorganic nutrient leaching represents the net balance between nutrient inputs to the soil solution (e.g. by organic matter mineralization or dissolution) and outputs from the soil solution (e.g. due to uptake by plants, or immobilization in microbial biomass), as well as nutrient exchange with the soil matrix (i.e. via (de)sorption to minerals). Management, such as fertilization and grazing, changes these relations by increasing both inputs and outputs of nutrients from the ecosystem. In the past, grasslands were considered to have low N leaching fluxes, and introducing temporary (mowed) grasslands in arable crop rotations can even reduce nitrate leaching (Kunrath et al., 2015). However, studies investigating the effects of grassland management on soil nitrate show increased leaching risk in pastures compared to meadows (Ryden et al., 1984), and when broader management gradients are included, both fertilization and grazing equally increase nitrate leaching risk (Klaus et al., 2018). Grassland intensification can affect nutrient cycling *directly* by inputs and outputs (i.e. fertilization, grazing and harvest) (Rumpel et al., 2015) and *indirectly* by changing plant communities (e.g. loss of diversity or functional change) and altering interactions between soils, plants and soil microorganisms (de Vries et al., 2012; Klaus et al., 2018). However, at present, it is unclear if management intensity predominantly drives leaching risk directly and/or indirectly by changing plant and/or microbial communities.

Grassland management, mainly fertilization, mowing and grazing, influences plant and microbial properties (Herold et al., 2014; Kleinebecker et al., 2014; Boeddinghaus et al., 2019), and, thus, alter the decomposition of organic matter (Solly et al. 2014), with potential effects on nutrient leaching. For instance, fertilization increases nutrient concentrations in plant biomass, and it reduces plant diversity and root litter decomposition (Gilhaus et al., 2017; Klaus et al., 2011; Solly et al., 2014). Many studies have reported that a high plant species diversity reduces N and P availability and leaching in grasslands (de Vries et al., 2016; Leimer et al., 2015 and 2016; Oelmann et al., 2011; Tilman et al., 1996), especially when compared to monocultures (Scherer-Lorenzen et al., 2003). Furthermore, effects of plant community composition, e.g. the abundance of functional groups or specific traits, can also play an important role in grassland nutrient cycling (Boeddinghaus et al 2019; de Vries and Bardgett, 2016). However, plant diversity effects on nitrate leaching are still debated and have not yet been comprehensively assessed up to now, as most of the respective studies were conducted in experimental grasslands or mesocosm experiments. Such studies are useful to gain a mechanistic understanding of the underlying processes controlling soil and plant interactions. However, they often do not consider realistic management and/or environmental gradients. Thus, they might fail to capture the complexity of real-world ecosystems (Klaus et al., 2020a) since, for instance, management is an important determinant of plant community diversity and composition (Busch et al., 2018; Midolo et al., 2018). To overcome these constraints, and gain knowledge transferable to sustainable grassland management, we need to investigate grassland ecosystems along broad gradients of management intensity and environmental conditions (Manning et al., 2019).

Recent evidence highlights the importance of soil microbial communities and their properties for N cycling (Grigulis et al., 2014; Pommier et al., 2017). Understanding, and even controlling, plant-microbe interactions could provide opportunities for enhanced N retention in grasslands, with desired effects both on biomass production and N leaching mitigation. Studies on element cycling including soil microbial properties are generally scarce compared to those addressing abiotic and/or plant effects (Pommier et al., 2017), and so are studies that simultaneously measure the cycling of multiple nutrients in managed grasslands. However, management also strongly changes microbial properties since, for instance, fertilization shifts the microbial community structure towards bacteria-dominated communities (de Vries et al., 2006; Donnison et al., 2000). To elucidate the drivers of ecosystem (dis)services like nutrient leaching, a holistic approach that includes management, but also soil, plant and microbial effects and their interactions is needed (Koncz et al., 2015; Pommier et al., 2017).

Reliable assessments of the drivers of nutrient leaching need to cover long time spans, for example due to the large variability in weather conditions and the timing of land management activities (de Vries et al., 2011; Klaus et al., 2018). This is even more important since recent evidence suggests that plant diversity effects on N leaching follow seasonal patterns, with more pronounced effects in winter (Leimer et al., 2015), a season that is often ignored as most studies focus on the growth period only. However, accounting for both high spatial and temporal variability imposes significant logistical constraints. Resin methods for the evaluation of nutrient availability and leaching have received increasing attention in the literature (Grahmann et al., 2018; Grunwald et al., 2020; Predotova et al., 2011; Willich and Buerkert, 2016), and are often successfully used to integrate the temporal effects of different drivers on nutrient leaching (Klaus et al., 2018; Klaus et al., 2020b).

In this study, we measured the annual downward flux of nitrate, ammonium, phosphate and sulphate with the percolating water, i.e. leaching, at a depth of 10 cm in 150 grassland sites along a gradient of management intensity from spring 2018 to spring 2019. The grassland sites were located in three regions in Germany as part of the *Biodiversity Exploratories* project (Fischer et al., 2010), and covered broad management intensity gradients within each region. We used a resin method, namely the Self-Integrating Accumulators (SIAs), to determine the cumulative annual leaching, and not just the availability of nutrients in the soil solution (Bischoff, 2009). Grassland management, here fertilization and grazing, has direct (via increased inputs and outputs) and indirect (via soils, plants and microbial biomass) effects on nitrate, ammonium, phosphate and sulphate leaching. We addressed the following hypotheses:

1. Grazing and fertilization intensities increase nutrient leaching risk from topsoils due to increased nutrient inputs (direct effects).
2. Grazing and fertilization intensities increase nutrient leaching risk by altering the stoichiometry of soils, plant communities and soil microbial biomass (indirect effects).
3. Grazing and fertilization intensities increase nutrient leaching risk i) by shifting the functional composition of plants from slow-growing, nutrient-efficient communities at extensively managed grasslands to fast growing communities in intensively managed grasslands, and ii) by reducing plant species richness (indirect effects).
4. Due to its various indirect effects, fertilization intensity has overall stronger effects on nutrient leaching risk than grazing (net effect).

4.2 Methods

4.2.1 Study regions

This study was conducted in the *Biodiversity Exploratories* project, which investigates land use effects on biodiversity and ecosystem functions and services in real-world ecosystems

(Fisher et al., 2010). The project includes three study regions in Germany; the Schwäbische-Alb (ALB), the Hainich-Dün (HAI) and the Schorfheide-Chorin (SCH). The study regions differed in their geology, topology, dominant soil types and climate (Table 3.1). Soils in ALB developed mainly on Jurassic limestone and were clay-rich Leptosols or Cambisols (IUSS Working Group WRB, 2014). In HAI, soils had a loamy or clayey texture due to the dominant geological substrate of loess over limestone and the main soil types were Cambisols, Stagnosols and Vertisols. Soils in SCH were drained Histosols with a loamy texture, Gleysols, Cambisols, Luvisols and Albeluvisols.

In each study region, 50 plots of 50 m × 50 m were established within agriculturally managed grasslands (Fisher et al., 2010). Information regarding the regional management practices as well as soil, plant and microbial properties is given in Table 4.1. Management intensity ranged from extensively managed grasslands to intensively grazed and/or fertilized pastures and meadows, covering a wide and realistic management intensity gradient. Fertilization, expressed as N additions, was applied as organic or mineral (chemical) fertilizers. Grazing, expressed as equivalent-livestock unit per area multiplied with grazing days (i.e. the days the animals are on the plot; Blüthgen et al., 2012), included mainly cattle or sheep grazing.

4.2.2 Soil properties

In May 2017, we collected 14 soil samples of the upper 10 cm along two intersecting 20 m transects in each of the 150 grasslands. Descriptions of the methods used for the determination of soil properties (SOC, soil C:N ratio, pH and silt content, fine root biomass) are given in *Chapter 3.2.2* (Table 3.2 and 4.1). In addition, soil bulk density was determined by drying a known volume of soil collected in May 2014 at 105°C to constant weight (Table 4.1). Soil oxalate extractable Al (in the composite samples collected in May 2011) was determined with ICP-OES (Optima 3300 DV, PerkinElmer) as a measure for non and poorly crystalline oxi-hydroxides. Soil inorganic (Olsen) P concentration was determined after extraction with 0.5 M $NaHCO_3$ with ICP-OES (Optima 5300 DV, PerkinElmer) in soil samples collected in May 2014 (Sorkau et al., 2018).

4.2.3 Microbial soil properties

Aliquots of sieved soil (from the composite samples collected in May 2017) were used for microbial analysis. We determined the microbial C and N concentration with the chloroform-fumigation-extraction method, according to Vance et al. (1987) and Keil et al. (2011). A detailed description of the method is given in *Chapter 3.2.3*.

Table 4.1 Mean values and standard deviation of management practices and plant, microbial and soil properties of the three study regions; Schwäbische-Alb =ALB, Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Fertilization, grazing and mowing intensity data were obtained from Ostrowski et al. (2020) and averaged for the period 2015-2018. Abbreviation: LU =Livestock Units; CWM =Community Weighted Mean.

Parameter	Study region		
	ALB	HAI	SCH
Management practices			
Fertilization (kg N ha^{-1} y^{-1})	58.2±87.8	39.1±52.0	3.0±13.0
Grazing (LU days ha^{-1} y^{-1})	77.8±117.4	101.5±138.9	186.6±197.5
Mowing (cuts y^{-1})	1.3±1.1	1.0±0.7	0.8±0.6
Plant properties			
Aboveground biomass (g m^{-2})	178.5±96.5	145.4±69.7	364.6±156.2
Aboveground N (g N kg^{-1})	18.4±2.9	18.6±2.0	17.4±3.4
Aboveground P (g P kg^{-1})	2.8±0.4	3.1±0.3	2.7±0.3
Species richness (per 16 m^2)	30.4±8.8	37.2±13.3	25.8±7.2
Cumulative legume species cover (%)	8.2±9.0	13.6±10.9	9.3±11.7
Cumulative grass species cover (%)	46.5±18.7	42.1±18.6	87.4±28.5
CWM plant traits			
Specific leaf area, SLA (mm^2 mg^{-1})	23.2±3.2	23.7±1.8	23.8±2.4
Leaf dry matter content, LDMC (g g^{-1})	0.26±0.03	0.25±0.02	0.26±0.02
Leaf N (mg g^{-1})	24.2±4.0	25.7±2.5	25.3±2.7
Leaf P (mg g^{-1})	2.3±0.5	2.4±0.3	2.4±0.4
Fine root diameter, fineRD (mm)	0.19±0.01	0.20±0.02	0.18±0.01
Root tissue density, RTD (mg cm^{-3})	53.1±12.7	51.1±9.7	50.2±11.3
Specific root length, SRL (m g^{-1})	436±48	414±43	452±63
Microbial properties			
Biomass carbon (µg C g^{-1})	816±152	729±159	583±353
Biomass nitrogen (µg N g^{-1})	137±39	140±41	136±97
Soil properties (at 0-10 cm depth)			
Bulk density (g cm^{-3})	0.76±0.11	0.79±0.11	0.85±0.4
Clay (g kg^{-1})	536.1±134.0	422.6±130.3	173.8±87.0
Silt (g kg^{-1})	406.6±114.6	520.1±122.7	372.1±191.7
Sand (g kg^{-1})	57.4±45.0	57.6±23.1	454.2±221.0
pH	6.3±0.6	6.9±0.5	6.5±0.9
Cation exch. capacity (cmol kg^{-1})	23.0±8.54	21.9±9.67	21.0±17.4
Aluminum (oxalate extraction, g kg^{-1})	0.56±0.73	0.36±0.22	0.17±0.21
Soil organic C (g C kg^{-1})	69.8±14.3	49.9±12.1	95.5±90.7
Soil N (g N kg^{-1})	6.8±1.5	4.9±1.2	10.4±9.0
Total (Olsen) P (mg P kg^{-1})	33.2±20.9	19.5±10.1	45.3±19.5
Inorg. (Olsen) P (mg P kg^{-1})	19.3±16.6	11.7±8.1	29.8±17.1
Total S (g S kg^{-1})	0.9±0.2	0.6±0.1	1.4±1.3
Depth (cm)	41.5±21.6	60.2±18.3	42.8±50.1

4.2.4 Plant properties

Vegetation information (plant species richness, aboveground plant biomass, aboveground plant C:N ratio and fine root biomass) was collected in May 2018 in fenced subplots to prevent livestock grazing or mowing prior harvest. A detailed description of the methods is given in *Chapter 3.2.4*.

Plant traits can be used to categorize plant species and communities as fast-growing, and exploitative or slow-growing and conservative (Bruelheide et al., 2018; Reich 2014; Diaz et al 2016). Exploitative plant communities have higher specific leaf area and leaf N (and P) and lower leaf dry matter content than conservative communities (Diaz et al., 2016; Wright et al., 2004). Regarding belowground traits, exploitative communities have higher specific root length and lower root tissue density and fine root diameter than the conservative communities (Bergmann et al., 2020). Plant, and especially root, traits are thus broadly indicative of nutrient acquisition strategies of plant species and these are relevant for nutrient cycling in soils (de Vries and Bardgett, 2016). For instance, a high specific leaf area indicates high photosynthetic capacity, which relates to fast growth rates, short tissue lifespan and high decomposability, and high needs for nutrients (de Vries and Bardgett, 2016). Moreover, a high specific root length indicates a better screening of soil for nutrients, and a low fine root diameter indicates high nutrient uptake efficiency (Bardgett et al., 2014; Freschet et al., 2021). In managed grasslands, plant community traits are influenced by management-induced shifts in plant community composition (Allan et al., 2015; Busch et al., 2018) and might explain the effects of vegetation change on nutrient leaching.

We used plant species identity, and their abundances in our grasslands, to calculate the Community Weighted Mean (CWM) traits, for four above- (specific leaf area, SLA; the leaf dry matter content, LDMC; leaf N and P) and three belowground (fine root diameter, fineRD; root tissue density, RTD; specific root length, SRL) traits (Table 4.1). Aboveground traits were extracted from the TRY database (Kattge et al., 2020), and belowground traits were measured in a greenhouse experiment on plant species from the Biodiversity Exploratories grasslands (Lachaise et al., 2021). First, we matched the species identities found in our grassland sites to the TRY database (including synonyms) and excluded i) duplicates, ii) non-mature plants, iii) non-healthy or dead plants, iv) measurement contributions from experimental manipulations, and v) measurement contributions where mean data were not reported. Then, for each species-trait combination, we averaged the trait value for each contributing dataset and excluded outlying datasets. We calculated average trait values for each species over all contributing datasets, weighted by the number of replicates in each dataset. Finally, the community weighted mean was calculated as the average trait values of all species, weighted by their relative abundance.

In our grasslands, the cover of legume species was negatively correlated with the CWM leaf dry matter content and specific root length, and, to some extent, it was positively correlated with leaf N and fine root diameter (Figure 4.1). Moreover, the cover of grass species was negatively correlated with fine root diameter and root tissue density, and positively correlated with specific root length. Specific leaf area was not related to the plant functional group of legumes or grasses. Grazing intensity was negatively correlated with specific root length and leaf dry matter content, and was positively related to leaf N (and P). Fertilization was positively correlated with specific leaf area, and, to some extent, negatively related to leaf dry matter content and root tissue density.

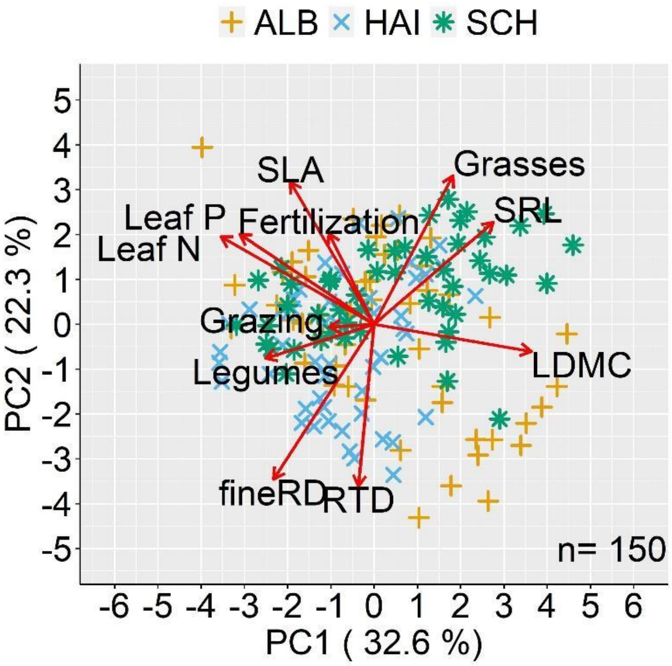


Figure 4.1 Principal component analysis among seven plant (community weighted mean) traits, two plant functional groups (i.e. cumulative cover of Legumes and Grasses) and management intensities (Grazing and Fertilization) for the three study regions; Schwäbische-Alb (ALB), Hainich-Dün (HAI) and Schorfheide-Chorin (SCH). Abbreviations: SLA =specific leaf area, LDMC =leaf dry matter content, fineRD =fine root diameter, RTD =root tissue density and SRL =specific root length.

4.2.5 Annual nutrient leaching

Annual, downward fluxes of nitrate, ammonium, phosphate and sulphate with the percolating water, i.e. leaching, were measured with the Self-Integrating Accumulators (SIAs) provided by the TerrAquat GmbH (Nürtingen, Germany, www.terraquat-gmbh.com; Bischoff, 2009). SIAs consisted of a PVC cylindrical cartridge (diameter and height: 10 cm) with a net at the lower end and were filled with a mixture of anion and cation exchange resin and fine silica sand. SIAs were installed below the upper 10 cm of soil, and we measured the leaching from the upper 10 cm of soil for three reasons. Firstly, we wanted our leaching data to match the soil and microbial properties data, which were also measured at the upper 10 cm of the soil (Table 4.1). Secondly, we wanted to follow a standardized methodology in every grassland, because the total soil depth varied among and within the three study regions (Table 4.1), and it was often ≤20 cm deep (in 58 out of the 150 grasslands). Finally, we were interested in biotic drivers on nutrient leaching, which are probably easier identified below the biologically most active topsoil layer than in the deeper subsoil, where geochemical drivers might become more important. Since we measured nutrient leaching from the upper 10 cm of soil, and not below the rooting zone, we cannot conclude on nutrient losses from the ecosystem, but our data indicate their loss risk.

We installed four SIAs in each of the 150 grasslands (total of 600 SIAs) in spring 2018, a year in which a summer drought occurred in central Europe (Figure S2.4 and S3.3). We selected four 1 m × 1 m subplots at a 2 m extension of the cardinal transects used for soil sampling. In each subplot, we opened a 40 cm × 40 cm pit down to 30 cm, and we selected the wall that was against the slope. On this wall, we opened a lateral cubic tunnel (side of 15 cm) at a depth of 10 to 25 cm, so that the percolating water would reach the SIAs from an undisturbed area. SIAs were saturated with deionized water from below and placed in the lateral tunnels. The empty space around the SIAs was filled with silica sand, and the pit was re-filled with the removed soil.

SIAs stayed in the soil for one year, until spring 2019. For the installation and removal, we visited the study regions in the same order. Installation and removal dates were recorded together with field observations (e.g. presence of roots and soil fauna) for a quality control of the samples. After removal, SIAs were stored at 4°C, and each SIA was separated to three resin samples by separating the resin column into three resin layers. The first resin layer accounted for nutrient leaching, and had a depth of 5 cm. The second resin layer was a control, and the third layer served as a buffer for upward nutrient movement, and they had a length of 1 and 4 cm, respectively. Each resin sample was mixed to ensure homogeneity, weighed, and stored at 4°C. To calculate leaching, we analysed the first two layers for their nutrient concentrations. If the second layer was contaminated with NO_3 (>10% of first layer NO_3-N concentration), then the SIA was excluded from our dataset. Out of the 600 SIAs, five SIAs were excluded due to disturbances by soil fauna, and 34 SIAs due to a contaminated second layer.

Resin samples of 15 g were weighed. After adding 100 mL of 1 M NaCl (Natriumchlorid zur Analyse Acs, Merck KGaA, Darmstadt, Germany) the resin was shaken for 45 minutes. The extracted solution was filtered by gravity using N-free filters (MN 619 DE 1/4, Macherey-Nagel Gmbh & Co, Germany), and the filtrate was transferred to a 15-mL falcon tube, which was stored at -20°C. Ammonium (NH_4-N), nitrite (NO_2-N) and nitrite and nitrate (NO_2-N + NO_3-N) and phosphate (PO_4-P) concentrations in the solution were determined by a flow-injection analyzer (Quickchem QC85S5, Lachat Instruments, Hach Company, Dreieich, Germany) after applying appropriate dilutions. Nitrate concentration was calculated as the difference of [NO_2-N + NO_3-N] from NO_2-N concentration. Sulphate (SO_4-S) concentration was determined by an ion chromatography system (Dionex-DX 500, Thermo Fisher Scientific GmbH, Dreieich, Germany), after applying appropriate dilutions. Nutrient concentrations of the first and second layer were used to calculate leaching fluxes.

4.2.6 Data analysis

We tested for differences of annual nutrient leaching in the three study regions using Analysis of Variance (ANOVA) models and the Tukey's (Honest Significant Difference) test. We use a backward stepwise function to assess the impact of i) study regions, ii) plant properties (aboveground biomass, species richness, legume cover and grass cover), iii) soil OC, iv) microbial biomass C and v) grassland management (fertilization and grazing intensities) on nutrient leaching based on the Akaike Information Criterion. Using the variables selected by the stepwise function, we modelled nutrient leaching with Analysis of Covariance (ANCOVA). ANCOVA models were performed using a type II sum of squares, which is not influenced by the order in which the explanatory variables are introduced in the model (Zuur et al., 2009). For every model, we used diagnostic plots to test the assumptions of linearity, normality and homoscedasticity of variance in the residuals, and to check for outliers. Heteroscedasticity issues were tackled with logarithmic or square-root transformations. To assess collinearities in the explanatory dataset, we checked the variance inflation factor, and values <3.0 were accepted. We followed the same approach to (separately) investigate the importance of several physico-chemical soil properties (Table S4.1) and CWM traits on nutrient leaching (Table S4.2) listed in Table 4.1.

We used Structural Equation Modeling (Shipley, 2016) to investigate how fertilization and grazing, and their impacts on soils, plants and soil microorganisms, affect nutrient leaching. Grazing and fertilization intensities were included as exogenous variables. Plant species richness, an important predictor of soil functions (Chen et al., 2019; Leimer et al., 2016) and sensitive to management intensification (Midolo et al., 2018), was included as an endogenous variable. Substrate stoichiometry controls the cycling of carbon and nutrients in soils (Manzoni et al., 2008; Schleuss et al., 2021), and so we used stoichiometric ratios (C:N, C:P or C:S) of soils, plants and soil microbial biomass as endogenous variables in our models. We removed the effects of study regions (with one-way ANOVA models) from all endogenous variables, and we used their residuals in our Structural Equation Models. Proposed causal structures with p-values >0.05 were accepted. In addition, we calculated the root mean square errors of approximation (RMSEAs) and the standardized root mean square residuals (SRMRs), which both should be <0.05. To tackle multivariate non-normality issues, we re-ran our models using bootstrapping, and monitored changes in the p-value of the causal structure. The p-values of bootstrapping models (not shown) were always similar to those without bootstrapping. The high number of observations compared with the number of estimated parameters in our models provided additional safety against multivariate non-normality issues. We followed the same approach to (separately) investigate the importance element pools (C, N, P or S, Figure S4.1) and plant CWM traits (selected based on Table S4.2) on nutrient leaching.

Statistical analysis was performed with the R statistical software (R Core Team, 2019). Figures were designed with the *ggplot2* package. ANOVA and ANCOVA models were performed with the *lm* function. For the backward stepwise elimination process the *step* function from the *stats* package was used. The variance inflation factor was calculated with the *vif* function of the *faraway* package. Tukey's test and PCA were performed with the *TukeyHSD* and the *prcomp* functions, respectively, of the *stats* package. The Structural Equation Modeling was performed with the *sem* function from the *lavaan* package (Rosseel, 2012).

4.3 Results

4.3.1 Annual nutrient leaching from the topsoil in grassland

The median nitrate (annual) leaching flux at a depth of 10 cm was 7.4 kg NO_3-N ha^{-1} (interquartile range: 2.2-22.2 kg NO_3-N ha^{-1}) over the three study regions and increased in the order ALB<SCH=HAI (Figure 4.2a). The leaching of the less mobile ammonium was 4.4 kg NH_4-N ha^{-1} (interquartile range: 3.8-6.0 kg NH_4-N ha^{-1}) and increased in the order HAI<ALB=SCH (Figure 4.2b). The cumulative inorganic N (NH_4-N+NO_3-N) leaching followed the patterns of nitrate leaching and increased in the order ALB<SCH=HAI, underlining the importance of nitrate for total N losses. The median phosphate leaching flux was 0.8 kg PO_4-P ha^{-1} (interquartile range: 0.6-1.7 kg PO_4-P ha^{-1}) over the three study regions

and there were no detectable differences among the three study regions (Figure 4.2c). Lastly, the median sulphate leaching flux was 21.8 kg SO_4-S ha^{-1} (interquartile range: 13.7-29.5 kg SO_4-S ha^{-1}) and leaching fluxes in SCH were significantly higher than ALB (Figure 4.2d), but not than HAI. Nutrient leaching fluxes differed among the three study regions and each nutrient followed a different leaching pattern. The three study sites differ in atmospheric N inputs, which can be relevant for inorganic N leaching since, for instance, the annual bulk deposition of total N in SCH (11.8±1.7 kg N ha^{-1}, Schwarz et al., 2014) is nearly four times higher than the annual fertilization inputs (Table 4.1). Nutrient leaching patterns differed from the patterns of soil nutrient concentrations for nitrate, phosphate and sulphate, while ammonium leaching differences among the study regions matched those of soil N concentrations (Table 4.1).

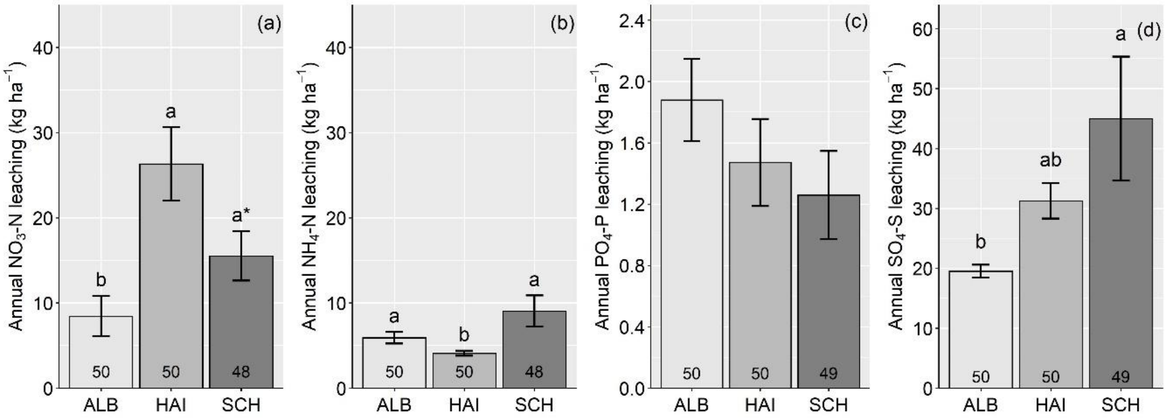


Figure 4.2 Annual leaching in a depth of 10 cm of (a) nitrate (NO_3-N), (b) ammonium (NH_4-N), (c) phosphate (PO_4-P) and (d) sulphate (SO_4-S) for the three study regions; Schwäbische-Alb (ALB), Hainich-Dün (HAI) and Schorfheide-Chorin (SCH). Bars show average values and error bars represent standard deviations. Numbers at the base of the bars show the number of observations. Lower case letters indicate significant (p-value <0.05), and asterisks indicate marginal (0.10< p-value ≤0.05) differences among the study regions based on ANOVA and Tukey's HSD models.

4.3.2 Management effects on nutrient leaching

Including management intensities and selected soil, plant and microbial properties in our ANCOVA models for nitrate, ammonium and phosphate annual leaching (Table 4.2) considerably increased the explained variance compared to models including only soil properties (Table S4.1). Our models explained 39, 27, 23 and 38% of the variance in nitrate, ammonium, phosphate and sulphate leaching, respectively (Table 4.2). Study regions were important predictors of leaching for every nutrient. Fertilization intensity increased nitrate, ammonium and phosphate annual leaching, while grazing had an increasing effect only on nitrate and phosphate leaching. Management was not important for sulphate leaching. Plant aboveground biomass was positively related to ammonium and phosphate leaching, but it had a negative effect on sulphate leaching. Plant species richness was negatively associated with nitrate leaching, and it was not important for the other nutrients. The relative cover of legumes was marginally (and positively) related to nitrate leaching, and that of grasses was negatively related to nitrate leaching. Soil OC was positively related to ammonium leaching. Finally, microbial biomass C was negatively associated with ammonium and phosphate leaching, but it was positively associated with sulphate leaching.

4.3.3 Management and stoichiometry effects on nutrient leaching

According to our Structural Equation Models, fertilization intensity had (direct) positive effects on nitrate, ammonium and phosphate leaching risk, but not on sulphate, and these effects were not explained by plant species richness or soil, plant and microbial stoichiometry (Figure 4.3). Similarly, grazing intensity had direct positive effects on nitrate and phosphate leaching, but not on ammonium or sulphate. Plant species richness was directly associated with ammonium and sulphate leaching, and these relationships were negative and positive, respectively. In contrast to our ANCOVA model (Table 4.2), plant species richness was not directly related to nitrate leaching (Figure 4.3a).

Interestingly, soil, plant and microbial C:N ratios were not associated with fertilization intensity directly, but rather indirectly through plant species losses caused by fertilization. In contrast, grazing intensity was not related to C:N ratios directly or indirectly. A higher plant species richness was associated with higher C:N ratios of soils, plants and microbial biomass. Both plant and microbial C:N ratios had a negative relationship with nitrate leaching, while soil and plant C:N ratios had a positive relationship with ammonium leaching. In a similar model with N pools instead of C:N ratios, microbial and plant N were positively linked to nitrate leaching, and plant N was negatively linked to ammonium (Figure S4.1a, b).

Grazing and fertilization intensities were negatively associated with C:P ratios of plants and soils and with microbial biomass C (no microbial P data available) (Figure 4.3c). Apart from management, soil C:P ratio was the only parameter related to phosphate leaching, and this relationship was negative. In a similar model with P pools instead of C:P ratios, soil inorganic P was positively related to phosphate leaching, while plant P and microbial C were not important (Figure S4.1c).

Table 4.2 ANCOVA models of nutrient annual leaching with management intensities and plant, microbial and soil properties. Full models were stepwise reduced. Study region effects are given relative to the Schwäbische-Alb region. Significant predictors are given in bold. Abbreviation: HAI =Hainich-Dün; SCH =Schorfheide-Chorin; Microbial C =Microbial carbon concentration; SOC =Soil organic carbon concentration.

Annual leaching	**NO$_3$-N**		**NH$_4$-N**	
	df=138	Model p	df=135	Model p
	R^2=0.39	<0.001	R^2=0.27	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	**3.51**	**<0.001**	**2.96**	**0.004**
Region HAI	**6.64**	**<0.001**	**-3.14**	**0.002**
Region SCH	**3.51**	**<0.001**	-1.11	0.270
Fertilization	**2.54**	**0.012**	**2.74**	**0.007**
Grazing	**2.21**	**0.029**	-	-
Plant abov. biomass	-	-	**3.19**	**0.002**
Plant species richness	**-2.89**	**0.004**	-	-
Legume cover	1.83	0.069	-	-
Grass cover	**-3.55**	**<0.001**	-	-
Microbial C	1.38	0.170	**-2.49**	**0.014**
Soil OC conc.	1.50	0.137	**1.99**	**0.048**
Annual leaching	**PO$_4$-P**		**SO$_4$-S**	
	df=142	Model p	df=142	Model p
	R^2=0.23	<0.001	R^2=0.38	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	0.89	0.374	**6.29**	**<0.001**
Region HAI	-1.30	0.195	**5.16**	**<0.001**
Region SCH	**-3.60**	**<0.001**	**3.46**	**<0.001**
Fertilization	**2.31**	**0.022**	-	-
Grazing	**2.57**	**0.011**	-	-
Plant abov. biomass	**3.06**	**0.003**	**-2.29**	**0.024**
Plant species richness	-	-	-	-
Legume cover	-	-	-1.63	0.105
Grass cover	-	-	1.69	0.094
Microbial C	**-2.20**	**0.030**	**6.46**	**<0.001**
Soil OC conc.	-	-	-	-

For S, no information on plants or microbes was available. Microbial C and plant species richness were (directly) positively related to sulphate leaching (Figure 4.3d). However, plant species richness was negatively linked to microbial C, and therefore its effect on sulphate leaching was offset. Soil C:S ratio was directly associated with fertilization or grazing intensities, and it was only marginally associated with sulphate leaching. In a similar model with soil S concentration instead of C:S ratio, soil S was positively related to sulphate leaching, and there were no other effects from management, plants or microbial biomass on sulphate leaching (Figure S4.1d). The total (net direct and indirect) effects of management on sulphate leaching were negative and rather small (Table S4.3).

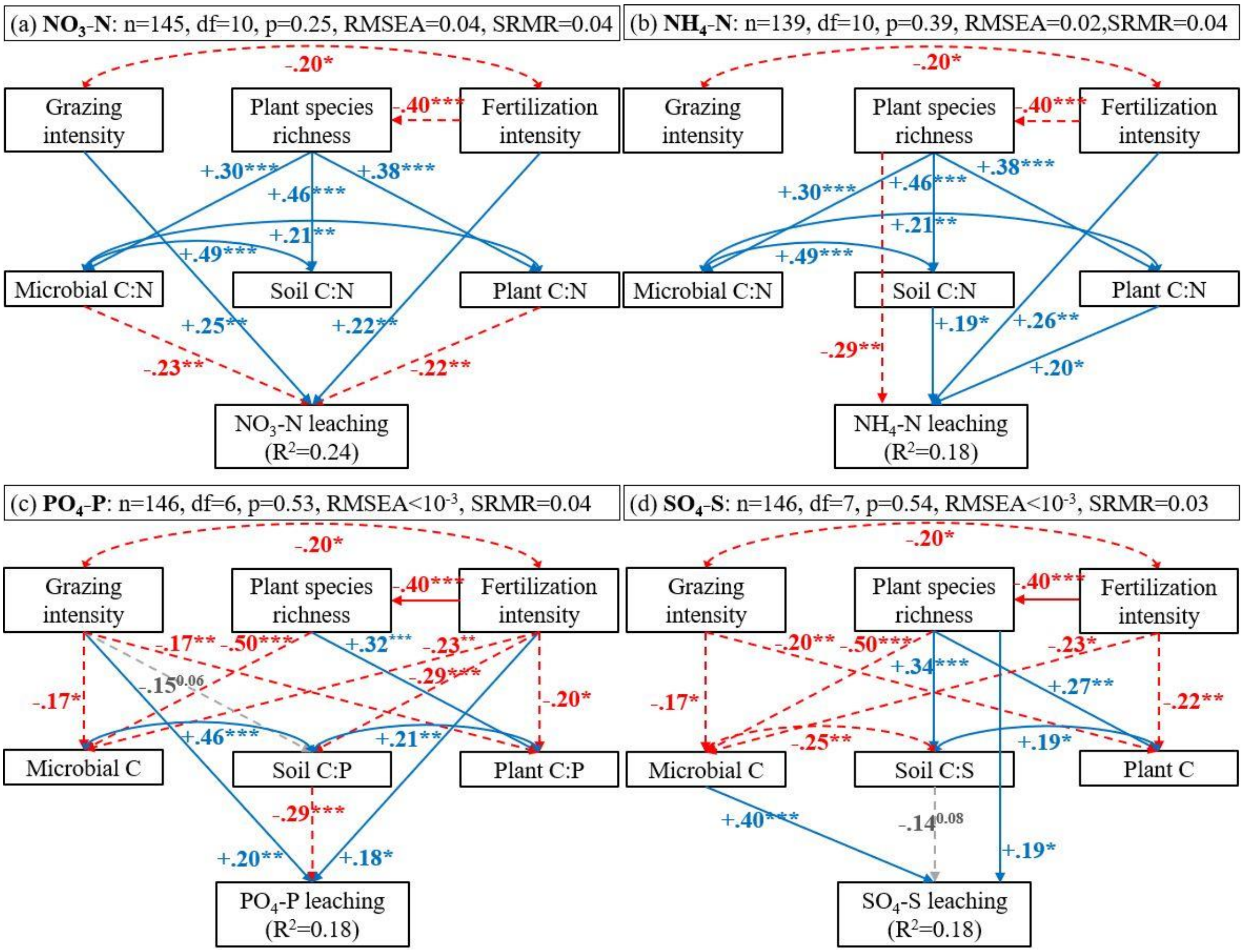


Figure 4.3 Structural Equation Models for (a) nitrate, (b) ammonium, (c) phosphate and (d) sulphate annual leaching explained by fertilization and grazing intensities, plant species richness and stoichiometry of soils, plants and microbial biomass. Endogenous variables are included as residuals after removing the effect of study regions. Single-headed arrows indicate direct causal paths and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Grey arrows indicate paths of marginal significance. Standardized path coefficients are given together with their significance level (*p <0.050; **p <0.010; ***p <0.001). Coefficients of determination are given for the response variables.

4.3.4 Effects of plant community trait on nutrient leaching

Based on backwards stepwise analyses and ANCOVA models, we identified CWM plant traits that were important for nutrient leaching (Table S4.2). Each nutrient had a different set of important CWM plant traits, while for sulphate leaching none of the traits was important. Our Structural Equation Models show that fertilization and grazing intensities exhibited multiple indirect effects on nutrient leaching via CWM plant traits, in addition to their direct effects (Figure 4.4). Interestingly, while plant species richness was only affected by fertilization, CWM plant traits were, typically, influenced by both grazing and fertilization, though grazing acted more strongly on aboveground traits (with the exception of specific root length) than fertilization. Fertilization and grazing intensification seem to have shifted plant communities towards more resource acquisitive, fast growing species with higher leaf N and P, and lower leaf dry matter content and root tissue density (Figure 4.1). However, grazing was also negatively associated with specific root length.

Fine root diameter directly increased and leaf dry matter content directly decreased nitrate leaching (Figure 4.4a). Microbial N mediated positive indirect effects from fine root diameter and leaf dry matter content on nitrate leaching. Interestingly, plant species richness directly decreased nitrate leaching, even after including selected CWM plant traits and microbial N in our model. In contrast, plant and microbial C:N ratios (from field measurements) fully mediated the effects of plant species richness on nitrate leaching, but, in that model too, the total effect was negative (Figure 4.3a).

Root tissue density was marginally (and positively) linked to ammonium leaching, and leaf dry matter content was marginally (and negatively) linked to it (Figure 4.4b, Table S4.2). Plant species richness was again negatively associated with ammonium leaching. Leaf P increased phosphate leaching, and it mediated indirect positive effects from both fertilization and grazing intensities on phosphate leaching (Figure 4.4c). Specific root length was positively linked to sulphate leaching, and it mediated a indirect negative effect from grazing intensity on sulphate leaching (Figure 4.4d). Finally, leaf N marginally (and negatively) related to sulphate leaching, and it mediated indirect negative effects by both fertilization and grazing intensities on sulphate leaching.

4.4 Discussion

We measured the annual leaching of nitrate, ammonium, phosphate and sulphate from the upper 10 cm of soil in 150 temperate managed grasslands to assess effects of management, as well as interactive effects of management intensity with soil, plant and microbial properties. We show that all factors involved, i.e. management, soils, plants (species richness and functional composition) and soil microorganisms, are closely interlinked and jointly affect nutrient leaching.

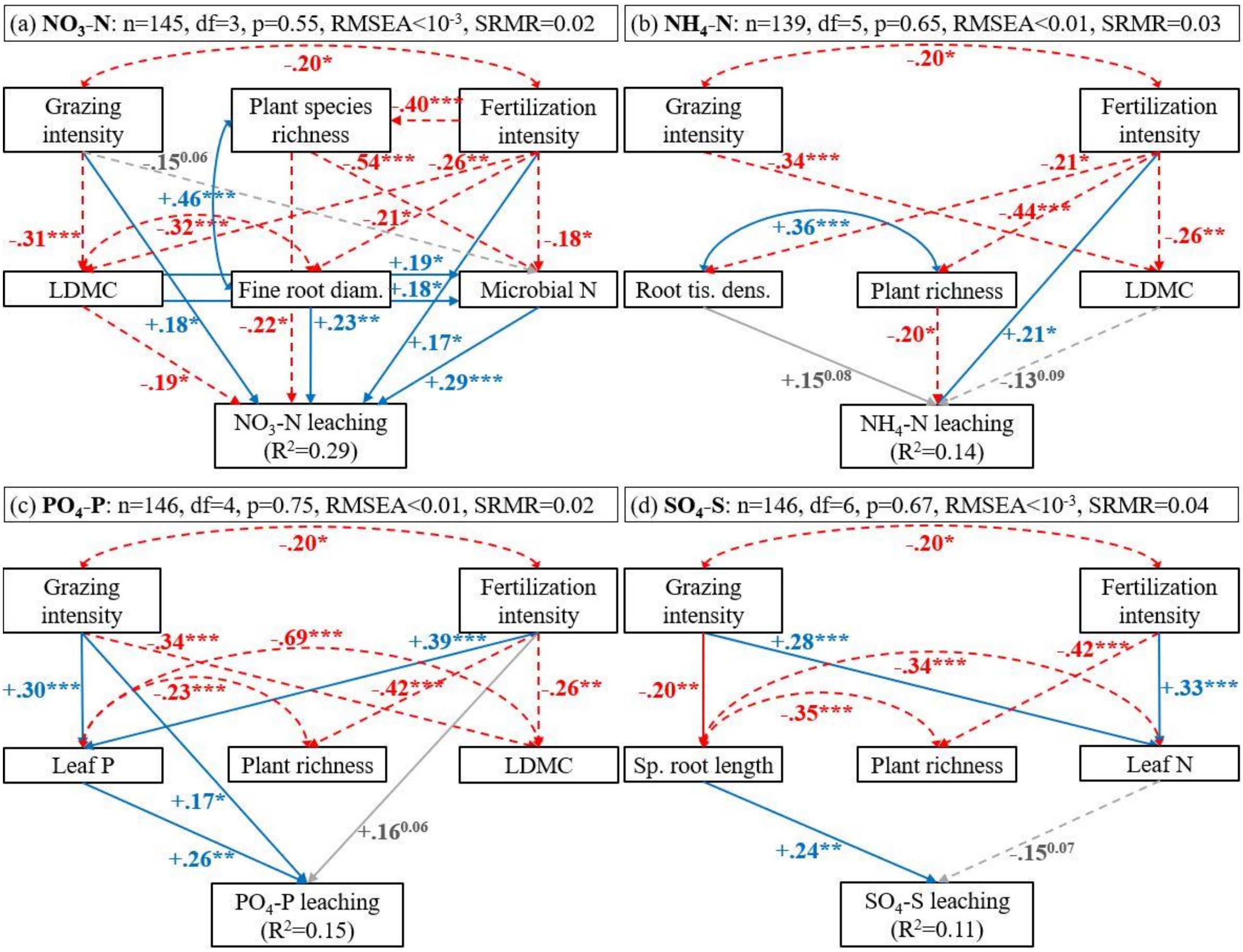


Figure 4.4 Structural Equation Models for (a) nitrate, (b) ammonium, (c) phosphate and (d) sulphate annual leaching explained by fertilization and grazing intensities, plant species richness and selected CWM plant traits (LDMC: leaf dry matter content; Fine root diam.: fine root diameter; Root tis. dens.: root tissue density; Leaf P or N: leaf phosphorus or nitrogen; Specific root length). Endogenous variables are included as residuals after removing the effect of study regions. Single-headed arrows indicate direct causal paths and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Grey arrows indicate paths of marginal significance. Standardized path coefficients are given together with their significance level (*$p < 0.050$; **$p < 0.010$; ***$p < 0.001$). Coefficients of determination are given for the response variables.

4.4.1 Management intensification directly increases nutrient leaching risk

Previous studies stressed the importance of grazing, and the associated dung and urine patches, for nitrate leaching (Ledgar et al., 2009), and they associated (grazed) pastures with higher nitrate leaching risk than (mown) meadows (Cameron et al., 2013; Ryden et al., 1984). In this study, nitrate leaching risk significantly increased with both fertilization and grazing intensity (Table 4.2, Figure 4.3). We observed that fertilized grasslands also have an elevated nitrate leaching risk, and we extended previous findings from mostly short-term observations (Klaus et al., 2018) to an annual timescale. In addition, ammonium annual leaching risk increased with fertilization intensity, but not with grazing (Table 4.2). This is possibly because grazing increases N inputs as urea, which is quickly oxidized to nitrate (in various soil types, Soulides and Clark, 1958; Tzanakakis et al., 2018), especially since management intensification increases nitrification rates (Stempfhuber et al., 2014). Considering both nitrate and ammonium, we conclude that fertilization intensity is more important than grazing for total inorganic N leaching across an annual timescale.

Agricultural activities are responsible for excess P losses via leaching, which contribute to the degradation of (surface) aquatic ecosystems due to eutrophication (SOER, 2020). We found that managed grasslands can also have a high P leaching risk, since annual phosphate leaching at 10 cm soil depth were frequently greater than 0.8 kg PO_4-P ha^{-1} (overall median) (Figure 4.2). Such fluxes are higher than leaching losses from the subsoil of other agricultural land-uses, like croplands (Leinweber et al., 1999). However, P leached from the topsoil, as measured in our study, can still be fixed in the subsoil, or be taken up by plants (Kuhlmann and Baumgärtel, 1991), and so it may not reach aquatic ecosystems. Therefore, our study can only identify an increased nutrient loss risk due to management intensification. However, the P sorption capacity in subsoils depends on soil texture and pH, and it could, at some point, be exceeded with long term excess P supply (Gocke et al., 2021; Werner et al., 2006), especially in sandy soils, such as those in SCH (Table 4.1). Overall, we found that phosphate leaching significantly increased with management intensity (Table 4.2), and that fertilization and grazing equally increased phosphate loss risk.

Compared to N and P, little is known for S cycling in temperate managed grasslands. Due to ample atmospheric S depositions in the past, temperate soils are typically considered to be S-rich. However, concerns that S might be a limiting resource for plant production and quality have been raised (Eriksen, 2009; Mathot et al., 2008). In this study, sulphate was the only nutrient for which management intensity reduced its leaching, and this operated indirectly (Figure S4.1d). Both grazing and fertilization reduced soil S concentrations, and, thus, sulphate leaching. A possible explanation is that management intensification increases plant productivity, which creates higher S demands and plant uptake (Eriksen, 2009), eventually leading to increased export of S from the ecosystem with harvest or grazing. This is supported by a negative relationship between aboveground plant biomass and sulphate leaching in our grasslands (Table 4.2). Another explanation for management-dependent reductions in soil S is the desorption of sulphate from soil minerals due to phosphate additions, since phosphate is adsorbed more strongly than sulphate (Bolan et al., 1988). However, this process should have led to increased sulphate leaching risk with increased fertilization, which we did not observe. The lack of information on P and S fertilization rates might obscure additional effects from fertilization on sulphate leaching.

4.4.2 Management changes in soil, plant and microbial stoichiometry and their effects on nutrient leaching risk

Soil and plant stoichiometry were indirectly affected by fertilization, since their C:N ratios were negatively associated with fertilization-induced reductions in plant species richness (Figure 4.3a). In addition, both grazing and fertilization had a direct negative relationship with soil N, while fertilization was indirectly associated with soil N and plant N in a positive way, via plant species richness (Figure S4.1a). This suggests that fertilizer N might be first taken up by plants and afterwards returned to the soil as litter, controlling thus soils nutrient pools and stoichiometry.

The positive effect of plant N on nitrate leaching (Figure S4.1a) indicates faster mineralization of N-rich litter under high management intensity. Meanwhile the negative effect of plant N on ammonium leaching (Figure S4.1b) could be similarly explained by higher nitrification rates in intensively managed grasslands (Stempfhuber et al., 2014), or, alternatively, by a preference for ammonium uptake by plants. In contrast to plant N, plant biomass production, which increases with management intensification (Klaus et al., 2018), was positively related to ammonium leaching (Table 4.2). This could be explained by increased above- and belowground litter inputs, and thus increased soil organic N (Leimer et al., 2016), which are then mineralized to ammonium.

In contrast to ammonium, nitrate leaching risk was also controlled by microbial properties. Microbial nitrification, following soil organic N mineralization, could explain the positive relationship between microbial biomass N and nitrate leaching. Management intensity, and especially fertilization, was shown to increase the nitrification potential of the studied soils (Meyer et al. 2013; Stempfhuber et al., 2014). Therefore, fast nitrification of ammonium at fertilized sites with low C:N ratios could be the reason for the observed reduction of ammonium leaching with increasing microbial biomass C (Table 4.2), and for the observed increase of ammonium leaching risk with increasing soil C:N ratio (Figure 4.3b).

Interestingly, the microbial C:N ratio was negatively linked with nitrate leaching risk (Figure 4.3a), indicating that leaching was not only affected by changes in plant communities, but also microbial communities (Figure S4.2). This may be because the less nutrient-demanding, more fungal-dominated communities at extensively managed, botanically diverse grasslands are capturing N more effectively, and prevent leaching (de Vries et al., 2006). Alternatively, it may be that the turnover and decomposition of fungal-derived, N-poor necromass is low. In contrast to the latter, evidence from short-term observations suggests that microbial communities that are not N-limited, increase N retention in the plant-soil system (de Vries and Bardgett, 2016). To our knowledge, our work is among the few studies showing such a clear relationship between microbial biomass and annual nitrate leaching in managed grasslands under field conditions.

Microbial biomass N had a positive effect on nitrate leaching risk (Figure S4.1a). In experimental grasslands, the negative effect of microbial biomass on the leaching of dissolved organic N has been attributed to enhanced mineralization (Leimer et al., 2016), which could explain higher soil nitrate concentrations and, thus, leaching. Previous work has shown that N additions can increase or decrease microbial biomass (Dietrich et al., 2017; Lu et al., 2015). Soil microbial communities are also influenced by plant communities, mainly through changes in N availability (Moreau et al., 2015), and perhaps to a greater extent than by management intensity in our study sites (Boeddinghaus et al., 2019). In our study, management intensity directly reduced microbial biomass N (and C), but the net effect was still close to neutral, because the fertilization-induced reduction in species richness increased it again (Figure S4.1a). This suggests that relations between long-term fertilizer additions and microbial biomass in managed grasslands are modified by plant communities and that the observed responses of microbial activity to short-term fertilizer additions to soils in the laboratory, where plant communities are absent, might not be representative of field conditions.

Phosphate leaching risk was mainly associated with soil inorganic P and C:P ratio, which were both positively related to plant P and C:P ratios (Figure 4.3c, S4.1c). Accordingly, fertilization-induced reductions in plant species richness increased phosphate leaching risk, due to an increasing effect on soil inorganic P. In contrast to N, phosphate leaching was negatively related to soil pH (Table S4.1), indicating high P immobilization as calcium phosphate in alkaline soils.

4.4.3 Effects of plant functional groups and community traits on nutrient leaching risk

We observed that the functional group of legumes was positively (but marginally) associated with annual nitrate leaching risk, while the group of grasses was negatively (and significantly) associated (Table 4.2). Neither of them was as important for ammonium, phosphate and sulphate leaching risk. A positive relationship between legumes and nitrate leaching can be explained by increased N availability caused by their N-fixing ability and N rich tissues (Leimer et al., 2015). Grasses decrease nitrate leaching due to increased N acquisition and/or more efficient use of N and water (de Vries and Bardgett, 2016; Leimer et al., 2016) and due to their, typically, lower tissue N content. Indeed, we observed that legume cover was positively correlated with CWM leaf N, and that grass cover was negatively correlated with CWM fine root diameter (Figure 4.1), which could, at least partly, explain the functional group effects on nutrient leaching.

Plant traits give hints on the importance of plant nutrient acquisition for nutrient leaching. In accordance with the observation that phosphate and sulphate leaching risk were more related to soil properties than inorganic N (Table S4.1), plant traits were less important for them, too (Table S4.2). We identified the CWM leaf dry matter content as an important predictor of inorganic N leaching (Table S4.2, Figure 4.4), reducing both, nitrate and ammonium leaching risk. This effect could be explained by a slower and more efficient N cycling in conservative than exploitative plant communities (Kleinebecker et al., 2014), and/or by lower N inputs in the soil system since leaf dry matter content was negatively related to i) fertilization, ii) legume cover and iii) CWM leaf N (Figure 4.1). The positive relationship between CWM fine root diameter and nitrate leaching risk could be explained by a reduced nutrient uptake efficiency with coarser roots. For ammonium, leaching risk was related to root tissue density (Figure 4.4b), where a higher root tissue density would indicate coarser fine roots (Figure 4.1) and a reduced nutrient uptake potential.

To date, it is not clear whether conservative, slow-growing plant communities, or exploitative, fast-growing plant communities lead to a greater N retention in the soil, plant and soil microorganisms' system. For instance, conservative plant communities can lead to decreased N retention due to low N acquisition rates (de Vries and Bardgett, 2016), and exploitative plant communities can lead to enhanced N cycling and high N availability, and eventually high leaching risk (de Vries et al., 2012; Grigulus et al., 2013; Robson et al., 2010). We observed a differentiation between the effects of above- and belowground traits on inorganic N leaching risk, similar to findings on N-related ecosystem functions by de Vries and Bardgett (2016). Conservative aboveground plant traits reduced inorganic N leaching risk, while conservative belowground traits increased it (Figure 4.4). Regarding our third hypothesis, we suggest that management intensity, and especially fertilization, shifted mostly the aboveground plant traits towards values more representative of exploitative communities (Figure 4.1; Allan et al., 2015; Busch et al., 2018; Zobel et al., 2007), which are characterized by a fast tissue turnover and decomposition (Lavorel et al., 2010), and, thus, influenced their association with annual N leaching risk.

4.4.4 Plant diversity reduces inorganic N leaching risk

Previous studies have reported a negative relationship between plant diversity and nitrate availability or leaching in managed and experimental grasslands (Leimer et al., 2016; Niklaus et al., 2001), but effects on ammonium leaching are less often described (Leimer et al 2015). Here, plant species richness was negatively associated both with nitrate and ammonium leaching risk, even after considering fertilization intensity (Table 4.2, Figure 4.3b). Interestingly, the negative effect of plant species richness on nitrate leaching was completely mediated by higher plant and microbial C:N ratios (and lower N) in more botanically diverse grasslands (Figure 4.3a, S4.1a), while aboveground plant biomass was not selected as a predictor for nitrate. In contrast to nitrate, the negative effect of plant species richness on ammonium leaching was not completely mediated by stoichiometry, element pools or CWM plant traits (Figure 4.3b, S4.1b, 4.4b). However, it could be explained by the negative relationship between plant (aboveground) productivity and species richness in managed grasslands (Klaus et al., 2018) since plant productivity was positively associated with ammonium leaching (Table 4.2). Alternatively, it could be explained by niche complementarity and ammonium uptake by plants in species-rich grasslands, as shown for plant species identity and functional groups (Kahmen et al., 2006). In addition, the negative relationship between plant species richness and plant N led to a positive indirect effect on ammonium leaching risk (Figure S4.1b). The negative relationship between plant species richness and plant N could be explained by fertilization that simultaneously causes plant biomass N enrichment and plant species losses, and/or by an increased plant N use efficiency with increasing plant diversity in grasslands (Dias et al., 2010; Fargione et al., 2007). To conclude, in terms of net effects, plant species richness was negatively associated both with nitrate and ammonium leaching risk, and the stoichiometry of soils, plants and soil microorganisms, as well as several CWM plant traits, mediated most of the effects.

4.4.5 Fertilization has a stronger impact on inorganic N leaching than grazing

In line with our hypotheses, management intensification shifted the stoichiometry of soils, plants and soil microorganisms, as well as plant communities' functional traits, in ways that increased the leaching risk of nitrate, ammonium and, to a lesser extent, phosphate, but not of sulphate. Plant species richness was significantly reduced by fertilization, and mediated most of the indirect effects of fertilization intensity on nutrient leaching, but it was not affected by grazing. Comparing net effects (Table S4.3), we found that fertilization had a stronger impact on inorganic N leaching risk compared to grazing, but not on phosphate or sulphate.

4.5 Conclusions

We identified that management intensity, and especially fertilization, not only promote nutrient leaching risk directly, but also indirectly by changing the properties of soils, plant and microbes. Most of the indirect effects of management on nutrient leaching were mediated by fertilization-induced reductions in plant species richness. While ceasing fertilization will reduce its direct effect on N and P leaching over a relatively short period in time, some of these changes, such as a loss in plant diversity and functional changes in plant communities, are not immediately reversible. Thus, management intensification changes the risk of nutrient leaching on the long-term and active (restoration) measures to maintain diverse plant communities are needed, which might, in return, mitigate the increased leaching risk that management intensification imposes on grasslands. Finally, we showed that, when considering both direct and indirect effects, fertilization leads to higher inorganic N leaching risk in the topsoil than grazing, while both practices increase phosphate leaching risk. As such, shifting grassland management towards (mowed) meadows instead of (grazed) pastures should not be considered as a management strategy for N leaching mitigation, as long as meadows are fertilized.

4.6 Supplementary material

Table S 4.1 ANCOVA models (sum of squares type II) of nitrate, ammonium, phosphate and sulphate annual leaching with soil properties. Full models were stepwise reduced. Study region effects are given relative to the Schwäbische-Alb region. Significant predictors are given in bold. Abbreviation: HAI =Hainich-Dün; SCH =Schorfheide-Chorin; OC =Organic Carbon; Al =aluminum.

Annual leaching	**NO_3-N**		**NH_4-N**	
	df=136	Model p	df=137	Model p
	R^2=0.24	<0.001	R^2=0.17	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	**4.36**	**<0.001**	**2.57**	**0.011**
Region HAI	**4.99**	**<0.001**	**-2.57**	**0.011**
Region SCH	1.66	0.099	0.54	0.593
Silt content	**-2.01**	**0.046**	-	-
Clay content	-	-	-	-
Bulk density	-	-	**2.49**	**0.014**
Soil pH	-	-	-	-
Cation Exch. Capacity	**3.22**	**0.002**	-	-
Soil OC conc.	-	-	1.45	0.149
Soil Al conc.	**-2.69**	**0.008**	-	-

Annual leaching	**PO_4-P**		**SO_4-S**	
	df=138	Model p	df=135	Model p
	R^2=0.12	<0.001	R^2=0.40	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	**3.54**	**<0.001**	**5.05**	**<0.001**
Region HAI	-	-	**3.94**	**<0.001**
Region SCH	-	-	**3.51**	**<0.001**
Silt content	-	-	1.96	0.052
Clay content	-	-	**2.21**	**0.029**
Bulk density	**2.29**	**0.023**	**-3.54**	**<0.001**
Soil pH	**-2.44**	**0.016**	-	-
Cation Exch. Capacity	-	-	-	-
Soil OC conc.	-	-	-1.49	0.140
Soil Al conc.	-1.24	0.219	-	-

Table S 4.2 ANCOVA models (sum of squares type II) of nitrate, ammonium, phosphate and sulphate annual leaching with plant species richness and seven plant (community weighted mean) traits. Full models were stepwise reduced. Traits that were never selected in the final models are not shown. Study region effects are given relative to the Schwäbische-Alb region. Significant predictors are given in bold. Abbreviation: HAI =Hainich-Dün; SCH =Schorfheide-Chorin; Leaf N =leaf nitrogen; Leaf P =leaf phosphorus.

Annual leaching	**NO_3-N**		**NH_4-N**	
	df=142	Model p	df=133	Model p
	R^2=0.31	<0.001	R^2=0.22	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	0.86	0.391	**6.39**	**<0.001**
Region HAI	**5.11**	**<0.001**	**-2.77**	**0.006**
Region SCH	**2.43**	**0.017**	-0.06	0.950
Plant species richness	**-4.19**	**<0.001**	**-3.12**	**0.002**
Leaf dry matter content	**-2.23**	**0.027**	**-1.99**	**0.049**
Leaf N	-	-	-	-
Fine root diameter	**2.60**	**0.010**	-	-
Root tissue density	-	-	1.58	0.117
Specific root length	-	-	-	-

Annual leaching	**PO_4-P**		**SO_4-S**	
	df=144	Model p	df=143	Model p
	R^2=0.17	<0.001	R^2=0.16	<0.001
Parameter	t-value	p-value	t-value	p-value
Intercept	1.40	0.164	1.49	0.139
Region HAI	-1.82	0.070	**2.99**	**0.003**
Region SCH	**-2.67**	**0.008**	**2.41**	**0.017**
Plant species richness	-	-	-	-
Leaf dry matter content	-1.44	0.152	-	-
Leaf P (or N)	**2.23**	**0.028**	-1.82	0.071
Fine root diameter	-	-	-	-
Root tissue density	-	-	-1.71	0.090
Specific root length	-	-	1.92	0.057

Table S 4.3 Net effect of fertilization intensity, grazing intensity and plant species richness on nitrate, ammonium, phosphate and sulphate annual leaching according to the Structural Equation Models with stoichiometric ratios (Figure 4.3) and CWM plant traits (Figure 4.4). Net effects and p-values were calculated with the lavaan package in R. Significant net effects are given in bold.

Structural Equation Models with	Grazing	Fertilization	Plant species richness
Stoichiometry			
Nitrate leaching	**+0.25 (p=0.001)**	**+0.28 (p<0.001)**	**-0.15 (p<0.001)**
Ammonium leaching	0	+0.**32 (p<0.001**)	-0.13 (p=0.133)
Phosphate leaching	**+0.25 (p=0.001)**	**+0.26 (p=0.001)**	0
Sulphate leaching	-0.05 (p=0.087)	-0.07 (p=0.158)	-0.04 (p=0.664)
CWM plant traits			
Nitrate leaching	**+0.18 (p=0.019)**	**+0.25 (p=0.002)**	**-0.38 (p<0.001)**
Ammonium leaching	+0.04 (p=0.117)	**+0.30 (p<0.001)**	**-0.20 (p=0.035)**
Phosphate leaching	**+0.26 (p=0.001)**	**+0.24 (p=0.002)**	0
Sulphate leaching	**-0.09 (p=0.008)**	-0.05 (p=0.091)	0

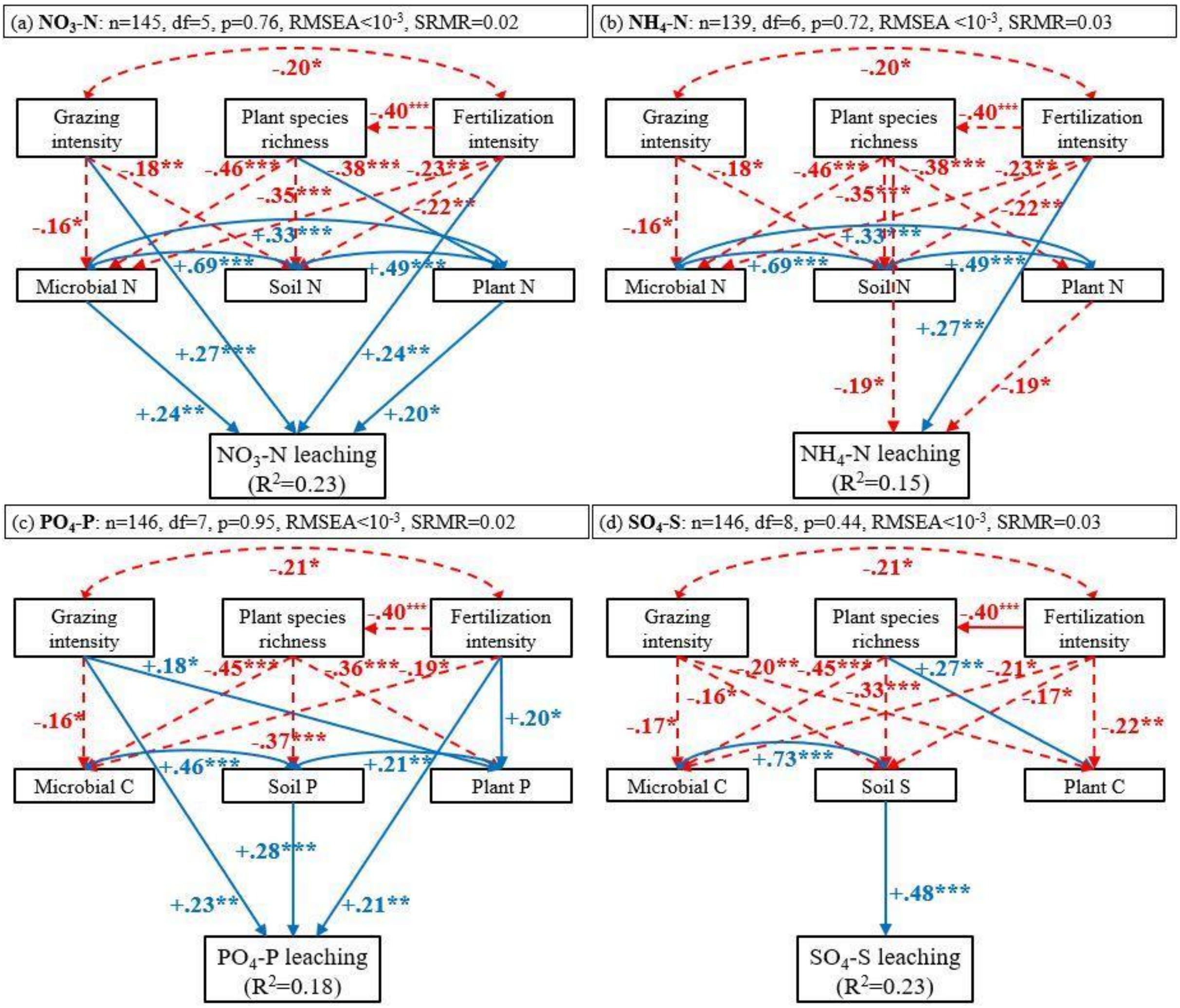


Figure S 4.1 Structural Equation Models for (a) nitrate, (b) ammonium, (c) phosphate and (d) sulphate annual leaching explained by fertilization and grazing intensities, plant species richness and C, N, P or S concentrations of soils, plants and microbial biomass. Endogenous variables are included as residuals after removing the effect of study regions. Single-headed arrows indicate direct causal paths and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Grey arrows indicate paths of marginal significance. Standardized path coefficients are given together with their significance level (*p <0.050; **p <0.010; ***p <0.001). Coefficients of determination are given for the response variables.

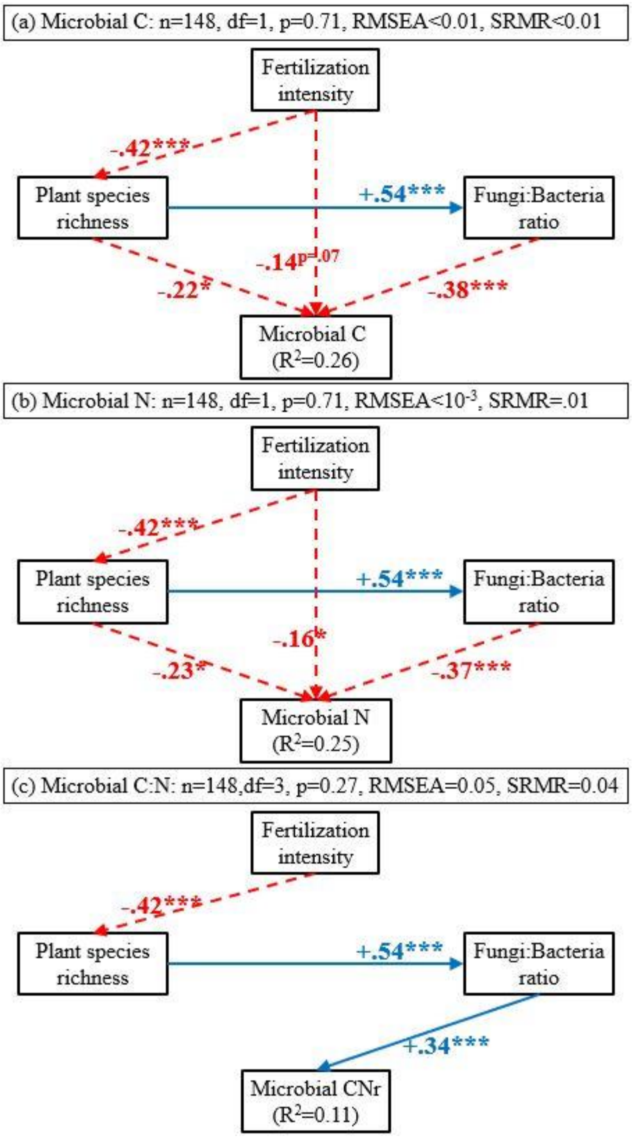


Figure S 4.2 Structural Equation Models for microbial biomass (a) carbon, (b) nitrogen and (c) C:N ratio explained by fertilization intensity, plant species richness and Fungi:Bacteria ratio. Endogenous variables are included as residuals after removing the effect of study regions. Single-headed arrows indicate direct causal paths and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects and dashed, red arrows indicate negative effects. Grey arrows indicate paths of marginal significance. Standardized path coefficients are given together with their significance level (*p <0.050; **p <0.010; ***p <0.001). Coefficients of determination are given for the response variables. Fungi:Bacteria ratio is calculated from Phospholipid-derived fatty acids data obtained by Boeddinghaus et al. (2019; https://doi.org/10.1111/1365-2745.13182).

5 General discussion and synthesis

5.1 In-situ soil respiration in temperate ecosystems

5.1.1 The role of land-use

Soil respiration varies between different land-use types, like forests and grasslands. For instance, early summer in-situ soil respiration ranges from 0.9 to 4.9 gCO_2-C m^{-2} d^{-1} in temperate forests (Borken and Beese, 2005; Borken et al., 2002; Berger et al., 2010; Kukumägi et al., 2017; Vesterdal et al., 2012; Søe and Buchmann, 2005), while values >5.0 gCO_2-C m^{-2} d^{-1} are frequent in temperate grasslands (Bahn et al., 2008; Burri et al., 2014; Koncz et al., 2015; Moinet et al., 2019). In line with the literature, in this study, in-situ soil respiration in grasslands was significantly higher than in forests for every study region and year, with the exception of HAI in 2018 where the two land-uses did not differ (Figure 5.1a).

Soil respiration differs between temperate forests and grasslands because different land-use types shape distinct environmental conditions in the ecosystems. Regarding the climatic conditions, grasslands had, on one hand, always higher soil temperature compared to forests (Figure 5.1b), which can result in a higher metabolic activity of soil organisms (Alvarez et al., 1995). However, on the other hand, grasslands had often lower soil water content compared to forests, as found in HAI and SCH (considering only mineral soils; Figure 5.1c), which strongly limits microbial activity (Moyano et at al., 2013). Differences between soil temperature and water content in forests and grasslands can possibly be explained by i) the (overstory and understory) canopy shading effect in forests compared to grasslands, ii) the biomass removal in grasslands that exposes soil surface to the sunlight, and iii) the ability of trees to acquire water from deeper soil.

Besides climatic conditions, soil properties can also contribute to differences in soil respiration rates between forests and grasslands. In the *Biodiversity Exploratories* project, grasslands have higher soil pH and organic carbon concentration in the mineral soil than forests and lower soil C:N ratio than forests (Table 2.2 and 3.2; Herold et al., 2014b; Solly et al., 2014), which can lead to higher microbial activity and heterotrophic respiration (Hobbie et al., 2007). Compared to forests, grasslands have higher decomposition rates of above- and belowground plant litter (Solly et al., 2014; Zhang et al., 2008). Similarly, in an incubation study, temperate grasslands exhibited higher mineralization rates of OC compared to forests, which was attributed to higher microbial biomass in the former (Gan, 2019). In addition, grasslands have

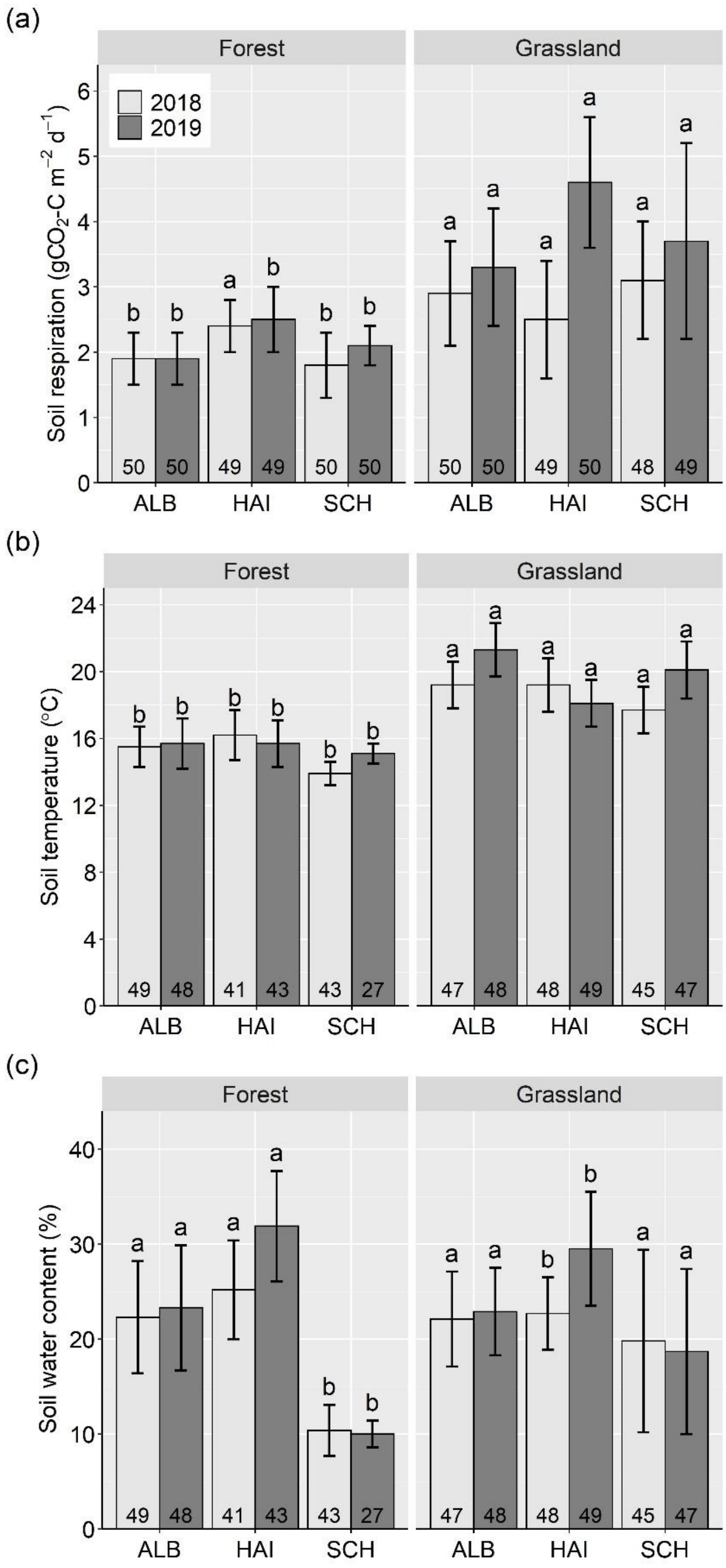


Figure 5.1 (a) Soil respiration, (b) temperature (at 5 cm soil depth) and (c) volumetric water content (at 10 cm soil depth) in 2018 and 2019 for the three study regions; Schwäbische-Alb =ALB; Hainich-Dün =HAI and Schorfheide-Chorin =SCH. Bars show mean values and error bars show standard deviations. Numbers at the base of the bars indicate the number of observations available in each study region and year. Lower-case letters indicate differences between forest and grassland ecosystems in a given study region and year.

higher fine root biomass than forests (in the upper 10 cm of mineral soil, Solly et al., 2014), which can lead to higher autotrophic respiration rates in grasslands. Overall, this evidence supports the higher in-situ soil respiration rates observed in temperate grasslands compared to forests, but it is unclear if drought would influence the two land-use types in a similar, or differential, way.

Both forests and grasslands had in-situ soil respiration values that matched the lower part of the aforementioned literature range (i.e. <4.9 gCO_2-C m^{-2} d^{-1} in temperate forests and >5.0 gCO_2-C m^{-2} d^{-1} in temperate grasslands), indicating that drought events in 2018 and 2019 suppressed soil respiration in both land-use types. This study cannot conclude which of the two land-use types was more severely impacted by drought in terms of C cycling, because in-situ soil respiration data from wetter summers were not available. However, it is interesting that grasslands exhibited, in general, higher in-situ soil respiration fluxes compared to forests, despite the often-lower soil water content in grasslands with mineral soils (Figure 5.1a, c). Based on the higher C losses via respiration in grasslands than in forests, even under the extreme conditions of a drought, I suggest that grasslands might provide more opportunities for reductions in CO_2 emissions from soils through sustainable management than (more) near-natural ecosystems, like forests.

5.1.2 The role of land management

Forest properties, as shaped by silvicultural management, influence in-situ soil respiration (Figure 2.3). After examining seven different forest properties, mean diameter at breast height (both in 2018 and 2019) and conifer share (only in 2018) were important predictors of soil respiration under the prevailing dry conditions (Figure 2.4). In more detail, this study found that forest stands with low mean diameter at breast height and/or stands with high conifer share have lower summer soil respiration compared to stands with bigger trees and/or stands with more deciduous trees. However, droughts impose a strong disturbance on biological processes such as the soil microbial and root activity (Nikolova et al., 2009), and, thus, the dry conditions in this study might have masked effects from other factors, such as the tree species richness, on soil respiration and/or altered the magnitude of their effects.

Similar to silvicultural management, this study shows that grassland management influences in-situ soil respiration indirectly. In more detail, grazing and fertilization intensities were not important predictors of in-situ soil respiration (Table 3.3), but when indirect effects through soil and plant properties were considered by means of Structural Equation Modeling, we found that fertilization caused several positive (i.e. increasing) effects on soil respiration (Figure 3.2a, c). Fertilization resulted in plant species loss which decreased the C:N ratios of soils and plants, and soil (both in 2018 and ’19) and plant (only in 2019) C:N ratios were negatively associated with soil respiration (Figure 3.2a, c). In addition, and in accordance with previous findings on basal respiration (Dietrich et al., 2017), this study reports a dependence of fertilization-induced effects on soil respiration from the climatic conditions, with stronger effects under wetter (here 2019) than drier (here 2018) years (Figure 3.2b, d). Similar to earlier studies (De Deyn et al., 2011), this Thesis suggests that C mineralization depends on the climatic conditions, i.e. soil water availability, and the quality of plant litter and soil organic matter.

Silvicultural and grassland management follow distinct practices and time scales. For this reason, a direct comparison of the effects of management on in-situ soil respiration between forests and grasslands is impractical. I could tentatively suggest that, while soil respiration in grasslands increased with fertilization intensity, silvicultural intensification seemed to lead to lower fluxes. For the latter, this is shown by a positive link between mean diameter at breast height and soil respiration and a negative link between conifer share and soil respiration. However, in contrast to grasslands, I observed that forest in-situ soil respiration i) was not related to (organic layer and mineral) soil organic carbon concentrations and C:N ratios, and ii) it was positively related to fine root biomass. This indicates that forest in-situ soil respiration might originate mainly from autotrophic respiration under the conditions of a summer drought. For this reason, I cannot conclude on the importance of ecosystem management for C cycling between the two land-use types.

5.1.3 The role of soil, plant and microbial properties

In temperate forests, and in accordance with the literature (Luan et al., 2011; Søe and Buchmann, 2005), fine root biomass was positively associated with soil respiration. In addition, fine root biomass was the strongest and most reliable predictor of soil respiration between the two sampling years, and it mediated effects from mean diameter at breast height, conifer share and silt content (Table 2.5 and Figure 2.4). The strong relationship between fine root biomass and soil respiration indicates the importance of autotrophic respiration (i.e. CO_2 respired by root, mycorrhiza and rhizospheric microorganism; Bond-Lamberty et al., 2004; Hanson et al., 2000; Subke et al., 2006) for total in-situ soil respiration in early summer. Based on this observation, together with the lack of a contribution from the organic layer in soil respiration (Figure 2.2) and the lack of a relationship between soil organic carbon stocks (Table 2.5), I suggest that forest soil respiration possibly originated from autotrophic respiration, while the heterotrophic respiration was suppressed under the extreme conditions of a summer drought.

In contrast to temperate forests, in-situ soil respiration in grasslands was not related to fine root biomass (Table 3.3), and it was negatively related to soil and plant C:N ratios (Figure 3.2), which suggests a dependency on the quality of the substrate for the heterotrophic respiration. The main indication of suppressed respiration in grasslands was a positive relationship with soil water content which suggests water-limiting conditions. The relative contribution of autotrophic and heterotrophic respiration to total soil respiration, and their response to drought, depends on the land-use type, management and environmental conditions (Huang et al., 2018; Nikolova et al., 2009; Sun et al., 2019). So, droughts presumably suppressed heterotrophic respiration to a lesser in grasslands than in forests.

Both in temperate forests and grasslands, in-situ soil respiration was positively related to soil water content and silt content (Table 2.3 and 3.3). A positive relationship between soil respiration and soil water content can indicate water-limiting conditions both for heterotrophic and autotrophic respiration (Moyano et at al., 2013; Sun et al., 2019). Soil texture is important for water and gas fluxes in soils, and the positive effect of silt on respiration is presumably related to this. Apart from the ecological significance of silt content for soil water availability (Salter and Williams, 1965), silt effects on soil respiration could be related to texture effects on root distribution, soil organic carbon stability, or residual differences between the three study regions. For example, in 2019, silt content effects on forest in-situ soil respiration was completely mediated by soil water content (Figure 2.5), while, under the incubation conditions, silt content effects were completely mediated by the mineral soil organic carbon concentration (Figure 2.4e).

5.2 Nutrient leaching risk in temperate ecosystems

5.2.1 The role of land management

In Central Europe, groundwater bodies are characterized by high concentration of nitrate which represents the loss of an important agricultural resource, and, at the same time, a potential threat to human health and aquatic ecosystems (EEA, 2018; ESF, 2011). In the period 2012-2015, about 25% of the sampled groundwater bodies in Germany had nitrate concentrations >50 mg L^{-1} (EU-Nitrate Directive, 2018), which is the threshold established by World Health Organization for potable water (WHO, 2011). Agriculture is considered as a main contributor of nutrients, and especially nitrate, in groundwater and surface water bodies, and this can be deteriorated by improper management (EU-Nitrate Directive, 2018). Fertilization increases N inputs in grasslands and increases the fraction of N in soils that are available for leaching (Klaus et al., 2018; Rumpel et al., 2015). Grazing partially removes N from the ecosystem with the animal biomass, but it also returns N in soils as dung and urine patches, in a form that is more soluble than in plant biomass and residues (Chardon et al., 2007; Ledgar et al., 2009). Management intensification in temperate grasslands imposes a considerable risk for nutrient losses via leaching.

Considering the difference between nitrogen inputs (i.e. fertilization) and outputs (i.e. mowing), fertilized grasslands can have a positive or a negative N balance, while unfertilized grasslands tended to have a negative N balance (Figure 5.2d). Moreover, while N inputs increase plant biomass N (Figure 5.2b), unfertilized and low-input (e.g. <40 kg N ha^{-1}) grasslands can still have considerable N outputs with biomass mowing (Figure 5.2e). Based on this, it was expected that unfertilized (and mostly grazed) grasslands would have lower inorganic N leaching fluxes than intensively fertilized grasslands, due to a lower N availability. However, grasslands with higher N outputs than inputs showed a wide range of inorganic N leaching (from almost 0 to about 120 kg N ha^{-1}) (Figure 5.2f, left panel). In addition, while higher N inputs (e.g. 40-140 kg N ha^{-1}) increased inorganic N leaching especially in HAI, further increase in N inputs (150 to 350 kg N ha^{-1}) did not result in increased inorganic N leaching, presumably due to higher N outputs with mowing, as found in ALB. Unreasonably high N inputs (i.e. >400 kg N ha-1) resulted in very high leaching fluxes (Figure 5.2f, right panel). While previous studies associated pastures with a higher risk for nitrate leaching (Cameron et al., 2013; Ryden et al., 1984), in this study, both fertilization and grazing intensities increased inorganic N leaching risk.

Temperate grasslands are considered to have low P leaching compared to more intensive agricultural land-uses, like croplands. This study reports phosphate leaching values comparable to P losses from croplands (Leinweber et al., 1999), which could potentially lead to environmental pollution issues. Similar to inorganic N, grassland management intensification can lead to high P leaching risk in temperate regions (Chardon et al., 2007; Heathwaite et al., 1997; Rumpel et al. 2015). Here, too, both fertilization and grazing intensities increased phosphate leaching almost to the same extent (Table 4.2). Interestingly, fertilization and grazing effects on phosphate leaching were found to be mostly direct ones, or mediated by soil properties, but not mediated by plant or microbial properties (Figure 4.3). Therefore, the reported fluxes were measured within the rooting zone (at 10 cm soil depth) and do not necessarily represent P losses from the ecosystem.

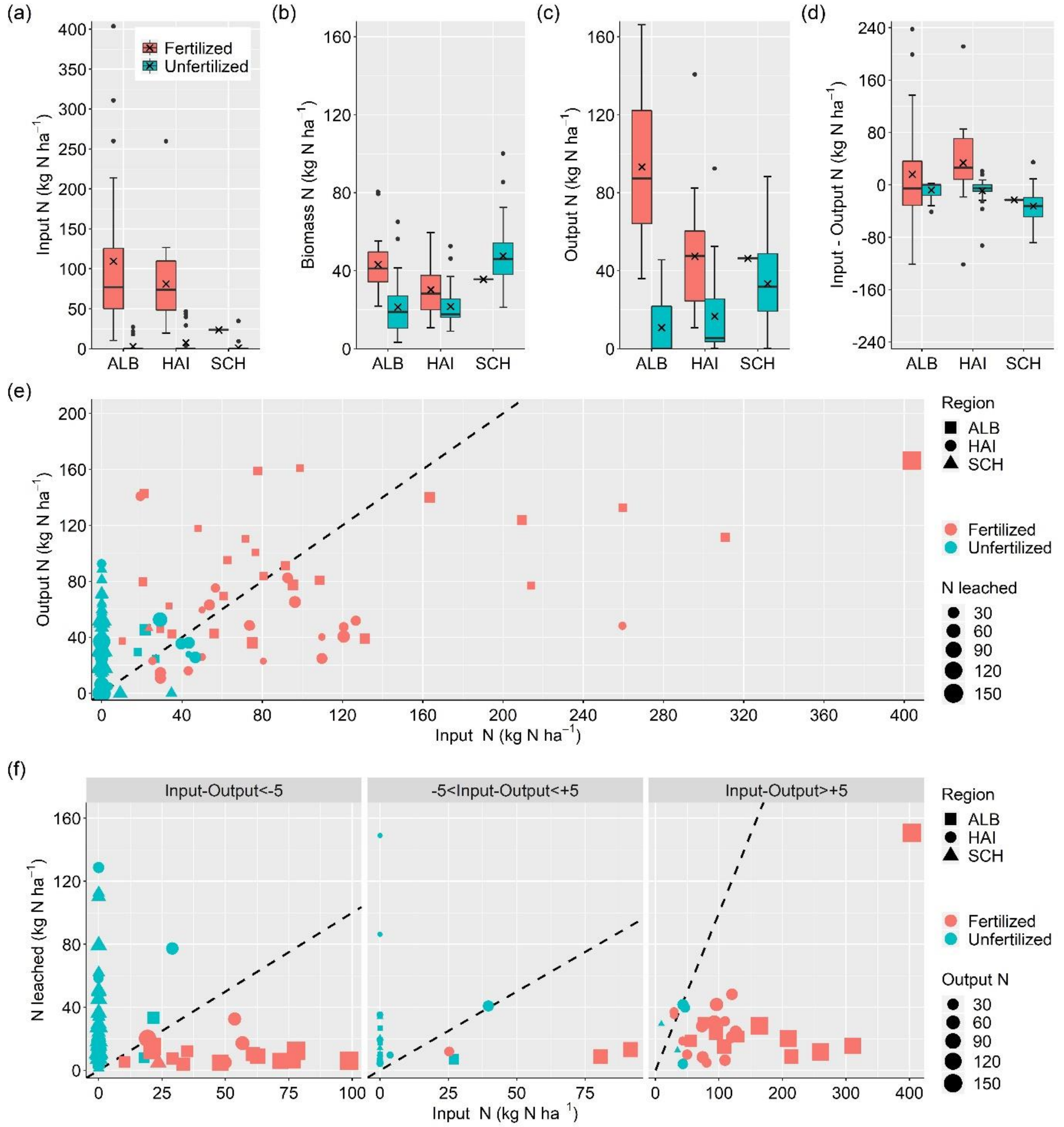


Figure 5.2 (a) Mean nitrogen inputs (fertilization) from 2015 to 2018 for the three study regions: ALB =Schwäbische-Alb, HAI =Hainich-Dün, SCH =Schorfheide-Chorin. (b) Mean nitrogen in plant biomass from 2015 to 2018 for the regions. (c) Mean nitrogen output (only mowing, not grazing) from 2015 to 2018 for the regions. (d) Difference of mean nitrogen inputs and outputs from 2015 to 2018 for the regions. (e) Relationship between nitrogen outputs and inputs for the three study regions. Point size indicates annual inorganic nitrogen leaching in 2018. Dashed lines indicate the 1:1 line. (f) Relationship between annual inorganic nitrogen leaching in 2018 and mean nitrogen inputs from 2015 to 2018. Point size indicates mean nitrogen output from 2015 to 2018. The three panels represent positive (>+5 kg N ha^{-1}), neutral (>-5 kg N ha^{-1} and <+5 kg N ha^{-1}) and negative (<-5 kg N ha^{-1}) differences between nitrogen inputs and outputs.

The leaching of S has received less attention than that of inorganic N and P in temperate grasslands, despite the agricultural and environmental importance of S (Eriksen, 2009; Gallejones et al., 2012). In this Thesis, fertilization and grazing intensities did not seem to affect sulphate leaching (Table 4.2). Interestingly, when indirect effects through soil properties were considered, sulphate was the only of the studied nutrients that exhibited a negative relationship with fertilization and grazing intensities, even though indirectly. These effects of management were mediated mostly by soil S (Figure S4.1), and possibly represent increased outputs of S from the ecosystem due to higher biomass removal (i.e. mowing or grazing) in intensively compared to extensively managed grasslands.

5.2.2 The role of soil, plant and microbial properties

Studies that investigate the leaching of multiple nutrients while considering soil, plant and soil microbial properties are, in general, scarce (de Vries et al., 2012; Grigulus et al., 2013; Klaus et al., 2018). In this Thesis, soil properties were important for phosphate and sulphate leaching, and higher soil inorganic P (and lower C:inorganic P ratios) and S concentrations were associated with higher leaching risk for the respective nutrient (Figure 4.3 and S4.2). There was no correlation between soil organic P and phosphate leaching (data not shown), while soil inorganic P was positively associated with phosphate leaching. This, possibly, indicates that available P for leaching mainly originates from management activities, and not from the mineralization of soil organic matter.

Similar to phosphate and sulphate, ammonium leaching was also associated with soil C:N ratio (Figure 4.3b). However, ammonium leaching was also positively related to plant C:N ratio. Nitrate leaching did not seem to me associated with soil properties, and it was negatively associated with plant C:N ratio (Figure 4.3a). Finally, and in contrast to the other nutrients, nitrate leaching was strongly linked to microbial properties, as found in previous studies (Grigulis et al., 2013; Pommier et al., 2017). However, more field research is needed in real-world ecosystems to understand management effects on soil microbes and ecosystem functions, and their interaction with plant diversity.

5.2.3 The role of plant diversity

Management intensification shifted both plant species richness and community functional traits in a way that increased inorganic N leaching risk. While plant species richness was severely impacted only by fertilization intensity, plant community functional traits were associated both with fertilization and grazing intensity (Figure 4.1 and 4.4). Aboveground plant CWM traits were influenced by both management practices in the same direction. For instance, LDMC decreased with increasing grazing and fertilization intensity. In contrast, belowground traits were mostly associated with fertilization intensity. Therefore, functional traits might be more sensitive indicators for grassland management effects on soil functions than plant species richness.

To date, it is not clear whether conservative, slow-growing plant communities, or exploitative, fast-growing plant communities lead to a greater N retention in the soil, plant and soil microorganisms' system. Based on the presented results (Chapter 4), I suggest that CWM aboveground plant traits in intensively managed grasslands were more indicative of exploitative plant communities compared to extensively managed grasslands (Figure 4.1; Allan et al., 2015; Busch et al., 2018; Zobel et al., 2007), which are characterized by a fast tissue turnover and decomposition (Lavorel et al., 2010), and, thus, influenced their association with annual N leaching risk.

Plant species richness was negatively associated with inorganic N leaching (Table 4.2 and S4.3) as shown in previous studies (Klaus et al., 2018; Leimer et al., 2016). However, here, it was also found that plant species richness was indirectly linked with inorganic N leaching (Figure 4.3 and S4.1) and that it mediated several effects from fertilization on inorganic N leaching through soil, plant and microbial properties. Overall, while there is strong evidence that plant diversity is positively associated with N retention in grasslands, species richness might not be the driving force of plant diversity effects on N-related ecosystem functions.

Plant functional traits could potentially explain the effects of plant species richness on inorganic N leaching. However, when plant CWM functional traits and plant species richness were included in a model (and linked via covariances), both plant functional traits and plant species richness had direct effects on inorganic N leaching. There might be two methodological explanations for this. First, both above- and belowground plant CWM traits were calculated based on aboveground species abundance, which is not necessarily indicative of belowground abundances. This could lead to biased belowground CWM functional traits (discussed in de Vries and Bardgett, 2016). Second, management intensification can influence plant community traits i) by shifting plant species presence and abundance and ii) by shifting the properties of plant species. However, the way that plant CWM traits were calculated from the TRY database accounts only for the first managemental effect. To this end, more field studies should try to explain the effects of plant species richness and functional traits on N-related ecosystem functions, and field observations of functional traits, instead of calculation based on databases, might provide useful insights.

5.3 Common drivers of soil respiration and nutrient leaching in temperate grasslands

In the 150 temperate grasslands of this study, in-situ soil respiration fluxes were determined as single-in-time measurements integrated over three days in early summer in 2018 and 2019. In contrast, in-situ nutrient leaching was determined as an annual cumulative flux from spring 2018 to spring 2019. These methodological differences did not allow a direct comparison between in-situ soil respiration and nutrient leaching. More specifically, linear models of nitrate, ammonium and phosphate leaching with soil respiration had p-value >0.36, while sulphate leaching with soil respiration had a positive but marginal relationship (p-value =0.08). However, apart from the direct comparison of these two major soil functions, we can gain interesting insights about grassland management by comparing their drivers and their relative importance.

It is well documented that C and nutrient cycles are tightly coupled (see discussions in Gan, 2019). However, it is still unclear how nutrient additions would affect C cycling. There are two prominent hypotheses, namely the stoichiometric decomposition and the microbial N mining (Craine et al., 2017), that describe contradicting effects of nutrient additions in C cycling. *Stoichiometric decomposition* suggests that increased nutrient availability would increase soil organic matter mineralization, while *microbial N mining* proposes that limiting N availability would increase organic matter mineralization. Understanding C and nutrient coupling is especially important for grassland management, where soil organic carbon and nutrient retention depend simultaneously on management practices and organic matter dynamics (i.e. plant litter decomposition and soil organic matter mineralization) (Conant, 2017; Lavorel et al., 2010; Rumpel et al., 2015).

In the same 150 grasslands, Gan (2019) found that high microbial activities (i.e. high respiration) were linked to high mineralized C:N ratios and low net mineralized N in an incubation experiment, supporting the *microbial N mining* hypothesis. In contrast, my field observations seem to support the *stoichiometric decomposition* hypothesis, since substrate (plant and soil) C:N ratios were negatively linked to soil respiration and nitrate leaching (Figure 3.2 and 4.3a). However, field observations integrate effects of multiple biotic and abiotic factors. This, on one hand, is more representative of real-world conditions, but also makes it difficult to disentangle confounded effects. Differences observed between field and lab observations might stem from plant-related processes, like water and nitrate acquisition, or climatic factors which are especially important under droughts.

Previously, I suggested that management practices are mainly responsible for phosphate leaching, based on its positive relationship with soil inorganic P (Figure 4.3c) and a lack of association with soil organic P (p-value =0.145, data not shown). This is further supported by grassland soil respiration that was not associated with soil C:P ratio (Figure S3.6), or with soil organic P (p-value =0.542, data not shown). In sum, phosphate leaching originates from soluble P added with fertilization or animal dungs, and not from the mineralization of organic matter.

In sum, I found that low-input, plant species-rich grasslands lead to slower C and N cycling compared to high-input, plant species-poor grasslands, and, thus, have lower soil respiration and inorganic N leaching fluxes. Management intensification changes the risk of soil C and N losses in grasslands on the long-term and active restoration measures to maintain diverse plant communities are needed. Diverse communities, in return, might mitigate the increased leaching risk that management intensification imposes on grasslands. However, maintaining diverse plant communities in managed grasslands is a great ecological challenge (Freitag et al., 2020), and more research in this direction is needed.

5.4 Main findings

Here, I summarize the main findings and highlights of my Thesis. Regarding in-situ soil respiration in temperate forests:

- Forest in-situ soil respiration mainly originates from autotrophic respiration (i.e. CO_2 respired by roots, mycorrhiza and rhizospheric microorganisms) under the dry conditions of a summer drought.
- Forest properties are important drivers of in-situ soil respiration at the landscape scale even under the conditions of a summer drought, however, droughts might obscure effects from other factors.
- Forest stands with low diameter at breast height and/or stands with high conifer share have lower summer soil respiration compared to stands with bigger trees and/or stands with more deciduous trees under dry conditions.

Regarding in-situ soil respiration in temperate grasslands:

- Grassland fertilization intensity severely reduces plant species richness, while grazing intensity does not seem to have an effect.
- Grassland fertilization intensity increases in-situ soil respiration indirectly by promoting plant species losses, while grazing intensity does not seem to have an effect on soil respiration.

- Extensively managed, plant species-rich grasslands are characterized by higher soil and plant C:N ratios compared to intensively managed grasslands, which leads to lower in-situ soil respiration in the former under the dry conditions of a summer drought.
- Summer drought leads to low in-situ soil respiration both in temperate forests and grasslands, but fluxes in grasslands remain higher than forests.

Regarding nutrient leaching in temperate grasslands:

- Grazing and fertilization intensities both increase the leaching risk of nitrate and phosphate, while ammonium leaching risk is only increased by fertilization intensity, and sulphate is generally not affected by management.
- Considering inorganic N leaching risk, fertilization intensity is a stronger driver than grazing.
- Extensively managed, plant species-rich grasslands have higher C:N ratios of soils, plants and soil microorganisms, which reduces the leaching risk of nitrate and phosphate, but not of ammonium or sulphate.
- Grazing and fertilization intensification shifts plant communities towards more exploitative (community weighted mean) functional traits, which reduces the leaching risk of inorganic N.
- Plant species richness is negatively associated with inorganic N leaching risk, but, in managed grasslands, this relationship is driven by fertilization intensity and, eventually, it is mediated by the C:N ratios of soils, plants and soil microorganisms.

5.5 Future research

Based on my PhD work, I identified the following topics that future research should address:

- There is evidence that forest properties and tree species diversity influence in-situ soil respiration (Jewell et al., 2017; Rodeghiero et al., 2005), however studies that examine these effects under drought are, in general, scarce. The present study identifies effects from forest properties on in-situ soil respiration under dry summer conditions, however the ability to form conclusions is limited by the lack of observations on wetter summers. Due to climate change, the intensity and frequency of extreme climatic events might change in the near future. Therefore, future research should investigate the effects of forest properties, as shaped by management, on carbon cycling in soils during and after droughts to ensure the sequestration potential of temperate forests.

- According to the present study, fertilization effects on in-situ soil respiration depend on environmental conditions, and especially on soil water. Therefore, grazing and fertilization effects on in-situ soil respiration should be investigated under different water availability levels. This is even more important for fertilization, since, in exceptionally dry years, grasslands receive higher amounts of organic fertilizers (Klaus et al., 2020b).
- Management intensification changes the risk of soil C and N losses in grasslands on the long-term and active (restoration) measures to maintain diverse plant communities are needed. Diverse plant communities can potentially mitigate the increased C and N losses from soils that management intensification imposes on grasslands. However, maintaining diversity plant communities in managed grasslands is a great ecological challenge (Freitag et al., 2020), and more research in this direction is needed.
- Plant functional traits have a high potential to explain carbon and nutrient related ecosystem functions (de Vries and Bardgett, 2016). Including both above- and belowground functional traits to explain ecosystem functions is, in general, a new approach. Field and mesocosm studies should focus on plant functional traits, in an attempt to understand the actual mechanisms behind plant diversity effects on ecosystem functions.

6 Summary/ Zusammenfassung

In English

Land-use and land management is known to influence the coupled cycles of carbon and nutrients in soils. However, studies that investigate the carbon and nutrient cycling in multiple land-uses over multiple study regions with distinct environmental conditions and across broad management gradients are scarce. Therefore, many questions regarding management, biotic and abiotic effects on carbon and nutrient cycling, remain open in real-world temperate ecosystems. Some of these research questions are: i) how forest properties affect soil respiration: through their effects on soil properties or on climatic conditions?, ii) how grassland management influences soil respiration, and that is the role of plant diversity? and iii) what is the relative importance of direct and indirect effects of grassland management on nutrient leaching, and how are the indirect effects mediated by soil, plant and microbial properties?

For this reason, I measured in-situ soil respiration and nutrient leaching (of nitrate, ammonium, phosphate and sulphate) in 150 forests and 150 grasslands over three temperate regions in Germany which were part of the *Biodiversity Exploratories* project. In-situ soil respiration was measured with soda-lime method as single-in-time measurements over a period of 3-7 days, depending on the land-use, in early summer in 2018 and 2019. Nutrient leaching was determined at 10 cm soil depth with a resin method as the cumulative downward flux over a year (from spring 2018 to spring 2019). The main objectives of my Thesis were to: i) determine management effects on these two major soil functions, ii) determine their main biotic and abiotic drivers, and iii) compare these carbon and nutrient fluxes between the two land-use types.

Chapter 2 focuses on the effects of forest properties, as shaped by silvicultural management, on in-situ soil respiration. Forest properties explained a large portion (>30%) of the soil respiration in both years, and mean diameter at breast height and conifer share were positively and negatively linked to soil respiration, respectively. Organic C stocks (of organic layer and mineral soil) were not related to soil respiration, while fine root biomass was positively associated with it in both years. In addition, there were no differences between soil respiration from total soil (organic layer and mineral soil) and mineral soil (organic layer removed) examined in a subset of 29 forests. In sum, the presented results suggest that autotrophic respiration was possibly the main contributor to total in-situ soil respiration under the prevailing dry summer conditions in 2018 and 2019.

Chapter 3 focuses on the effects of grassland management on in-situ soil respiration through changes on soil, plant and microbial stoichiometry. In early summer 2018 and 2019, in-situ soil respiration was suppressed due to limited water availability caused by naturally occurring droughts. Under the prevailing environmental conditions, grazing intensity did not seem to affect in-situ soil respiration, while fertilization intensity indirectly increased it. More specifically, fertilization-induced plant species loss reduced the C:N ratios of soils and plants, leading to increased soil respiration, presumably due to substrate of higher quality and decomposability. In conclusion, extensively managed, plant species-rich grasslands are characterized by higher soil and plant C:N ratios compared to intensively managed grasslands, which leads to lower in-situ soil respiration in the former under the dry conditions of a summer drought.

Chapter 4 focuses on the effects of grassland management on in-situ annual nutrient leaching of four nutrients. Fertilization intensity was positively linked to nitrate, ammonium and phosphate leaching risk, and grazing intensity was positively associated only to nitrate and phosphate. Fertilization intensity seemed to increase inorganic N leaching risk, not only directly, but also by changing the plant species richness and the stoichiometry of soils, plants and soil microorganisms. In addition, both grazing and fertilization intensities shifted plant communities towards an exploitative functional composition (characterized by high tissue turnover rates), and, thus, further promoted the leaching of inorganic N. Plant species richness was associated with lower inorganic N leaching risk, even after accounting for correlated effects of plant functional groups or community traits. In contrast to other nutrients, sulphate leaching risk was not directly related to management intensity and it was indirectly reduced by management, presumably due to increased biomass outputs. Therefore, maintaining and restoring diverse plant communities might mitigate the increased leaching risk that management intensity imposes upon grasslands.

Chapter 5 provides a synthesis of the previous three research *Chapters* of the Thesis. Comparing *Chapter 2* and *3*, grassland ecosystems exhibited higher fluxes than (the more near-to-natural) forests in a regional scale, even under the conditions of natural summer droughts. Hence, grasslands might provide more opportunities to reduce C losses as CO_2 emissions via management extensification than forests even in a drier future. Comparing *Chapter 3* and *4*, intensively managed, species-poor grasslands are characterized by faster C and nutrient cycling than extensively managed, species-rich grasslands. In detail, intensively managed grasslands have a higher risk of C and inorganic N losses, as CO_2 emissions and leaching respectively, than extensively managed grasslands, not only because of direct management effects, but also due to management-induced changes in soils, plants and soil microorganisms. Therefore, while management extensification could reduce the direct effects of management on ecosystem functioning over a relatively short period in time, some of these changes, such as a loss in plant diversity and functional changes in plant communities, are not immediately reversible. Management intensification changes ecosystem functioning on the long-term, and active (restoration) measures to maintain diverse plant communities are needed.

Auf Deutsch

Landnutzung und Bewirtschaftung beeinflussen die Kohlenstoff (C) und Nährstoffkreisläufe in Böden. Nur wenige Studien untersuchen gleichzeitig die C- und Nährstoffkreisläufe in verschiedenen Landnutzungen über breite Managementgradienten in Regionen mit unterschiedlichen Umweltbedingungen. Viele Fragen zur Bewirtschaftung sowie zu den biotischen und abiotischen Auswirkungen auf den C und Nährstoffkreislauf in gemäßigten Ökosystemen bleiben offen. Einige dieser Forschungsfragen sind: i) Wie beeinflussen Waldeigenschaften die Bodenatmung: durch ihre Auswirkungen auf die Bodeneigenschaften oder auf die klimatischen Bedingungen?, ii) Wie beeinflusst die Bewirtschaftung von Grünland die Bodenatmung, und welche Rolle spielt dabei die Pflanzenvielfalt? und iii) wie beeinflusst die Bewirtschaftung von Grünland die Nährstoffauswaschung: direkt oder durch Boden-, Pflanzen- und Mikrobeneigenschaften?

Hier wurden die Bodenatmung und die Nährstoffauswaschung (von Nitrat, Ammonium, Phosphat und Sulfat) in 150 Wäldern und 150 Grünlandflächen in drei gemäßigten Regionen in Deutschland gemessen, die Teil von dem *Biodiversity Exploratories* Projekt waren. Die Bodenatmung wurde mit der Kalk-Natron-Methode im Sommer 2018 und 2019 bestimmt. Die Nährstoffauswaschung wurde als kumulativer Abwärtsfluss mit dem Sickerwasser über ein Jahr (von Frühjahr 2018 bis Frühjahr 2019) bestimmt. Die Hauptziele dieser Dissertation waren: i) die Auswirkungen der Bewirtschaftung auf diese beiden wichtigen Bodenfunktionen zu ermitteln, ii) ihre wichtigsten biotischen und abiotischen Einflussfaktoren zu bestimmen und iii) diese C und Nährstoffflüsse zwischen den beiden Landnutzungstypen zu vergleichen.

Kapitel 2 befasst sich mit den Auswirkungen der Waldeigenschaften auf die Bodenatmung. Der mittlere Brusthöhendurchmesser stand in einem positiven Zusammenhang mit der Bodenatmung, während der Anteil der Nadelbäume in einem negativen Zusammenhang mit ihr stand. Die organischen C-Vorräte standen in keinem Zusammenhang mit der Bodenatmung, während die Feinwurzelbiomasse positiv mit ihr korreliert war. Darüber hinaus gab es keine Unterschiede zwischen der Bodenatmung des gesamten Bodens und des Mineralbodens. Die autotrophe Atmung leistete während der Dürren in den Jahren 2018 und 2019 moglicherweise den Hauptbeitrag zur gesamten Bodenatmung.

Kapitel 3 befasst sich mit den Auswirkungen der Grünlandbewirtschaftung auf die Bodenatmung. Die Bodenatmung wurde aufgrund der begrenzten Wasserverfügbarkeit während der Dürren im Sommer 2018 und 2019 unterdrückt. Unter diesen Bedingungen stand die Beweidungsintensität in keinem Zusammenhang mit der Bodenatmung, während die Düngungsintensität indirekt positiv mit ihr korreliert war. Die Düngung führte zu einem Verlust an Pflanzenarten, der negativ mit dem C:N-Verhältnis von Boden und Pflanzen und positiv mit der Bodenatmung korreliert war. Extensiv bewirtschaftetes, pflanzenartenreiches Grünland im Vergleich zu intensiv bewirtschaftetem Grünland durch höhere C:N-Verhältnisse im Boden und in den Pflanzen gekennzeichnet ist, was zu einer geringeren Bodenatmung unter trockenen Sommerbedingungen führt.

Kapitel 4 befasst sich mit den Auswirkungen der Grünlandbewirtschaftung auf die jährliche Nährstoffauswaschung. Die Düngungsintensität stand in einem positiven Zusammenhang mit der Auswaschung von Nitrat, Ammonium und Phosphat. Die Beweidungsintensität wirkte sich nur auf die Nitrat und Phosphatverlagerung positiv aus. Die Düngungsintensität schien die Auswaschung von anorganischem N sowohl direkt als auch indirekt zu erhöhen. Indirekte Effekte wurden durch den Pflanzenartenreichtum und die Stöchiometrie von Böden, Pflanzen und Bodenmikroorganismen vermittelt. Der Reichtum an Pflanzenarten wurde mit einer geringeren anorganischen N-Auswaschung in Verbindung gebracht. Die Sulfatauswaschung stand in keinem direkten Zusammenhang mit der Bewirtschaftungsintensität und wurde durch die Bewirtschaftung indirekt verringert. Die Erhaltung und Wiederherstellung vielfältiger Pflanzengemeinschaften könnte das erhöhte Auswaschungsrisiko von Nährstoffen, welches durch die intensive Bewirtschaftung des Grünlandes hervorgerufen wird, abmildern.

Kapitel 5 enthält eine Zusammenfassung der Arbeit. Ein Vergleich von *Kapitel 2* und *3* zeigt, dass Grünlandökosysteme bei sommerlichen Trockenperioden eine höhere Bodenatmung aufweisen als Wälder. Daher kann die Reduzierung der Bewirtschaftungsintensität in Grünländern in trockenen Jahren stärker zur Verringerung der CO2-Emissionen beitragen als die Reduzierung der Bewirtschaftung in Wäldern. Ein Vergleich der *Kapitel 3 und 4* zeigt, dass intensiv bewirtschaftetes, artenarmes Grünland durch schnellere C und Nährstoffkreisläufe gekennzeichnet ist als extensiv bewirtschaftetes Grünland. Im Einzelnen haben intensiv bewirtschaftete Grünland ein höheres Risiko von C-und anorganischen N-Verlusten als extensiv bewirtschaftete Grünland. Dies ist nicht nur auf direkte Bewirtschaftungseffekte zurückzuführen, sondern auch auf bewirtschaftungsbedingte Veränderungen der Bödeneigenschaften, sowie der Pflanzen und Bodenmikroorganismen. Die Extensivierung der Bewirtschaftung kann die Beeinträchtigung von Ökosystemfunktionen über einen relativ kurzen Zeitraum hinweg verringern. Einige der indirekten Auswirkungen, wie z.B. der Verlust der Pflanzenvielfalt, sind jedoch nicht sofort reversibel. Die Intensivierung der Bewirtschaftung verändert die Funktionsweise der Ökosysteme langfristig, und es sind aktive (Wiederherstellungs-)Maßnahmen zur Erhaltung vielfältiger Pflanzengemeinschaften erforderlich.

7 Bibliography

Achat, D.L., Fortin, M., Landmann, G., Ringeval, B., Augusto, L., 2015. Forest soil carbon is threatened by intensive biomass harvesting. Sci. Rep. 5, 1–10. https://doi.org/10.1038/srep15991

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., Böhm, S., Grassein, F., Hölzel, N., Klaus, V.H., Kleinebecker, T., Morris, E.K., Oelmann, Y., Prati, D., Renner, S.C., Rillig, M.C., Schaefer, M., Schloter, M., Schmitt, B., Schöning, I., Schrumpf, M., Solly, E., Sorkau, E., Steckel, J., Steffen-Dewenter, I., Stempfhuber, B., Tschapka, M., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Fischer, M., 2015. Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. Ecol. Lett. 18, 834–843. https://doi.org/10.1111/ele.12469

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. (Ted., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. 259, 660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Alvarez, R., Santanatoglia, O.J., Garcîa, R., 1995. Effect of temperature on soil microbial biomass and its metabolic quotient in situ under different tillage systems. Biol. Fertil. Soils 19, 227–230. https://doi.org/10.1007/BF00336164

Ammer, C., Wagner, S., 2005. An approach for modelling the mean fine-root biomass of Norway spruce stands. Trees - Struct. Funct. 19, 145–153. https://doi.org/10.1007/s00468-004-0373-4

Angst, G., Mueller, K.E., Eissenstat, D.M., Trumbore, S., Freeman, K.H., Hobbie, S.E., Chorover, J., Oleksyn, J., Reich, P.B., Mueller, C.W., 2019. Soil organic carbon stability in forests: Distinct effects of tree species identity and traits. Glob. Chang. Biol. 25, 1529–1546. https://doi.org/10.1111/gcb.14548

Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. Ann. For. Sci. 59, 233–253. https://doi.org/10.1051/forest:2002020

Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drösler, M., Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J., Wohlfahrt, G., Cernusca, A., 2008. Soil respiration in European grasslands in relation to climate and assimilate supply. Ecosystems 11, 1352–1367. https://doi.org/10.1007/s10021-008-9198-0

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, W.J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Funct. Ecol. 13, 650–660. https://doi.org/10.1046/j.1365-2435.1999.00362.x

Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: Root traits as drivers of ecosystem processes. Trends Ecol. Evol. 29, 692–699. https://doi.org/10.1016/j.tree.2014.10.006

Bastos, A., Ciais, P., Friedlingstein, P., Sitch, S., Pongratz, J., Fan, L., Wigneron, J.P., Weber, U., Reichstein, M., Fu, Z., Anthoni, P., Arneth, A., Haverd, V., Jain, A.K., Joetzjer, E., Knauer, J., Lienert, S., Loughran, T., McGuire, P.C., Tian, H., Viovy, N., Zaehle, S., 2020. Direct and seasonal legacy effects of the 2018 heat wave and drought on European ecosystem productivity. Sci. Adv. 6, 1–14. https://doi.org/10.1126/sciadv.aba2724

Bengtsson, J., Bullock, J.M., Egoh, B., Everson, C., Everson, T., O’Connor, T., O’Farrell, P.J., Smith, H.G., Lindborg, R., 2019. Grasslands—more important for ecosystem services than you might think. Ecosphere 10. https://doi.org/10.1002/ecs2.2582

Berg, B., McClaugherty, C., 2004. Plant Litter: Decomposition, Humus Formation, Carbon Sequestration. Springer

Berger, T. W., Inselsbacher, E., & Zechmeister-Boltenstern, S., 2010. Carbon dioxide emissions of soils under pure and mixed stands of beech and spruce, affected by decomposing foliage litter mixtures. Soil Biology and Biochemistry, 42(6), 986–997. https://doi.org/10.1016/j.soilbio.2010.02.020

Bergmann, J., Weigelt, A., Van Der Plas, F., Laughlin, D.C., Kuyper, T.W., Guerrero-Ramirez, N., Valverde-Barrantes, O.J., Bruelheide, H., Fresche, G.T., Iversen, C.M., Kattge, J., McCormack, M.L., Meier, I.C., Rillig, M.C., Roumet, C., Semchenko, M., Sweeney, C.J., Van Ruijven, J., York, L.M., Mommer, L., 2020. The fungal collaboration gradient dominates the root economics space in plants. Sci. Adv. 6, 1–10. https://doi.org/10.1126/sciadv.aba3756

Bierbaß, P., Gutknecht, J.L.M., Michalzik, B., 2015. Nest-mounds of the yellow meadow ant (Lasius flavus) at the “Alter Gleisberg”, Central Germany: Hot or cold spots in nutrient cycling? Soil Biol. Biochem. 80, 209–217. https://doi.org/10.1016/j.soilbio.2014.09.020

Bischoff, W. A., 2009. Development and applications of the self-integrating accumulators: A method to quantify the leaching losses of environmentally relevant substances. Hohenheimer Bodenkundliche Hefte, Heft 91, Editors: Kandeler, E.; Kuzyakov, Y.; Stahr, K.; Streck, T.; Kaupenjohann, M., University of Hohenheim, Stuttgart. 145 p.

Bittman, S., Forge, T.A., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem. 37, 613–623. https://doi.org/10.1016/j.soilbio.2004.07.038

Blüthgen, N., Dormann, C.F., Prati, D., Klaus, V.H., ..., Weisser, W.W., 2012. A quantitative index of land-use intensity in grasslands: Integrating mowing, grazing and fertilization. Basic Appl. Ecol. 13, 207–220. https://doi.org/10.1016/j.baae.2012.04.001

Boeddinghaus, R.S., Marhan, S., Berner, D., Boch, S., Fischer, M., Hölzel, N., Kattge, J., Klaus, V.H., Kleinebecker, T., Oelmann, Y., Prati, D., Schäfer, D., Schöning, I., Schrumpf, M., Sorkau, E., Kandeler, E., Manning, P., 2019. Plant functional trait shifts explain concurrent changes in the structure and function of grassland soil microbial communities. J. Ecol. 107, 2197–2210. https://doi.org/10.1111/1365-2745.13182

Bolan, N.S., Syers, J.K., Tillman, R.W. and Scotter, D.R., 1988. Effect of liming and phosphate additions on sulphate leaching in soils. Journal of Soil Science, 39, 493-504. https://doi.org/10.1111/j.1365-2389.1988.tb01234.x

Bond-Lamberty, B., Wang, C., Gower, S.T., 2004. A global relationship between the heterotrophic and autotrophic components of soil respiration? Glob. Chang. Biol. 10, 1756–1766. https://doi.org/10.1111/j.1365-2486.2004.00816.x

Borken, W., Beese, F., 2005. Soil respiration in pure and mixed stands of European beech and Norway spruce following removal of organic horizons. Can. J. For. Res. Can. Rech. For. 35, 2756–2764. https://doi.org/10.1139/x05-192

Borken, W., Savage, K., Davidson, E.A., Trumbore, S.E., 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Glob. Chang. Biol. 12, 177–193. https://doi.org/10.1111/j.1365-2486.2005.01058.x

Borken, W., Xu, Y.J., Davidson, E. a, Beese, F., 2002. Site and temporal variation of soil respiration in European beech, Norway spruce, and Scots pine forests. Glob. Chang. Biol. 8, 1205–1216. https://doi.org/10.1046/j.1365-2486.2002.00547.x

Bruelheide, H., Dengler, J., Purschke, O., ..., Jandt, U., 2018. Global trait–environment relationships of plant communities. Nat. Ecol. Evol. 2, 1906–1917. https://doi.org/10.1038/s41559-018-0699-8

Burri, S., Niklaus, P.A., Grassow, K., Buchmann, N., Kahmen, A., 2018. Effects of plant productivity and species richness on the drought response of soil respiration in temperate grasslands. PLoS One 13, 1–18. https://doi.org/10.1371/journal.pone.0209031

Burri, S., Sturm, P., Prechsl, U.E., Knohl, A., Buchmann, N., 2014. The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO2 efflux in a temperate grassland. Biogeosciences 11, 961–975. https://doi.org/10.5194/bg-11-961-2014

Busch, V., Klaus, V.H., Penone, C., Schäfer, D., Boch, S., Prati, D., Müller, J., Socher, S.A., Niinemets, Ü., Penuealas, J., Hölzel., N., Fischer, M., Kleinebecker, T., 2018. Nutrient stoichiometry and land use rather species richness determine plant functional diversity. Ecology and Evolution 8, 601-616. https://doi.org/10.1002/ece3.3609

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: A review. Ann. Appl. Biol. 162, 145–173. https://doi.org/10.1111/aab.12014

Casals, P., Lopez-Sangil, L., Carrara, A., Gimeno, C., Nogués, S., 2011. Autotrophic and heterotrophic contributions to short-term soil CO 2 efflux following simulated summer precipitation pulses in a Mediterranean dehesa. Global Biogeochem. Cycles 25, 1–12. https://doi.org/10.1029/2010GB003973

Chang, C.T., Sperlich, D., Sabaté, S., Sánchez-Costa, E., Cotillas, M., Espelta, J.M., Gracia, C., 2016. Mitigating the stress of drought on soil respiration by selective thinning: Contrasting effects of drought on soil respiration of two oak species in a mediterranean forest. Forests 7. https://doi.org/10.3390/f7110263

Chardon, W.J., Aalderink, G.H., van der Salm, C., 2007. Phosphorus Leaching from Cow Manure Patches on Soil Columns. J. Environ. Qual. 36, 17–22. https://doi.org/10.2134/jeq2006.0182

Chen, H., Mommer, L., van Ruijven, J., de Kroon, H., Fischer, C., Gessler, A., Hildebrandt, A., Scherer-Lorenzen, M., Wirth, C., Weigelt, A., 2017. Plant species richness negatively affects root decomposition in grasslands. J. Ecol. 105, 209–218. https://doi.org/10.1111/1365-2745.12650

Chen, S., Zou, J., Hu, Z., Chen, H., Lu, Y., 2014. Global annual soil respiration in relation to climate, soil properties and vegetation characteristics: Summary of available data. Agric. For. Meteorol. 198, 335–346. https://doi.org/10.1016/j.agrformet.2014.08.020

Chen, X., Chen, H.Y.H., 2019. Plant diversity loss reduces soil respiration across terrestrial ecosystems. Glob. Chang. Biol. 25, 1482-1492. https://doi.org/10.1111/gcb.14567

Christiansen, J.R., Vesterdal, L., Callesen, I., Elberling, B., Schmidt, I.K., Gundersen, P., 2010. Role of six European tree species and land-use legacy for nitrogen and water budgets in forests. Glob. Chang. Biol. 16, 2224–2240. https://doi.org/10.1111/j.1365-2486.2009.02076.x

Conant, R.T., Cerri, C.E.P., Osborne, B.B., Paustian, K., 2017. Grassland management impacts on soil carbon stocks: a new synthesis. Ecol. Appl. 27, 662–668. https://doi.org/10.1002/eap.1473

Craine J.M., Wedin, D.A., Reich, P.B., 2001. The response of soil CO2 flux to changes in atmospheric CO2, nitrogen supply and plant diversity. Glob. Chang. Biol. 7, 947-953. https://doi.org/10.1046/j.1354-1013.2001.00455.x

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1

De Deyn, G.B., Shiel, R.S., Ostle, N.J., Mcnamara, N.P., Oakley, S., Young, I., Freeman, C., Fenner, N., Quirk, H., Bardgett, R.D., 2011. Additional carbon sequestration benefits of grassland diversity restoration. J. Appl. Ecol. 48, 600–608. https://doi.org/10.1111/j.1365-2664.2010.01925.x

de Vries, F.T., Bardgett, R.D., 2012. Plant-microbial linkages and ecosystem nitrogen retention: Lessons for sustainable agriculture. Front. Ecol. Environ. 10, 425–432. https://doi.org/10.1890/110162

de Vries, F.T., Bardgett, R.D., 2016. Plant community controls on short-term ecosystem nitrogen retention. New Phytol. 210, 861–874. https://doi.org/10.1111/nph.13832 de Vries, F.T., Bloem, J., Quirk, H., Stevens, C.J., Bol, R., Bardgett, R.D., 2012. Extensive Management Promotes Plant and Microbial Nitrogen Retention in Temperate Grassland. PLoS One 7, 1–12. https://doi.org/10.1371/journal.pone.0051201

de Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biol. Biochem. 38, 2092–2103. https://doi.org/10.1016/j.soilbio.2006.01.008

de Vries, F.T., van Groenigen, J.W., Hoffland, E., Bloem, J., 2011. Nitrogen losses from two grassland soils with different fungal biomass. Soil Biol. Biochem. 43, 997–1005. https://doi.org/10.1016/j.soilbio.2011.01.016

Dias, A.T.C., van Ruijven, J., Berendse, F., 2010. Plant species richness regulates soil respiration through changes in productivity. Oecologia 163, 805–813. https://doi.org/10.1007/s00442-010-1569-5

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, ..., Gorné, L.D., 2016. The global spectrum of plant form and function. Nature 529, 167–171. https://doi.org/10.1038/nature16489

Dietrich, P., Buchmann, T., Cesarz, S., Eisenhauer, N., Roscher, C., 2017. Fertilization, soil and plant community characteristics determine soil microbial activity in managed temperate grasslands. Plant Soil 419, 189–199. https://doi.org/10.1007/s11104-017-3328-4

DIN ISO 11277, 2002. Soil quality - determination of particle size distribution in mineral soil material - method by sieving and sedimentation, Beuth, Berlin.

Donnison, L.M., Griffith, G.S., Hedger, J., Hobbs, P.J., Bardgett, R.D., 2000. Management influences on soil microbial communities and their function in botanically diverse haymeadows of northern England and Wales. Soil Biol. Biochem. 32, 253–263. https://doi.org/10.1016/S0038-0717(99)00159-5

Ehbrecht, M., Schall, P., Ammer, C., Fischer, M., Seidel, D., 2019. Effects of structural heterogeneity on the diurnal temperature range in temperate forest ecosystems. For. Ecol. Manage. 432, 860–867. https://doi.org/10.1016/j.foreco.2018.10.008

Eriksen, J., 2009. Chapter 2 Soil Sulfur Cycling in Temperate Agricultural Systems. Adv. Agron. 102, 55–89. https://doi.org/10.1016/S0065-2113(09)01002-5

European Science Foundation (ESF), 2011. Report European science foundation. Available in: http://archives.esf.org/media-centre/esf-in-the-media/archives-2011.html

EU-Nitrate Directive, 2018. Report from the commission to the council and the European Parliament.

European Environment Agency (EEA), 2018. Environmental indicator report 2018. In support to the monitoring of the Seventh Environment Action Programme.

FAO, 2010. Challenges and opportunities for carbon sequestration in grassland systems: A technical report on grassland management and climate change mitigation. Integrated Crop Management, vol. 9-2010.

Fargione, J., Tilman, D., Dybzinski, R., Lambers, J.H.R., Clark, C., Harpole, W.S., Knops, J.M.H., Reich, P.B., Loreau, M., 2007. From selection to complementarity: Shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proc. R. Soc. B Biol. Sci. 274, 871–876. https://doi.org/10.1098/rspb.2006.0351

Felipe-Lucia, M.R., Soliveres, S., Penone, C., Manning, P., van der Plas, F., Boch, S., Prati, D., Ammer, C., Schall, P., Gossner, M.M., Bauhus, J., Buscot, F., Blaser, S., Blüthgen, N., de Frutos, A., Ehbrecht, M., Frank, K., Goldmann, K., Hänsel, F., Jung, K., Kahl, T., Nauss, T., Oelmann, Y., Pena, R., Polle, A., Renner, S., Schloter, M., Schöning, I., Schrumpf, M., Schulze, E.D., Solly, E., Sorkau, E., Stempfhuber, B., Tschapka, M., Weisser, W.W., Wubet, T., Fischer, M., Allan, E., 2018. Multiple forest attributes underpin the supply of multiple ecosystem services. Nat. Commun. 9, 4839 (2018). https://doi.org/10.1038/s41467-018-07082-4

Finér, L., Helmisaari, H.S., Lõhmus, K., Majdi, H., Brunner, I., Børja, I., Eldhuset, T., Godbold, D., Grebenc, T., Konôpka, B., Kraigher, H., Möttönen, M.R., Ohashi, M., Oleksyn, J., Ostonen, I., Uri, V., Vanguelova, E., 2007. Variation in fine root biomass of three European tree species: Beech (Fagus sylvatica L.), Norway spruce (Picea abies L. Karst.), and Scots pine (Pinus sylvestris L.). Plant Biosyst. 141, 394–405. https://doi.org/10.1080/11263500701625897

Finér, L., Ohashi, M., Noguchi, K., Hirano, Y., 2011. Factors causing variation in fine root biomass in forest ecosystems. For. Ecol. Manage. 261, 265–277. https://doi.org/10.1016/j.foreco.2010.10.016

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., ..., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic Appl. Ecol. 11, 473–485. https://doi.org/10.1016/j.baae.2010.07.009

Freitag, M., Klaus, V.H., Bolliger, R., Hamer, U., Kleinebecker, T., Prati, D., Schäfer, D., Hölzel, N., 2021. Restoration of plant diversity in permanent grassland by seeding: Assessing the limiting factors along land-use gradients. J. Appl. Ecol. 58, 1681–1692. https://doi.org/10.1111/1365-2664.13883

Freschet, G.T., Roumet, C., Comas, …, Stokes, A., 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. New Phytol. https://doi.org/10.1111/nph.17072

Gallejones, P., Castellón, A., del Prado, A., Unamunzaga, O., Aizpurua, A., 2012. Nitrogen and sulphur fertilization effect on leaching losses, nutrient balance and plant quality in a wheat-rapeseed rotation under a humid Mediterranean climate. Nutr. Cycl. Agroecosystems 93, 337–355. https://doi.org/10.1007/s10705-012-9520-2

Gan, H.Y., 2019. The effects of land use and management on belowground carbon and nutrient interactions. PhD Thesis. Friedrich-Schiller-Universität Jena.

Gan, H.Y., Schöning, I., Schall, P., Ammer, C., Schrumpf, M., 2020. Soil Organic Matter Mineralization as Driven by Nutrient Stoichiometry in Soils Under Differently Managed Forest Stands 3, 1–15. https://doi.org/10.3389/ffgc.2020.00099

Gilhaus, K., Boch, S., Fischer, M., Hölzel, N., Kleinebecker, T., Prati, D., Rupprecht, D., Schmitt, B., Klaus, V.H., 2017. Grassland management in Germany: Effects on plant diversity and vegetation composition. Tuexenia 37, 379–397. https://doi.org/10.14471/2017.37.010

Gitay, H., Brown, S., Easterling, W., Jallow, B., Antle, J., Apps, M., Beamish, R., Chapin, T., Cramer, W., Frangi, J., Laine, J., Erda, L., Magnuson, J., Noble, I., Price, J., Prowse, T., Root, T., Schulze, E.-D., Sirotenko, O., Sohngen, B., Soussana, J., 2001. Chapter 5: Ecosystems and Their Goods and Services. Clim. Chang. 2001 Impacts Adapt. Vulnerability Contrib. Work. Gr. II to Third Assess. Rep. Intergov. Panel Clim. Clim. Chang. 235–342.

Gocke, M.I., Don, A., Heidkamp, A., Schneider, F., Amelung, W., 2021. The phosphorus status of German cropland—An inventory of top- and subsoils. J. Plant Nutr. Soil Sci. 184, 51–64. https://doi.org/10.1002/jpln.202000127

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food Security : The Challenge of 327, 812–818. https://doi.org/DOI: 10.1126/science.1185383

Grahmann, K., Verhulst, N., Palomino, L.M., Bischoff, W.A., Govaerts, B., Buerkert, A., 2018. Ion exchange resin samplers to estimate nitrate leaching from a furrow irrigated wheat-maize cropping system under different tillage-straw systems. Soil Tillage Res. 175, 91–100. https://doi.org/10.1016/j.still.2017.08.013

Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R.D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn, M., Clément, J.C., 2013. Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J. Ecol. 101, 47–57. https://doi.org/10.1111/1365-2745.12014

Grogan, P., 1998. CO2 flux measurement using soda lime: correction for water formed during CO2 adsorption. Ecology 79, 1467-1468. https://doi.org/10.1890/0012-9658(1998)079[1467:CFMUSL]2.0.CO;2

Grüneberg E., Schöning I., Riek W., Ziche D., Evers J. (2019) Carbon Stocks and Carbon Stock Changes in German Forest Soils. In: Wellbrock N., Bolte A. (eds) Status and Dynamics of Forests in Germany. Ecological Studies (Analysis and Synthesis), 237. Springer, https://doi.org/10.1007/978-3-030-15734-0_6

Grüneberg, E., Schöning, I., Hessenmöller, D., Schulze, E.D., Weisser, W.W., 2013. Organic layer and clay content control soil organic carbon stocks in density fractions of differently managed German beech forests. For. Ecol. Manage. 303, 1–10. https://doi.org/10.1016/j.foreco.2013.03.014

Grunwald, D., Panten, K., Schwarz, A., Bischoff, W.-A., Schittenhelm, S., 2020. Comparison of maize, permanent cup plant and a perennial grass mixture with regard to soil and water protection. GCB Bioenergy 12, 694-705. https://doi.org/10.1111/gcbb.12719

Gundersen, P., Schmidt, I.K., Raulund-Rasmussen, K., 2006. Leaching of nitrate from temperate forests – effects of air pollution and forest management. Environ. Rev. 14, 1–57. https://doi.org/10.1139/a05-015

Haines-Young, R., Potschin, M., 2018. CICES V5. 1. Guidance on the Application of the Revised Structure. Cices 53.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Seperating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48, 115–146.

Heathwaite, A.L., Griffiths, P., Haygarth, P.M., Jarvis, S.C., Parkinson, R.J., 1997. Phosphorus loss from grassland soils: Implications of land management for the quality of receiving waters. IAHS-AISH Publ. 243, 177–186.

Herold, N., Schöning, I., Berner, D., Haslwimmer, H., Kandeler, E., Michalzik, B., Schrumpf, M., 2014. Vertical gradients of potential enzyme activities in soil profiles of European beech, Norway spruce and Scots pine dominated forest sites. Pedobiologia 57, 181–189. https://doi.org/10.1016/j.pedobi.2014.03.003

Herold, N., Schöning, I., Gutknecht, J., Alt, F., Boch, S., Müller, J., Oelmann, Y., Socher, S.A., Wilcke, W., Wubet, T., Schrumpf, M., 2014a. Soil property and management effects on grassland microbial communities across a latitudinal gradient in Germany. Appl. Soil Ecol. 73, 41–50. https://doi.org/10.1016/j.apsoil.2013.07.009

Herold, N., Schöning, I., Michalzik, B., Trumbore, S., Schrumpf, M., 2014b. Controls on soil carbon storage and turnover in German landscapes. Biogeochemistry 119, 435–451. https://doi.org/10.1007/s10533-014-9978-x

Heuck, C., Weig, A., Spohn, M., 2015. Soil microbial biomass C: N: P stoichiometry and microbial use oforganic phosphorus. Soil Biol. Biochem. 85, 119–129. https://doi.org/10.1016/j.soilbio.2015.02.029

Hinko-Najera, N., Fest, B., Livesley, S.J., Arndt, S.K., 2015. Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broadleaved evergreen forest. Agric. For. Meteorol. 200, 66–77. https://doi.org/10.1016/j.agrformet.2014.09.013

Hobbie, S.E., Ogdahl, M., Chorover, J., Chadwick, O.A., Oleksyn, J., Zytkowiak, R., Reich, P.B., 2007. Tree species effects on soil organic matter dynamics: The role of soil cation composition. Ecosystems 10, 999–1018. https://doi.org/10.1007/s10021-007-9073-4

Hoover, D.L., Wilcox, K.R., Young, K.E., 2018. Experimental droughts with rainout shelters: A methodological review. Ecosphere 9. https://doi.org/10.1002/ecs2.2088

Huang, S., Ye, G., Lin, J., Chen, K., Xu, X., Ruan, H., Tan, F., Chen, H.Y.H., 2018. Autotrophic and heterotrophic soil respiration responds asymmetrically to drought in a subtropical forest in the Southeast China. Soil Biol. Biochem. 123, 242–249. https://doi.org/10.1016/j.soilbio.2018.04.029

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. https://doi.org/10.1017/CBO9781107415324

Isbell, F., Reich, P.B., Tilman, D., Hobbie, S.E., Polasky, S., Binder, S., 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. Proc. Natl. Acad. Sci. U. S. A. 110, 11911–11916. https://doi.org/10.1073/pnas.1310880110

IUSS Working Group WRB, 2014. World Reference Base for Soil Resources 2014, Update 2015. International Soil Classification System for Naming Soils and Creating Legends for soil Maps. World Soil Resources Reports No. 106, Rome: FAO.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.D., 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108, 389–411. https://doi.org/10.1007/BF00333714

Jagodzinski, A.M., Ziolkowski, J., Warnkowska, A., Prais, H., 2016. Tree age effects on fine root biomass and morphology over chronosequences of fagus sylvatica, quercus robur and alnus glutinosa stands. PLoS One 11, 1–25. https://doi.org/10.1371/journal.pone.0148668

Janssens, I. a., Kowalski, A.S., Longdoz, B., Ceulemans, R., 2000. Assessing forest soil CO2 efflux: an in situ comparison of four techniques. Tree Physiol. 20, 23–32. https://doi.org/10.1093/treephys/20.1.23

Janssens, I., Ceulemans, R., 1998. Spatial variability in forest soil CO2 efflux assessed with a calibrated soda lime technique. Ecol. Lett. 1, 95–98. https://doi.org/10.1046/j.1461-0248.1998.00026.x

Janssens, I., Kowalski, A.S., Longdoz, B., Ceulemans, R., 2000. Assessing forest soil CO2 efflux: an in situ comparison of four techniques. Tree Physiol. 20, 23–32. https://doi.org/10.1093/treephys/20.1.23

Jewell, M.D., Shipley, B., Low-Décarie, E., Tobner, C.M., Paquette, A., Messier, C., Reich, P.B., 2017. Partitioning the effect of composition and diversity of tree communities on leaf litter decomposition and soil respiration. Oikos 126, 959–971. https://doi.org/10.1111/oik.03868

Jia, X., Shao, M., Wei, X., 2012. Responses of soil respiration to N addition, burning and clipping in temperate semiarid grassland in northern China. Agric. For. Meteorol. 166–167, 32–40. https://doi.org/10.1016/j.agrformet.2012.05.022

Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423–436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Johnson, D., Phoenix, G.K., Grime, J.P., 2008. Plant community composition, not diversity, regulates soil respiration in grasslands. Biol. Lett. 4, 345–348. https://doi.org/10.1098/rsbl.2008.0121

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2005. Greenhouse gas emissions from a managed grassland. Glob. Planet. Change 47, 201–211. https://doi.org/10.1016/j.gloplacha.2004.10.011

Kattge, J., Bönisch, G., …, Wirth, C., 2020. TRY plant trait database – enhanced coverage and open access. Glob. Chang. Biol. 26, 119–188. https://doi.org/10.1111/gcb.14904

Keil, D., Meyer, A., Berner, D., Poll, C., Schützenmeister, A., Piepho, H.P., Vlasenko, A., Philippot, L., Schloter, M., Kandeler, E., Marhan, S., 2011. Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. FEMS Microbiol. Ecol. 77, 95–106. https://doi.org/10.1111/j.1574-6941.2011.01091.x

Keith, H., Wong, S.C., 2006. Measurement of soil CO2 efflux using soda lime absorption: both quantitative and reliable. Soil Biol. Biochem. 38, 1121–1131. https://doi.org/10.1016/j.soilbio.2005.09.012

Klaus, V.H., Friedritz, L., Hamer, U., Kleinebecker, T., 2020b. Drought boosts risk of nitrate leaching from grassland fertilization. Sci. Total Environ. 726, 137877. https://doi.org/10.1016/j.scitotenv.2020.137877

Klaus, V.H., Hölzel, N., Prati, D., Schmitt, B., Schöning, I., Schrumpf, M., Solly, E.F., Hänsel, F., Fischer, M., Kleinebecker, T., 2016. Plant diversity moderates drought stress in grasslands: Implications from a large real-world study on 13C natural abundances. Sci. Total Environ. 566–567, 215–222. https://doi.org/10.1016/j.scitotenv.2016.05.008

Klaus, V.H., Kleinebecker, T., Busch, V., Fischer, M., Hölzel, N., Nowak, S., Prati, D., Schäfer, D., Schöning, I., Schrumpf, M., Hamer, U., 2018. Land use intensity, rather than plant species richness, affects the leaching risk of multiple nutrients from permanent grasslands. Glob. Chang. Biol. 24, 2828–2840. https://doi.org/10.1111/gcb.14123

Klaus, V.H., Kleinebecker, T., Hölzel, N., Blüthgen, N., Boch, S., Müller, J., Socher, S.A., Prati, D., Fischer, M., 2011. Nutrient concentrations and fibre contents of plant community biomass reflect species richness patterns along a broad range of land-use intensities among agricultural grasslands. Perspect. Plant Ecol. Evol. Syst. 13, 287–295. https://doi.org/10.1016/j.ppees.2011.07.001

Klaus, V.H., Whittingham, M.J., Báldi, A., Eggers, S., Francksen, R.M., Hiron, M., Lellei-Kovács, E., Rhymer, C.M., Buchmann, N., 2020a. Do biodiversity-ecosystem functioning experiments inform stakeholders how to simultaneously conserve biodiversity and increase ecosystem service provisioning in grasslands? Biol. Conserv. 245, 108552. https://doi.org/10.1016/j.biocon.2020.108552

Klaus, V.H.,Friedritz, L., Hamer, U., Kleinebecker, T., 2020. Drought boosts risk of nitrate leaching from grassland fertilisation. Sci. Total Environ. 726. https://doi.org/10.1016/j.scitotenv.2020.137877

Kleinebecker T., Klaus V.H., Hölzel N., 2011. Reducing Sample Quantity and Maintaining High Prediction Quality of Grassland Biomass Properties with near Infrared Reflectance Spectroscopy. Journal of Near Infrared Spectroscopy 19, 495-505. https://doi.org/10.1255/jnirs.957

Kleinebecker, T., Hölzel, N., Prati, D., Schmitt, B., Fischer, M., Klaus, V.H., 2014. Evidence from the real world: 15N natural abundances reveal enhanced nitrogen use at high plant diversity in Central European grasslands. J. Ecol. 102, 456–465. https://doi.org/10.1111/1365-2745.12202

Kleinebecker, T., Klaus, V.H., Hölzel, N., 2011. Reducing sample quantity and maintaining high prediction quality of grassland biomass properties with near infrared reflectance spectroscopy. J. Near Infrared Spectrosc. 19, 495–505. https://doi.org/10.1255/jnirs.957

Koncz, P., Balogh, J., Papp, M., Hidy, D., Pintér, K., Fóti, S., Klumpp, K., Nagy, Z., 2015. Higher soil respiration under mowing than under grazing explained by biomass differences. Nutr. Cycl. Agroecosystems 103, 201–215. https://doi.org/10.1007/s10705-015-9732-3

Kuhlmann, H., Baumgärtel, G., 1991. Potential importance of the subsoil for the P and Mg nutrition of wheat. Plant Soil 137, 259–266. https://doi.org/10.1007/BF00011204

Kukumägi, M., Ostonen, I., Uri, V., Helmisaari, H.S., Kanal, A., Kull, O., Lõhmus, K., 2017. Variation of soil respiration and its components in hemiboreal Norway spruce stands of different ages. Plant Soil 414, 265–280. https://doi.org/10.1007/s11104-016-3133-5

Kunrath, T.R., de Berranger, C., Charrier, X., Gastal, F., de Faccio Carvalho, P.C., Lemaire, G., Emile, J.C., Durand, J.L., 2015. How much do sod-based rotations reduce nitrate leaching in a cereal cropping system? Agric. Water Manag. 150, 46–56. https://doi.org/10.1016/j.agwat.2014.11.015

Lachaise, T., Bergmann, J., Rillig, M.C., van Kleunen, M., 2021. Below- and aboveground traits explain local abundance, and regional, continental and global occurrence frequencies of grassland plants. Oikos 130, 110–120. https://doi.org/10.1111/oik.07874

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nat. Commun. 6. https://doi.org/10.1038/ncomms7707

Lavorel, S., Grigulis, K., Lamarque, P., Colace, M.P., Garden, D., Girel, J., Pellet, G., Douzet, R., 2011. Using plant functional traits to understand the landscape distribution of multiple ecosystem services. J. Ecol. 99, 135–147. https://doi.org/10.1111/j.1365-2745.2010.01753.x

Ledgard, S., Schils, R., Eriksen, J., Luo, J., 2009. Environmental impacts of grazed clover/grass pastures. Irish J. Agric. Food Res. 48, 209–226.

Leimer, S., Oelmann, Y., Eisenhauer, N., Milcu, A., Roscher, C., Scheu, S., Weigelt, A., Wirth, C., Wilcke, W., 2016. Mechanisms behind plant diversity effects on inorganic and organic N leaching from temperate grassland. Biogeochemistry 131, 339–353.

Leimer, S., Oelmann, Y., Wirth, C., Wilcke, W., 2015. Time matters for plant diversity effects on nitrate leaching from temperate grassland. Agric. Ecosyst. Environ. 211, 155–163.

Leinweber, P., Meissner, R., Eckhardt, K.U., Seeger, J., 1999. Management effects on forms of phosphorus in soil and leaching losses. Eur. J. Soil Sci. 50, 413–424. https://doi.org/10.1046/j.1365-2389.1999.00249.x

Le Provost, G., Thiele, J., Westphal, C., …, Manning, P., 2021. Contrasting responses of above- and belowground diversity to multiple components of land-use intensity. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-23931-1

Li, Y., Liu, Y., Wu, S., Niu, L., Tian, Y., 2015. Microbial properties explain temporal variation in soil respiration in a grassland subjected to nitrogen addition. Sci. Rep. 5, 1–11. https://doi.org/10.1038/srep18496

Lu, M., Zhou, X., Luo, Y., Yang, Y., Fang, C., Chen, J., Li, B., 2011. Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. Agric. Ecosyst. Environ. 140, 234–244. https://doi.org/10.1016/j.agee.2010.12.010

Luan, J., Liu, S., Wang, J., Chang, S.X., Liu, X., Lu, H., Wang, Y., 2018. Tree species diversity promotes soil carbon stability by depressing the temperature sensitivity of soil respiration in temperate forests. Sci. Total Environ. 645, 623–629. https://doi.org/10.1016/j.scitotenv.2018.07.036

Lucas-Borja, M.E., Hedo, J., Cerdá, A., Candel-Pérez, D., Viñegla, B., 2016. Unravelling the importance of forest age stand and forest structure driving microbiological soil properties, enzymatic activities and soil nutrients content in Mediterranean Spanish black pine (Pinus nigra Ar. ssp. salzmannii) Forest. Sci. Total Environ. 562, 145–154. https://doi.org/10.1016/j.scitotenv.2016.03.160

Luo, Q., Gong, J., Zhai, Z., Pan, Y., Liu, M., Xu, S., Wang, Y., Yang, L., Baoyin, T. tao, 2016. The responses of soil respiration to nitrogen addition in a temperate grassland in northern China. Sci. Total Environ. 569–570, 1466–1477. https://doi.org/10.1016/j.scitotenv.2016.06.237

Luo, Y., Zhou, X., 2006. Soil respiration and the environment, 1st ed. Elsevier.

Manning, P., Loos, J., Barnes, A.D., ..., Tscharntke, T., 2019. Chapter Ten - Transferring biodiversity-ecosystem function research to the management of 'real-world' ecosystems. Adv. Ecol. Res. 61, 323–356. https://doi.org/10.1016/bs.aecr.2019.06.009

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. Science (80-.). 321, 684–686. https://doi.org/10.1126/science.1159792

Mathot, M., Mertens, J., Verlinden, G., Lambert, R., 2008. Positive effects of sulphur fertilisation on grasslands yields and quality in Belgium. European Journal of Agronomy 28, 655-658. https://doi.org/10.1016/j.eja.2007.12.004

McCoy, K., Zimbron, J., Sale, T., Lyverse, M., 2015. Measurement of Natural Losses of LNAPL Using CO2 Traps. Groundwater 53, 658–667. https://doi.org/10.1111/gwat.12240

Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold, G., Knorr, M.A., Grandy, A.S., 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. Science (80). 358, 101–105. https://doi.org/10.1126/science.aan2874

Meyer, A., Focks, A., Radl, V., Keil, D., Welzl, G., Schöning, I., Boch, S., Marhan, S., Kandeler, E., Schloter, M., 2013. Different land use intensities in grassland ecosystems drive ecology of microbial communities involved in nitrogen turnover in soil. Plos One 8(9). https://doi.org/10.1371/journal.pone.0073536

Midolo, G., Alkemade, R., Schipper, A.M., Benítez-López, A., Perring, M.P., De Vries, W., 2019. Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. Glob. Ecol. Biogeogr. 28, 398–413. https://doi.org/10.1111/geb.12856

Moinet, G.Y.K., Midwood, A.J., Hunt, J.E., Rumpel, C., Millard, P., Chabbi, A., 2019. Grassland management influences the response of soil respiration to drought. Agronomy 9. https://doi.org/10.3390/agronomy9030124

Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L., Mougel, C., 2015. Plant traits related to nitrogen uptake influence plantmicrobe competition. Ecology 96, 2300–2310. https://doi.org/10.1890/14-1761.1

Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem. 59, 72–85. https://doi.org/10.1016/j.soilbio.2013.01.002

Muhr, J., Borken, W., 2009. Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil. J. Geophys. Res. Biogeosciences 114, 1–11. https://doi.org/10.1029/2009JG000998

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. https://doi.org/10.1111/j.1365-2486.2008.01695.x

Näthe, K., Levia, D.F., Tischer, A., Michalzik, B., 2018. Low-intensity surface fire effects on carbon and nitrogen cycling in soil and soil solution of a Scots pine forest in central Germany. Catena 162, 360–375. https://doi.org/10.1016/j.catena.2017.10.026

Niklaus, P.A., Kandeler, E., Leadley, P.W., Schmid, B., Tscherko, D., Korner, C., 2001. A link between plant diversity, elevated CO2 and soil nitrate. Oecologia 127, 540–548. https://doi.org/10.1007/s004420000612

Nikolova, P.S., Raspe, S., Andersen, C.P., Mainiero, R., Blaschke, H., Matyssek, R., Häberle, K.H., 2009. Effects of the extreme drought in 2003 on soil respiration in a mixed forest. Eur. J. For. Res. 128, 87–98. https://doi.org/10.1007/s10342-008-0218-6

Oelmann, Y., Richter, A.K., Roscher, C., Rosenkranz, S., Temperton, V.M., Weisser, W.W., Wilcke, W., 2011. Does plant diversity influence phosphorus cycling in experimental grasslands? Geoderma 167-168, 178-187. https://doi.org/10.1016/j.geoderma.2011.09.012

Ostrowski, A., Lorenzen, K., Petzold, E., Schindler, S., 2020. Land use intensity index (LUI) calculation tool of the Biodiversity Exploratories project for grassland survey data from three different regions in German since 2006. Zenodo. https://doi.org/10.25504/FAIRsharing.6AmTXC

Pan, Y., Birdsay, R.A., Fang, J., Houghton, R., Kauppi, P., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A Large and Persistent Carbon Sink in the World's Forests. Science 333, 988-993. DOI: 10.1126/science.1201609

Pangle, R.E., Hill, J.P., Plaut, J.A., Yepez, E.A., Elliot, J.R., Gehres, N., McDowell, N.G., Pockman, W.T., 2012. Methodology and performance of a rainfall manipulation experiment in a piñon–juniper woodland. Ecosphere 3, 1-20. https://doi.org/10.1890/ES11-00369.1

Pommier, T., Cantarel, A.A.M., Grigulis, K., Lavorel, S., Legay, N., Baxendale, C., Bardgett, R.D., Bahn, M., Poly, F., Clément, J.C., 2018. The added value of including key microbial traits to determine nitrogen-related ecosystem services in managed grasslands. J. Appl. Ecol. 55, 49–58. https://doi.org/10.1111/1365-2664.13010

Powers, M., Kolka, R., Bradford, J., Palik, B., Jurgensen, M., 2017. Forest floor and mineral soil respiration rates in a northern Minnesota red pine chronosequence. Forests 9, 1–15. https://doi.org/10.3390/f9010016

Predotova, M., Bischoff, W.A., Buerkert, A., 2011. Mineral-nitrogen and phosphorus leaching from vegetable gardens in Niamey, Niger. J. Plant Nutr. Soil Sci. 174, 47–55. https://doi.org/10.1002/jpln.200900255

Purahong, W., Kapturska, D., Pecyna, M.J., Jariyavidyanont, K., Kaunzner, J., Juncheed, K., Uengwetwanit, T., Rudloff, R., Schulz, E., Hofrichter, M., Schloter, M., Krüger, D., Buscot, F., 2015. Effects of Forest Management Practices in Temperate Beech Forests on Bacterial and Fungal Communities Involved in Leaf Litter Degradation. Microb. Ecol. 69, 905–913. https://doi.org/10.1007/s00248-015-0585-8

R Core Team, 2019. R: a language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL: https://www.R-project.org/

Raich, J.W., Tufekciogul, A., 2000. Vegetation and soil respiration: correlations and controls [review]. Biogeochemistry 48, 71–90. https://doi.org/10.1023/A:1006112000616

Reich, P.B., 2014. The world-wide "fast-slow" plant economics spectrum: A traits manifesto. J. Ecol. 102, 275–301. https://doi.org/10.1111/1365-2745.12211

Reichstein, M., Rey, A., Freibauer, A., ..., Yakir, D., 2003. Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices. Global Biogeochem. Cycles 17, 1104. https://doi.org/10.1029/2003gb002035

Robson, T.M., Baptist, F., Clément, J.C., Lavorel, S., 2010. Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics. J. Ecol. 98, 62–73. https://doi.org/10.1111/j.1365-2745.2009.01609.x

Rodeghiero, M., Cescatti, A., 2005. Main determinants of forest soil respiration along an elevation/temperature gradient in the Italian Alps. Glob. Chang. Biol. 11, 1024–1041. https://doi.org/10.1111/j.1365-2486.2005.00963.x

Rosseel, Y., 2012. lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software 48. DOI: 10.18637/jss.v048.i02

Rumpel, C., Crème, A., Ngo, P.., Velásquez, G., Mora, M.., Chabbi, A., 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. J. soil Sci. plant Nutr. 15, 0–0. https://doi.org/10.4067/S0718-95162015005000034

Ryden, J.C., Ball, P.R., Garwood, E.A., 1984. Nitrate leaching from grassland. Nature 311, 10. https://doi.org/10.1038/311010a0

Salter, P.J., Williams, J.B., 1965. The influence of texture on the moisture characteristics of soils. II. Available-water capacity and moisture release characteristics. J. Soil Sci. 16, 310–317.

Schall, P., Ammer, C., 2013. How to quantify forest management intensity in Central European forests. Eur. J. For. Res. 132, 379–396. https://doi.org/10.1007/s10342-013-0681-6

Schall, P., Schulze, E.D., Fischer, M., Ayasse, M., Ammer, C., 2018. Relations between forest management, stand structure and productivity across different types of Central European forests. Basic Appl. Ecol. 32, 39–52. https://doi.org/10.1016/j.baae.2018.02.007

Scherer-Lorenzen, M., Palmborg, C., Prinz, A., Schulze, E., 2003. The Role of Plant Diversity and Composition for Nitrate Leaching in Grasslands Ecology 84, 1539–1552. https://doi.org/10.1890/0012-9658(2003)084[1539:TROPDA]2.0.CO;2

Schindlbacher, A., Wunderlich, S., Borken, W., Kitzler, B., Zechmeister-Boltenstern, S., Jandl, R., 2012. Soil respiration under climate change: Prolonged summer drought offsets soil warming effects. Glob. Chang. Biol. 18, 2270–2279. https://doi.org/10.1111/j.1365-2486.2012.02696.x

Schleuss, P.M., Widdig, M., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2021. Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils. Soil Biol. Biochem. 155, 108168. https://doi.org/10.1016/j.soilbio.2021.108168

Schwarz, M.T., Bischoff, S., Blaser, S., Boch, S., Schmitt, B., Thieme, L., Fischer, M., Michalzik, B., Schulze, E.D., Siemens, J., Wilcke, W., 2014. More efficient aboveground nitrogen use in more diverse Central European forest canopies. For. Ecol. Manage. 313, 274–282. https://doi.org/10.1016/j.foreco.2013.11.021

Selsted, M.B., van der Linden, L., Ibrom, A., Michelsen, A., Larsen, K.S., Pedersen, J.K., Mikkelsen, T.N., Pilegaard, K., Beier, C., Ambus, P., 2012. Soil respiration is stimulated by elevated CO2and reduced by summer drought: Three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). Glob. Chang. Biol. 18, 1216–1230. https://doi.org/10.1111/j.1365-2486.2011.02634.x

Shabaga, J.A., Basiliko, N., Caspersen, J.P., Jones, T.A., 2015. Seasonal controls on patterns of soil respiration and temperature sensitivity in a northern mixed deciduous forest following partial-harvesting. For. Ecol. Manage. 348, 208–219. https://doi.org/10.1016/j.foreco.2015.03.022

Shipley, B., 2016. Cause and Correlation in Biology. Cambridge. https://doi.org/10.1017/CBO9781139979573

Socher, S.A., Prati, D., Boch, S., Müller, J., Klaus, V.H., Hölzel, N., Fischer, M., 2012. Direct and productivity-mediated indirect effects of fertilization, mowing and grazing on grassland species richness. J. Ecol. 100, 1391–1399. https://doi.org/10.1111/j.1365-2745.2012.02020.x

Søe, A.R.B., Buchmann, N., 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiol. 25, 1427–1436. https://doi.org/10.1093/treephys/25.11.1427

Solly, E.F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Müller, J., Zscheischler, J., Trumbore, S.E., Schrumpf, M., 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant Soil 382, 203–218. https://doi.org/10.1007/s11104-014-2151-4

Sorkau, E., Boch, S., Boeddinghaus, R.S., Bonkowski, M., Fischer, M., Kandeler, E., Klaus, V.H., Kleinebecker, T., Marhan, S., Müller, J., Prati, D., Schöning, I., Schrumpf, M., Weinert, J., Oelmann, Y., 2018. The role of soil chemical properties, land use and plant diversity for microbial phosphorus in forest and grassland soils. J. Plant Nutr. Soil Sci. 181, 185–197. https://doi.org/10.1002/jpln.201700082

Soulides, D.A., and Clark, F.A., 1958. Nitrification in grassland soils. Soil Science Society of America Journal 22. https://doi.org/10.2136/sssaj1958.03615995002200040011x

Spehn, E.M., Joshi, J., Schmid, B., Alphei, J., Körner, C., 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224, 217–230. https://doi.org/10.1023/A:1004891807664

Spohn, M., Kuzyakov, Y., 2013. Phosphorus mineralization can be driven by microbial need for carbon. Soil Biol. Biochem. 61, 69–75. https://doi.org/10.1016/j.soilbio.2013.02.013

State and Outlook Environmental Report, 2020. The European Environment. European Environment Agency

Steinbeiss, S., Beßler, H., Engels, C., Temperton, V.M., Buchmann, N., Roscher, C., Kreutziger, Y., Baade, J., Habekost, M., Gleixner, G., 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. Glob. Chang. Biol. 14, 2937–2949. https://doi.org/10.1111/j.1365-2486.2008.01697.x

Stempfhuber, B., Welzl, G., Wubet, T., Schöning, I., Marhan, S., Buscot, F., Kandeler, E., Schloter, M., 2014. Drivers for ammonia-oxidation along a land-use gradient in grassland soils. Soil Biol. Biochem. 69, 179-186. https://doi.org/10.1016/j.soilbio.2013.11.007

Strecker, T., Barnard, R.L., Niklaus, P.A., Scherer-Lorenzen, M., Weigelt, A., Scheu, S., Eisenhauer, N., 2015. Effects of plant diversity, functional group composition, and fertilization on soil microbial properties in experimental grassland. PLoS One 10, 1–16. https://doi.org/10.1371/journal.pone.0125678

Subke, J.A., Inglima, I., Cotrufo, M.F., 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Glob. Chang. Biol. 12, 921–943. https://doi.org/10.1111/j.1365-2486.2006.01117.x

Sun, S., Lei, H., Chang, S.X., 2019. Drought differentially affects autotrophic and heterotrophic soil respiration rates and their temperature sensitivity. Biol. Fertil. Soils 55, 275–283. https://doi.org/10.1007/s00374-019-01347-w

Tilman, D., Isbell, F. & Cowles, J.M. 2014. Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics, 45, 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 376, 718–720. https://doi.org/https://doi.org/10.1038/379718a0

Trenberth, K.E., Dai, A., Van Der Schrier, G., Jones, P.D., Barichivich, J., Briffa, K.R., Sheffield, J., 2014. Global warming and changes in drought. Nat. Clim. Chang. 4, 17–22. https://doi.org/10.1038/nclimate2067

Tzanakakis, V.A., Apostolakis, A., Nikolaidis, N.P., Paranychianakis, N.V., 2018. Ammonia oxidizing archea do not respond to ammonium or urea supply in an alkaline soil. Applied Soil Ecology 132, 194-198. https://doi.org/10.1016/j.apsoil.2018.08.002

United Nations, 2019. World population prospects 2019, Department of Economic and Social Affairs. World Population Prospects 2019.

Vance, E.D, Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707. https://doi.org/10.1016/0038-0717(87)90052-6

Vesterdal, L., Clarke, N., Sigurdsson, B.D., Gundersen, P., 2013. Do tree species influence soil carbon stocks in temperate and boreal forests? For. Ecol. Manage. 309, 4–18. https://doi.org/10.1016/j.foreco.2013.01.017

Vesterdal, L., Elberling, B., Christiansen, J.R., Callesen, I., Schmidt, I.K., 2012. Soil respiration and rates of soil carbon turnover differ among six common European tree species. For. Ecol. Manage. 264, 185–196. https://doi.org/10.1016/j.foreco.2011.10.009

Vitousek, P.M., 1994. Beyond global warming: Ecology and global change. Ecology 75, 1861–1876. https://doi.org/10.2307/1941591

Wang, W., Fang, J., 2009. Soil respiration and human effects on global grasslands. Glob. Planet. Change 67, 20–28. https://doi.org/10.1016/j.gloplacha.2008.12.011

Ward, D., Kirkman, K., Hagenah, N., Tsvuura, Z., 2017. Soil respiration declines with increasing nitrogen fertilization and is not related to productivity in long-term grassland experiments. Soil Biol. Biochem. 115, 415–422. https://doi.org/10.1016/j.soilbio.2017.08.035

Werner, W., Trimborn, M., Pihl, U., 2006. Prediction of the P-leaching potential of arable soils in areas with high livestock densities. J. Zhejiang Univ. Sci. B. 7, 515–520. https://doi.org/10.1631/jzus.2006.B0515

Widdig, M., Heintz-Buschart, A., Schleuss, P.M., Guhr, A., Borer, E.T., Seabloom, E.W., Spohn, M., 2020. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. Soil Biol. Biochem. 150. https://doi.org/10.1016/j.soilbio.2020.108041

Willich, M., Buerkert, A., 2016. Leaching of carbon and nitrogen from a sandy soil substrate: A comparison between suction plates and ion exchange resins. J. Plant Nutr. Soil Sci. 179, 609–614. https://doi.org/10.1002/jpln.201600036

Withers, P.J.A., Neal, C., Jarvie, H.P., Doody, D.G., 2014. Agriculture and eutrophication: Where do we go from here? Sustain. 6, 5853–5875. https://doi.org/10.3390/su6095853

World Health Organization (WHO), 2011. Nitrate and Nitrite in Drinking-water; Background documents for development of WHO Guidelines for Drinking-water Quality.

Wright, I., Reich, P.B., Westoby, M., ..., Villar, R., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol. Monogr. 85, 133–155. https://doi.org/10.1890/14-0777.1

Zhang, C., Niu, D., Hall, S.J., Wen, H., Li, X., Fu, H., Wan, C., Elser, J.J., 2014. Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in a semiarid grassland. Soil Biol. Biochem. 75, 113–123. https://doi.org/10.1016/j.soilbio.2014.04.013

Zhang, D., Hui, D., Luo, Y., Zhou, G., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. J. Plant Ecol. 1, 85–93. https://doi.org/10.1093/jpe/rtn002

Zhao, X., Li, F., Zhang, W., Ai, Z., Shen, H., Liu, X., Cao, J., Manevski, K., 2016. Soil respiration at different stand ages (5, 10, and 20/30 years) in coniferous (Pinus tabulaeformis Carrière) and deciduous (Populus davidiana Dode) plantations in a sandstorm Source Area. Forests 7, 1–15. https://doi.org/10.3390/f7080153

Zobel, R.W., Kinraide, T.B., Baligar, V.C., 2007. Fine root diameters can change in response to changes in nutrient concentrations. Plant Soil 297, 243–254. https://doi.org/10.1007/s11104-007-9341-2

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in Ecology with R. Springer, book series: Statistics for biology and health. DOI: 10.1007/978-0-387-87458-6

Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Ort, Datum Unterschrift des Verfassers