Nutr Cycl Agroecosyst (2022) 124:101–116
https://doi.org/10.1007/s10705-022-10224-2

ORIGINAL ARTICLE

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

Antonios Apostolakis · Ingo Schöning · Beate Michalzik · Valentin H. Klaus · Runa S. Boeddinghaus · Ellen Kandeler · Sven Marhan · Ralph Bolliger · Markus Fischer · Daniel Prati · Falk Hänsel · Thomas Nauss · Norbert Hölzel · Till Kleinebecker · Marion Schrumpf

Received: 31 May 2022 / Accepted: 31 July 2022 / Published online: 16 August 2022

Abstract Soil respiration is an important pathway of soil organic carbon losses in temperate grasslands; however, it is rarely studied across broad management intensity gradients in a landscape. Using the soda-lime method, we measured in-situ soil CO_2 efflux with single measurements of long exposure time (i.e. 3 day long) in 150 grasslands in three German regions in early summer 2018 and 2019. The grasslands ranged from unfertilized and grazed grasslands to intensively fertilized and frequently harvested ones. To assess effects of grazing and fertilization intensities and plant diversity on soil CO_2 efflux, we used Structural Equation Modeling to account for direct effects and indirect effects through soil and plant organic matter quantity and quality. Soil CO_2 efflux was suppressed by limited water availability caused by naturally occurring droughts in both study years. Under the prevailing environmental conditions, grazing intensity, plant biomass and plant C:N ratio were not related to soil CO_2 efflux. In contrast, fertilization intensity was positively associated with soil CO_2 efflux (standardized coefficient of net effect: + 0.04 in 2018 and + 0.03 in 2019). This was because fertilization led to lower plant species richness and, thus,

A. Apostolakis (✉) · I. Schöning · M. Schrumpf
Max-Planck-Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany
e-mail: apostolakis@bgc-jena.mpg.de

A. Apostolakis · B. Michalzik
Department of Soil Science, Friedrich Schiller University Jena, Löbdergraben 32, 07743 Jena, Germany

B. Michalzik
German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leibzig, Deutscher Platz 5e, 04103 Leipzig, Germany

V. H. Klaus
Institute of Agricultural Sciences, ETH Zürich, Universitätstr. 2, 8092 Zurich, Switzerland

R. S. Boeddinghaus · E. Kandeler · S. Marhan
Institute of Soil Science and Land Evaluation, Soil Biology Department, University of Hohenheim, Emil-Wolff-Str. 27, 70599 Stuttgart, Germany

R. Bolliger · M. Fischer · D. Prati
Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

F. Hänsel · T. Nauss
Environmental Informatics, Department of Geography, Phillips-Universität Marburg, Deutschhausstraße 12, 35037 Marburg, Germany

N. Hölzel
Institute of Landscape Ecology, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany

T. Kleinebecker
Institute of Landscape Ecology and Resource Management, Justus-Liebig-University Gießen, Heinrich-Buff-Ring 26-32, 35392 Gießen, Germany

T. Kleinebecker
Centre for International Development and Environmental Research (ZEU), Justus-Liebig-University Giessen, Senckenbergstrasse 3, 35390 Giessen, Germany

to lower C:N ratios in soils, which were associated with higher soil CO_2 efflux (plant species richness net effect: −0.09 in 2018 and −0.18 in 2019; soil C:N ratio direct effect: −0.23 in 2018 and −0.33 in 2019). Intensively managed grasslands have higher soil respiration than extensively managed, plant species-rich grasslands even under the extreme conditions of natural droughts.

Keywords Soil CO_2 efflux · Fertilization · Grazing · Organic matter quantity · Organic matter quality · Plant diversity

Introduction

Temperate grasslands store large amounts of carbon (C) in soils (Jobbagy and Jackson, 2000), and the fate of this C is determined by the balance between C inputs and losses due to decomposition. Grassland management intensification potentially threatens soil organic carbon (OC) stocks (Jacobs et al. 2020; Conant et al. 2017), since it can alter carbon fluxes, and especially soil respiration. Improving our understanding on the interactions among management, biotic drivers and soil respiration, while accounting for abiotic and environmental drivers (i.e. soil properties and climate), can provide important insights for sustainable grassland management and climate change mitigation (FAO 2010).

Intensification of grassland management, which mainly includes fertilization, mowing and grazing, increases soil respiration rates globally (Raich and Tufekcioglu 2000; Wang and Fang 2009). Fertilization, in general, increases soil respiration, but its effect also depends on the amounts and type of nitrogen (N) applied (Jones et al. 2006; Luo et al. 2016). Both mowing and grazing reduce soil respiration presumably by removing plant biomass (Bahn et al. 2008). 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, hampering our understanding of management effects on grassland soil respiration. Since the temporal patterns of soil respiration remain relatively constant during the growing season (Johnson et al. 2008), short-term measurement campaigns can be used to sample a high number of replicated plots (Dias et al. 2010) and cover broad management gradients.

Grassland management can influence soil, plant and microbial properties to various degrees (Boeddinghaus et al. 2019; 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 increases plant productivity (Socher et al. 2012) and the associated C inputs into the soil (da Silva et al. 2022), which increases the substrate for soil fauna and microbial activity. In addition, fertilization decreases soil C:N ratio and increases enzymatic activities (Herold et al. 2014a), possibly leading to increased C losses via decomposition. However, it is still not clear whether the quantity or the quality of organic matter, which are both influenced by management, mainly control soil respiration in temperate grasslands.

Fertilization is a main driver of plant diversity loss in grasslands (Blüthgen et al. 2012; Midolo et al. 2018), which might have an adverse effect on soil OC since recent evidence suggests that plant diversity increases C storage and decreases losses (Lange et al. 2015; Steinbeiss et al. 2008). Furthermore, fertilization of grasslands shifts soil microbial composition 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). In conclusion, responses of soil respiration to grassland management should not only be reported as direct effects, but also account for changes that management causes to the soil, plant and microbial systems (Apostolakis et al. 2022; Koncz et al. 2015).

When investigating the effects of plant diversity on soil respiration both positive (Chen and Chen 2019; Chen et al. 2014; Dias et al. 2010) and neutral (Dietrich et al. 2017) effects have been reported. For instance, plant diversity can increase plant biomass productivity, and exert a positive effect on soil respiration (Craine et al. 2001; Dias et al. 2010). In contrast, a higher N use efficiency found in species-rich grasslands (Kleinebecker et al. 2014) can lead to lower N concentrations in plant biomass and litter (Fargione et al. 2007) and, thus, to reduced decomposability. Dias et al. (2010), using Structural Equation Modeling, found that plant diversity increased soil respiration in experimental grasslands (1) by increasing aboveground plant biomass, and (2) by

alleviating the negative relationship between plant nitrogen on plant biomass. However, 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 experimental 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 interactive effects of management practices (Klaus et al. 2020a), which are, however, a main driving force of plant diversity and ecosystem functioning in real world ecosystems.

We obtained single soil CO_2 efflux measurements of long exposure time (i.e. 3 day long) from 150 grasslands in the *Biodiversity Exploratories* project, which cover a broad management intensity gradient. The 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, which affected our study regions. Our hypotheses were:

- Grassland soil respiration rates differ among the three study regions due to climatic and soil differences.
- Fertilization and grazing intensification indirectly increase soil respiration due to increased quantity (i.e. higher plant productivity and soil organic matter) and quality (i.e. lower C:N ratios in soils and plant biomass) of organic matter.
- Extensive, plant species-rich grasslands have lower soil respiration fluxes than intensively managed grasslands due to lower nutrient status.

Methods

Study regions

This study was conducted as part of the *Biodiversity Exploratories* project that includes three regions in Germany; the Schwäbische-Alb (ALB), the Hainich-Dün (HAI) and the Schorfheide-Chorin (SCH) (Fischer et al. 2010). The regions differ in their geology, climate and topology (Table S1). In 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 region, we selected 50 agriculturally managed permanent grasslands and, within each grassland, we established a plot of 50 m × 50 m (Fischer et al. 2010). Information regarding the regional management practices as well as the basic plant, microbial and soil properties are given in Table 1. Management ranged from unfertilized to heavily fertilized and intensively grazed and/or mowed grasslands, covering a wide management gradient. Fertilization, expressed as N additions, was applied as organic and/or mineral fertilizer. Grazing, expressed as equivalent-livestock units 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. Due to the high correlation between fertilization and harvesting in our grasslands, mowing (expressed as cuts per year) was not included as an explanatory variable.

Plant properties

Vegetation data were collected in May 2018 and 2019 at peak standing biomass. Aboveground biomass was measured in eight 0.5 m × 0.5 m subplots in each grassland. The subplots were fenced to prevent livestock grazing or mowing prior harvest. Vegetation was clipped at 20 mm height, dried at 80 °C for 48 h, weighed and ground for chemical analysis. In a nearby 4 m × 4 m subplot, we recorded all vascular plant species to determine plant species richness. We further determined the C and N 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.

Soil properties

One composite soil sample per grassland was prepared from fourteen soil subsamples of the upper

Table 1 Mean values and standard deviation of management practices and soil, plant and microbial properties of the three study regions

Parameter	Study region		
	Schwäbische-Alb (ALB)	Hainich-Dün (HAI)	Schorfheide-Chorin (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
Abovegr. biomass C:N ratio (2018)	24.4 ± 3.8ab*	23.7 ± 2.6b	26.4 ± 5.4a*
Abovegr. biomass C:N ratio (2019)	21.7 ± 4.9	21.7 ± 2.2	22.0 ± 5.6
Fine root biomass (g cm^{-3}, 2011)	1.3 ± 1.1b	6.0 ± 2.5a	6.9 ± 4.2a
Microbial properties			
Microbial carbon (µg C g^{-1})	815.7 ± 152a	729.1 ± 159.3a	582.7 ± 352.5b
Microbial nitrogen (µg N g^{-1})	137.4 ± 38.7	140.4 ± 41.4	136.3 ± 97.1
Soil properties			
Clay (g kg^{-1} soil)	536.1 ± 133.3a	422.6 ± 126.6b	173.8 ± 86.5c
Silt (g kg^{-1} soil)	406.6 ± 114.0b	520.122.0a	372.1 ± 190.7b
Sand (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
Soil organic carbon (g C kg^{-1})	69.8 ± 14.3b	49.9 ± 12.1b	95.5 ± 90.7a
Soil nitrogen (g N kg^{-1})	6.8 ± 1.5b	4.9 ± 1.2b	10.4 ± 9.0a
Soil C:N ratio	10.4 ± 0.7a	10.2 ± 0.5a	9.4 ± 1.4b

Fertilization, grazing and mowing intensity data were obtained from Ostrowski et al. (2020) and averaged for the period 2015–18 and 2016–19. Plant properties were measured both in 2018 and 2019. Microbial and soil properties were measured in 2017. Fine root biomass and soil texture were measured in 2011. Lower-case letters indicate differences among the three study regions in a given period. Asterisks indicate significant differences between 2018 and 2019 in a given study region

Differences were considered significant at p-value <0.050

0.10 m of the soil along two intersecting 20 m transects in May 2017. All soil samples were sieved to < 2 mm and air-dried, and an aliquot 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 C and total N 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 (OC) by exposing 250 mg of soil to 450 °C for 16 h. Soil OC 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.

Microbial properties

Aliquots of sieved soil (from the composite samples collected in May 2017, stored at -20 °C) were used for microbial analysis. Microbial C and N concentration was determined with the chloroform-fumigation-extraction method (CFE), according to Vance et al. (1987) and Keil et al. (2011). Fumigated sample replicates were incubated with ethanol-free $CHCl_3$ for 24 h. C and N were extracted from each fumigated and non-fumigated replicate (5 g each) with 40 ml 0.5 M K_2SO_4. The suspension was horizontally shaken (30 min, 150 rpm) and centrifuged (30 min, 4400 × 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.

Soil temperature and volumetric water content

Soil temperature (°C) and volumetric water content (%) were recorded in 30-min intervals in each grassland since 2008 using the ADL-MX Data Logger System (Meier-NT GmbH, Zwönitz, Germany). Instruments were installed in fenced areas (2 m × 2 m) within the managed grasslands. Soil temperature was monitored at 0.05 m depth below the surface of the mineral soil with the MNT-FExtension and water content at 0.10 m 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 study year.

Soil CO_2 efflux

Soil CO_2 efflux was measured with an absorption method using soda-lime as the absorption material along with an open and static chamber. The chamber design was based on previous work of Bierbaß et al. (2015) and Näthe et al. (2018). The chamber consisted of a PVC ring (internal diameter = 0.10 m and height = 0.12 m), a PVC lid and an O-ring to ensure air-tightness (Fig. S1). 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 from 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 installation time was about three days (i.e. 65 to 70 h). For each measurement, we used 12 g of dried soda-lime per installation day. We determined the absorbed CO_2 mass by the difference between the dried soda-lime mass before the field measurement and the dried soda-lime mass after the field measurement (i.e. weight gain, WG). The soil CO_2 efflux was calculated by the equation (Keith and Wong 2006):

$$R_s\left[gCO_2 - Cm^{-2}d^{-1}\right] = \frac{\mathrm{WG}_{\mathrm{sample}}\left[gCO_2\right] - \mathrm{WG}_{\mathrm{control}}\left[gCO_2\right]}{\mathrm{CA}\left[m^2\right]} \times \frac{24\left[\frac{h}{d}\right]}{T[h]} \times \frac{12\left[\frac{gC}{\mathrm{mol}}\right]}{44\left[\frac{gCO_2}{\mathrm{mol}}\right]} \times 1.69$$

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

Soil CO_2 efflux measurements were taken from June to July in 2018 and 2019 following the order HAI (from 28 of May to 20 of June), SCH (from 21 of June to 12 of July) and ALB (from 14 to 30 of

July). Four chambers, forming a square of 10 m side length, were installed for the measurement of soil CO_2 efflux 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 10–20 mm soil depth. Measurements (i.e. installation of soda-lime and PVC lids) 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 campaigns, we installed 1200 traps and 300 controls for the determination of soil CO_2 effluxes.

In our study, the soda-lime mass gain was lower than the proposed limit of 10% per dry mass (given a saturation point of about 28% per dry mass, Janssens et al. 2000) to avoid saturation of soda-lime (Table S2). In addition, 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, we did not detect significant differences between measurements taken with the two methods (Fig. S2).

Data analysis

Using ANOVA models and the Tukey's Honest Significant Difference (HSD) test, we tested for differences in in-situ soil CO_2 efflux, soil, plant and microbial properties and environmental conditions among the three study regions, and, when possible, between the two study years. Differences were considered significant at p-value < 0.05. We performed a variance partitioning to find the relative importance of (1) climate (i.e. soil temperature and moisture), (2) management (i.e. fertilization and grazing intensities), (3) plant properties (i.e. plant species richness, aboveground biomass, fine root biomass and plant C:N ratio), (4) microbial properties (microbial C and C:N ratio) and (5) soil properties (SOC, soil C:N ratio, pH and silt content) on soil CO_2 efflux. Then, we ran a (backward elimination) stepwise analysis to evaluate the importance of the individual variables on model performance based on the Akaike information criterion. Based on the selected variables from stepwise analysis, we examined the significance of these variables 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 applied to evaluate the assumptions of linearity, normality and homoscedasticity in the residuals, and to check for influential values. We applied logarithmic transformations on soil CO_2 efflux and plant species richness to tackle heteroscedasticity issues. In the selected set of independent variables for the ANCOVA models, we used the variance inflation ratio to test for collinearities. The (maximum) variance inflation ratios was ≤ 3.0 (in detail: 1.4 in 2018 and 1.6 in 2019) and our models were considered acceptable.

We used Structural Equation Modeling (SEM) to separate direct from indirect effects of grazing and fertilization intensities on soil respiration and, further, to test whether the indirect effects of grazing and fertilization are mediated by organic matter quantity and/or quality. For this, we developed an initial structure where fertilization intensity, grazing intensity and soil water content were included as independent variables, while plant species richness, soil organic carbon, soil C:N ratio, plant biomass and plant C:N ratio and soil CO_2 efflux were included as dependent variables (Fig. S3). However, plant biomass and plant C:N ratio were never associated with soil CO_2 efflux and, thus, they were not included in the final models. Using one-way ANOVA models, we removed the effects of study regions from each variable, and we used their residuals in the SEMs. Structures with p-values > 0.05 and root mean square errors of approximation (RMSEA) < 0.05 were considered acceptable. The high number of observations (i.e. 139 observations in 2018 and 147 observations in 2019 from 150 grasslands due to missing values) compared to estimated parameters provides stability against multivariate non-normality issues. In addition, we used bootstrapping (with 1000 bootstrap draws) for additional stability. Structural Equation Models represent associations between variables, and not necessarily causal relationships. However, we interpret these associations as evidence of management effects and, for simplicity, we often use terms such as 'effects' and 'drivers' hereafter.

Statistical analysis was performed with the R software (R Core Team 2020). ANOVA and ANCOVA

models were performed with the *lm* function. Tukey's HSD test was performed with the *TukeyHSD* of the *stats* package. For the backward elimination stepwise analysis the *step* function from the *stats* package was used. Variance inflation ratio was calculated with the *vif* function of the *faraway* package (Faraway 2016). Variance partitioning was performed with the *calc.relimp* function using the *lmg* method of the *relaimpo* package (Groemping 2006). Structural Equation Modeling was performed with the *sem* function from the *lavaan* package (Rosseel 2012).

Results

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 (Fig. 1a). 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 (Fig. 1b). Between the two study years, soil water content differed only in HAI, and it was higher 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 (Fig. S4). 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 (Fig. S5).

In-situ soil CO_2 efflux

In the three study regions, in-situ soil CO_2 efflux ranged from 0.5 to 6.9 in 2018 and 1.1 to 9.2 g CO_2-C m^{-2} d^{-1} in 2019. In 2018, soil CO_2 efflux did not differ among the study regions, while in 2019 it increased in the order ALB = SCH < HAI (Fig. 1c). Between the two study years, soil CO_2 efflux differed only in HAI, and it was higher in 2019 than 2018, following the increase observed in soil volumetric water content (Fig. 1b).

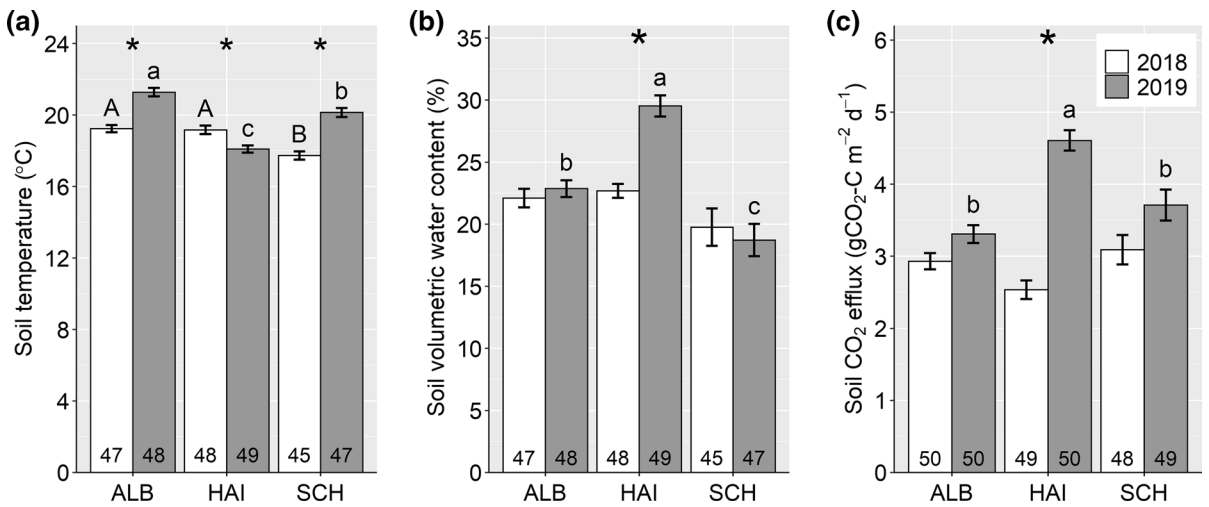

Fig. 1 **a** Soil temperature at 0.05 m soil depth, **b** soil volumetric water content at 0.10 m soil depth and **c** soil respiration in 2018 (white) and 2019 (grey) for the three study regions; Schwäbische-Alb = ALB; Hainich-Dün = HAI and Schorfheide-Chorin = SCH. Bars show mean values and error bars 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 (p-value < 0.05). Upper (for 2018) and lower-case letters (for 2019) indicate differences among the three study regions in the two years

Drivers of in-situ soil CO_2 efflux

Soil properties (i.e. SOC, soil C:N ratio, pH and silt content) explained 7% in 2018 and 14% in 2019 of soil CO_2 efflux variance, which was higher than the variance explained by climate, management, plant or microbial parameters (Fig. 2). In contrast, management (i.e. fertilization and grazing intensities) had the lowest explanatory power (~1%) in both years. Microbial properties (i.e. microbial C and C:N ratio) accounted for only 1% in 2018 and 4% in 2019 of soil CO_2 efflux variance, and plant properties (i.e. plant species richness, aboveground biomass, fine root biomass and plant C:N ratio) explained 5–6% in both years. Climate parameters (i.e. soil temperature and water content) accounted for 4% in 2018 and 14% in 2019 showing the largest change between the two years.

ANCOVA models explained 18% of the variation in in-situ soil CO_2 efflux in 2018 and 46% in 2019, respectively (Table 2). Study regions were important predictors of soil CO_2 efflux 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 did not affect soil respiration at least directly. Soil volumetric water content had a strong positive effect on soil CO_2 efflux, indicating water-limiting 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 CO_2 efflux, respectively. In 2019, microbial C and silt content had significant positive effects on soil CO_2 efflux. 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 CO_2 efflux in 2019. Finally, grazing intensity, soil temperature, plant biomass and C:N ratio, fine root biomass, soil OC concentration and microbial C:N ratio were not selected as predictors of soil CO_2 efflux in neither of the study years.

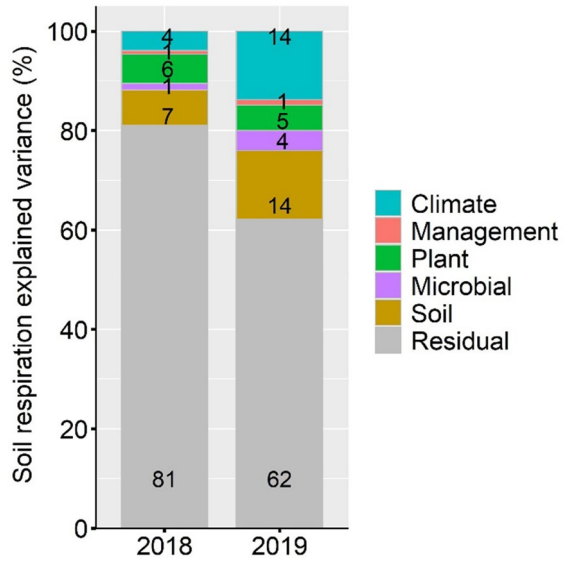

Fig. 2 Variance partitioning of in-situ soil respiration in 2018 and 2019 with five explanatory groups: (i) climate (soil temperature and volumetric water content), (ii) management (grazing and fertilization intensities), (iii) plant properties (plant species richness, aboveground plant biomass, plant C:N ratio and fine root biomass) (iv) microbial properties (microbial biomass C and C:N ratio) and (v) soil properties (soil OC concentration, soil C:N ratio, pH and silt content)

Table 2 Linear models (according to ANCOVA type II) of in-situ soil respiration in 2018 and 2019 with management intensities, soil water content, plant, microbial and soil properties

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	–	–	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**

Full models were stepwise reduced according to Akaike information criterion. Study region effects are given relative to the Schwäbische-Alb (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 (p-value <0.05). 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: df = degrees of freedom; HAI = Hainich-Dün; SCH = Schorfheide-Chorin

Indirect management intensity effects on in-situ soil CO_2 efflux

We used Structural Equation Modeling to investigate indirect effects of fertilization and grazing intensities on in-situ soil CO_2 efflux through organic matter quantity (expressed as plant biomass and soil organic carbon concentration) and quality (expressed as C:N ratios in plant biomass and soils) (Fig. S3). Plant biomass and C:N ratio were not related to soil CO_2 efflux and, thus, not included in the final models. Soil water content had a positive effect on soil CO_2 efflux in both years (Fig. 3). In both years, soil C:N ratio was directly and negatively associated with soil CO_2 efflux, while soil OC concentration was positively associated with soil CO_2 efflux only in 2019 (Fig. 3c). Plant species richness was indirectly and negatively associated with soil CO_2 efflux due to its positive correlation with soil C:N ratios in 2018 and, in addition, due to its negative correlation with soil OC concentration in 2019 (Fig. 3b, d). Grazing intensity remained unrelated to soil CO_2 fluxes in both study years. Finally, in both years, fertilization intensity was positively linked to soil CO_2 efflux by decreasing plant species richness and the associated decreased soil C:N ratio but, in 2019, this effect was offset by a positive indirect effect mediated by soil OC concentration.

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), short-term 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, using the soda-lime method, we determined in-situ soil CO_2 efflux with single measurements of long exposure time (i.e. 3 day long) in 150 temperate managed grasslands in three regions in Germany, to assess fertilization and grazing intensity effects on soil CO_2 efflux through changes in plant species richness and organic matter quantity and quality.

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. 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). Thus, compared to the literature, our summer soil respiration fluxes were relatively low. This, together with the strong correlation between soil respiration and soil water content (Table 2), indicates suppressed soil respiration because of the limiting water conditions in summers 2018 and 2019 (Fig. S5; Apostolakis 2022). In our first hypothesis, we expected that soil respiration would differ among the three study regions due to their differences in climatic, soil and ecological conditions (Table 1 and S1). 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 (Fig. 1c). This discrepancy between the two study years was possibly caused by a slightly less severe drought in 2019 compared to 2018 (Fig. S5), a condition that was more profound in HAI (Fig. 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 from soil properties, like the soil OC. In more detail, soil respiration did not correlate with soil OC concentration in 2018, but it did in 2019 (Fig. 3). Similarly, Dietrich et al. (2017) found that microbial basal respiration did not correlate with soil OC under a naturally occurring summer drought, while they correlated in the summer of the next year that was wetter. Some studies have suggested that droughts suppress heterotrophic respiration more than autotrophic respiration both in forests and grasslands (Apostolakis 2022; Moinet et al. 2019), but such observations are frequently reported together with strong correlations between soil respiration and root biomass. Here, using fine root biomass data measured in 2011, we did not observe a correlation between soil respiration and root biomass.

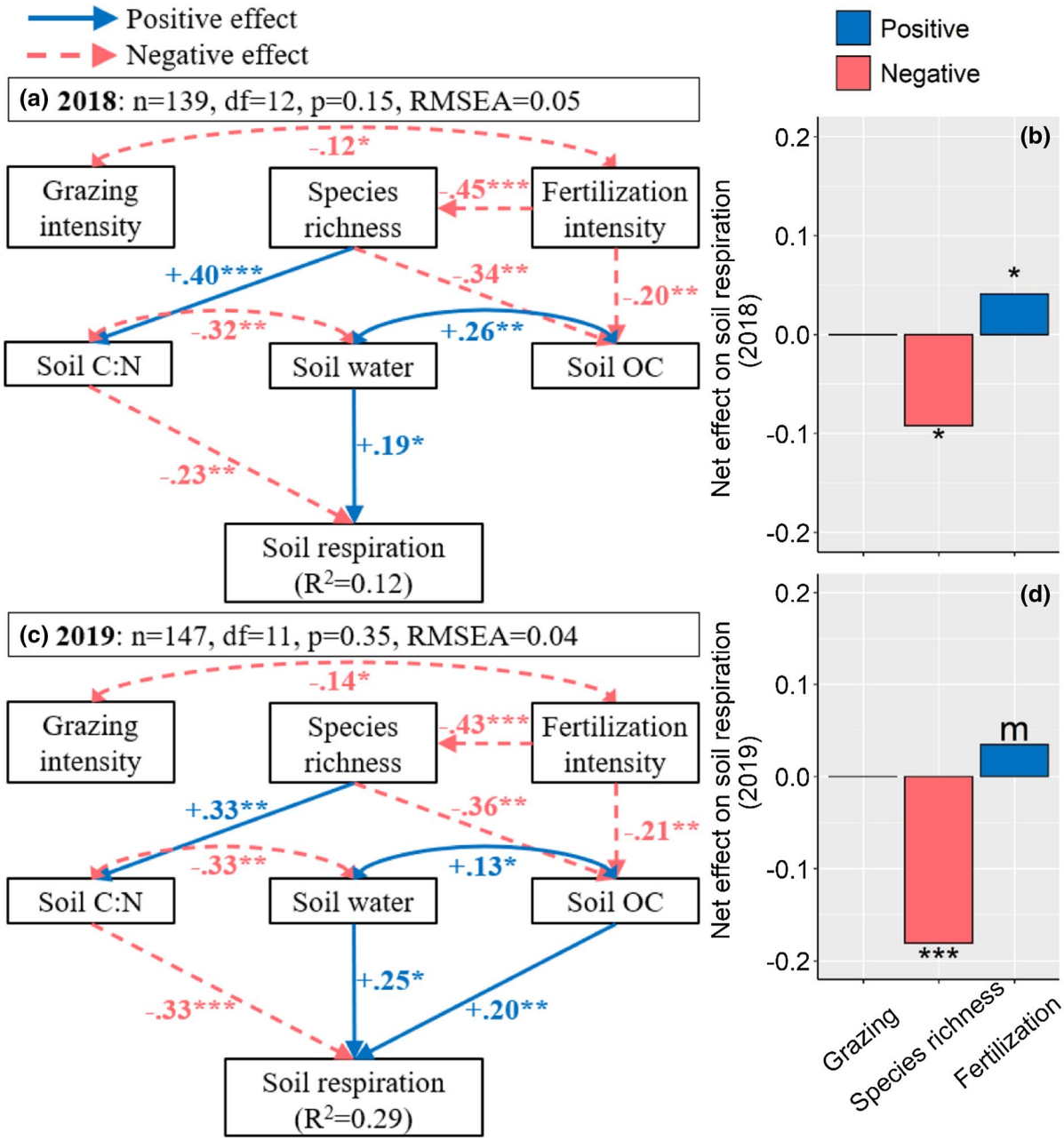

Fig. 3 **a**, **c** Structural Equation Models for soil respiration in 2018 and 2019 explained by grazing and fertilization intensities, soil volumetric water content, plant species richness and soil organic carbon concentration and soil C:N ratio. Single-headed arrows indicate direct paths and double-headed arrows indicate covariances. Solid, blue arrows indicate positive effects, and dashed, red arrows indicate negative effects. Number of observations (n), degrees of freedom (df), fitness statistics (p-value, RMSEA) and standardized path coefficients and their significance level ($0.100 > m > 0.050$; $*p < 0.050$; $**p < 0.010$; $***p < 0.001$) are given. Coefficients of determination (R^2) are given for soil respiration. **b**, **d** Net effect of grazing and fertilization intensities and plant species richness on soil respiration in 2018 and 2019. Blue bars indicate positive net effects and red bars indicate negative net effects

Fertilization intensity has positive but small effects on in-situ soil respiration

Grazing and fertilization intensities were not important predictors of in-situ soil respiration (Table 2), but, when we accounted for indirect effects (with Structural Equation Modeling), we found that fertilization was indirectly and mostly positively linked with soil respiration (Fig. 3a, c). Fertilization-induced effects on soil respiration were stronger in the drier (2018) than in the wetter (2019) year (Fig. 3b, d), which is in contrast with previous findings for fertilization effects on microbial basal respiration in temperate grasslands under dry and wet summers (Dietrich et al. 2017). The two successive years of drought, compared to the single dry year in the study of Dietrich et al. (2017), might have led to legacy effects in the plant and microbial communities, contributing to the discrepancy between the studies. In addition, organic fertilization can partly compensate for yield losses due to drought (Klaus et al. 2020b) which could lead to more pronounced effects from fertilization on soil respiration under drier conditions.

Studies investigating fertilization effects on soil respiration in grasslands often report contrasting results including both positive (Jia et al. 2012; Jones et al. 2005) and negative responses (Ward et al. 2017). Fertilization can influence soil respiration through many mechanisms, i.e. by causing changes in soil and plant properties. For instance, fertilization effects on plant properties involve (1) increases in aboveground productivity (Socher et al. 2012), (2) reductions in belowground productivity (Bardgett et al. 1999), and (3) plant species loss (Isbel et al. 2013; Midolo et al. 2018). In addition, fertilization alters the nutrient status of soils and plants (Apostolakis et al. 2022; Blüthgen et al. 2012; Dietrich et al. 2017; Herold et al. 2014a), 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. Partially in line with our second hypothesis, we found that fertilization intensity indirectly increased soil respiration by altering both the organic matter quantity and quality of soils, but not of plant biomass. The lack of an effect from plant biomass and quality on soil respiration might be because of the droughts: (1) droughts occurred in June and lasted until autumn in 2018 and in 2019 affecting our in-situ soil respiration measurements (measured in June and July in both years), but not the plant properties that were measured earlier (in mid-May in both years) and/or (2) plant residues possibly occurred in upper soil depths that were drier due to low rainfall and higher evapotranspiration than deeper soil depths, which inhibits microbial activity and decomposition.

Fertilization intensification was positively associated with soil respiration even though indirectly (Fig. 3). In detail, fertilization intensification was negatively associated with plant species richness, while plant species richness was positively linked to soil C:N ratio and negatively linked to soil OC concentration. On their turn, soil C:N ratio was negatively linked to in-situ soil respiration both in 2018 and in 2019, and soil OC was positively linked to in-situ soil respiration only in 2019. However, in 2019, these effects were offset by a negative effect from fertilization on soil OC concentration. Thus, in our grasslands, both soil organic matter quantity and quality are potentially important drivers of soil respiration, but soil organic matter quality is a stronger driver than quantity and its effects vary less inter-annually (i.e. depend less on the environmental conditions).

Grazed grasslands are associated with higher soil CO_2 efflux and soil microbial respiration compared to mowed grasslands, possibly due to a higher microbial biomass and more diverse and degradable C inputs into the soil as animal excretions in the grazed grasslands (Gilmullina et al. 2020; Ma et al. 2021; Owensby and Auen 2020). In this work, grazing intensity was not associated with in-situ soil respiration neither directly nor indirectly via soil organic matter quantity and quality (Fig. 3). Similarly, in the same grasslands, grazing was found to affect nutrient cycling mostly via plant properties, and not via soil properties (Apostolakis et al. 2022). In this study,

plant properties effects on soil respiration were presumably dampened by the severity or the timing of the droughts.

Extensive, plant species-rich grasslands associate with lower in-situ soil respiration compared to intensive, species-poor grasslands

Plant diversity effects on soil respiration have been found to be positive (Chen and Chen 2019; Dias et al. 2010) or neutral (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 decreased soil OC concentration and increased soil C:N ratio (Fig. 3). However, plant diversity effects on soil respiration were ultimately driven by fertilization, since fertilization intensity is a main driver of plant species richness in managed grasslands. As fertilization increases grassland productivity and reduces its biodiversity, fertilization intensity reverses the positive link between plant biomass and productivity observed in biodiversity experiments (Oelmann et al. 2021).

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; Lavorel et al. 2011). Apart from plant chemistry, plant species richness was also negatively associated with (aboveground) plant productivity in our grasslands (Socher et al. 2012). Plant productivity can increase soil respiration, possibly because of increased C inputs into the soil (Craine et al. 2001; Dias et al. 2010; Malhi et al. 2010). In our grasslands, the negative plant species richness gradient driven by fertilization intensification was positively associated with plant biomass and negatively associated with plant C:N ratio (Apostolakis et al. 2022), but these changes did not influence soil respiration (Fig. S3) in neither of the study years, presumably because of the droughts. In line with our third hypothesis, we suggest that the aforementioned characteristics of extensive, plant species-rich grasslands lead to slower C cycling compared to intensive, species-poor grasslands, which has also been proposed for root turnover and N and P cycling (Apostolakis et al. 2022; Oelmann et al. 2021; Solly et al. 2014) and, thus, to lower soil respiration fluxes.

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 of this study, fertilization intensity indirectly increased soil respiration, and its effects were accompanied by a plant species richness loss and both soil organic matter quantity and quality. Soil organic matter quality increasing effects on soil respiration were observed in both study years, while those of soil organic matter quantity only in one of the years. This suggests that organic matter quality has a stronger and more stable effect on soil respiration than organic matter quantity. 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.

Acknowledgements We thank Theresa Klötzing, Steffen Ferber and Marco Poehlmann for their contribution in lab and field work. We thank Martin Freitag and Verena Busch for organizing and conducting plant-related analyses. We 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 Biodiversity Exploratories project. We 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 land owners, for the excellent collaboration. Field work permits were issued by the responsible state

environmental offices of Baden-Württemberg, Thüringen, and Brandenburg.

Authors' contributions Antonios Apostolakis designed and carried out the field and laboratory work related to soil respiration measurements, analysed and discussed the data, and was responsible for writing this manuscript. Ingo Schöning, Beate Michalzik and Marion Schrumpf designed the experiment and reviewed and edited drafts of the manuscript. Runa S. Boeddinghaus, Ellen Kandeler and Sven Marhan contributed data related to microbial properties. Valentin H. Klaus, Ralph Bolliger, Markus Fischer, Daniel Prati, Norbert Hölzel, Till Kleinebecker contributed data related to plant properties. Falk Hänsel, Thomas Nauss contributed soil temperature and water content data. All authors contributed to the manuscript and approved the final version.

Funding Open Access funding enabled and organized by Projekt DEAL. The work has been funded by the DFG Priority Program 1374 "Biodiversity- Exploratories" (TR 1054/4–3 and SCHR 1181/2–3) and the Max Planck Society.

Data availability This work is based on data elaborated by the projects TR 1054/4–3 and SCHR 1181/2–3 of the Biodiversity Exploratories program (DFG Priority Program 1374). The datasets are publicly available in the Biodiversity Exploratories Information System (https://www.bexis.uni-jena.de/; datasets: 26,908, 23,846, 22,246, 14,448, 14,686, 19,366, 25,086, 25,408, 26,106, 26,466, 26,526).

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

References

Apostolakis A, Schöning I, Klaus VH, Michalzik B, Bischoff WA, Boeddinghaus RS, Bolliger R, Fischer M, Hölzel N, Kandeler E, Kleinebecker T, Manning P, Marhan S, Neyret M, Oelmann Y, Prati D, van Kleunen M, Schwarz A, Schurig E, Schrumpf M (2022) Direct and plant community mediated effects of management intensity on annual nutrient leaching risk in temperate grasslands. Nutr Cycl Agroecosystems. https://doi.org/10.1007/s10705-022-10209-1

Apostolakis A (2022) Land use effects on carbon and nutrient fluxes in soils. PhD thesis. Friedrich-Schiller-Universität Jena. https://nbn-resolving.org/urn:nbn:de:gbv:27-dbt-20220608-153910-001

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 RD, Mawdsley JL, Edwards S, Hobbs PJ, Rodwell JS, Davies WJ (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

Bierbaß P, Gutknecht JLM, 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

Blüthgen N, Dormann CF, Prati D, Klaus VH, Kleinebecker T, Hölzel N, Alt F, Boch S, Gockel S, Hemp A, Müller J, Nieschulze J, Renner SC, Schöning I, Schumacher U, Socher SA, Wells K, Birkhofer K, Buscot F, Oelmann Y, Rothenwöhrer C, Scherber C, Tscharntke T, Weiner CN, Fischer M, Kalko EKV, Linsenmair KE, Schulze ED, Weisser WW (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

Burri S, Sturm P, Prechsl UE, Knohl A, Buchmann N (2014) The impact of extreme summer drought on the short-term carbon coupling of photosynthesis to soil CO_2 efflux in a temperate grassland. Biogeosciences 11:961–975. https://doi.org/10.5194/bg-11-961-2014

Burri S, Niklaus PA, 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

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

Chen X, Chen HYH (2019) Plant diversity loss reduces soil respiration across terrestrial ecosystems. Glob Chang Biol. https://doi.org/10.1111/gcb.14567

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 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

Conant RT, Cerri CEP, Osborne BB, 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 JM, Wedin DA, Reich PB (2001) The response of soil CO_2 flux to changes in atmospheric CO_2, nitrogen supply and plant diversity. Glob Chang Biol 7:947–953. https://doi.org/10.1046/j.1354-1013.2001.00455.x

da Silva LS, Sollenberger LE, Kimberly Mullenix M, Kohmann MM, Dubeux JCB, Silveira ML (2022) Soil carbon and nitrogen stocks in nitrogen-fertilized grass and legume-grass forage systems. Nutr Cycl Agroecosyst 122:105–117. https://doi.org/10.1007/s10705-021-10188-9

de Vries FT, 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

Dias ATC, 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

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

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.

Faraway J (2016). faraway: Functions and Datasets for Books by Julian Faraway. R package version 1.0.7.https://CRAN.R-project.org/package=faraway

Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JMH, Reich PB, 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

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze ED, Weisser WW (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

Gilmullina A, Rumpel C, Blagodatskaya E, Chabbi A (2020) Management of grasslands by mowing versus grazing – impacts on soil organic matter quality and microbial functioning. Appl Soil Ecol 156:103701. https://doi.org/10.1016/j.apsoil.2020.103701

Groemping U (2006) Relative Importance for Linear Regression in R: The Package relaimpo. J Stat Softw 17(1):1–27. https://doi.org/10.18637/jss.v017.i01

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

Herold N, Schöning I, Gutknecht J, Alt F, Boch S, Müller J, Oelmann Y, Socher SA, 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

Isbell F, Reich PB, Tilman D, Hobbie SE, 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

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

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.

Jacobs A, Poeplau C, Weiser C, Fahrion-Nitschke A, Don A (2020) Exports and inputs of organic carbon on agricultural soils in Germany. Nutr Cycl Agroecosyst 118:249–271. https://doi.org/10.1007/s10705-020-10087-5

Janssens IA, Kowalski AS, 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

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 EG, Jackson RB (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 GK, Grime JP (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 SK, Rees RM, Skiba UM, Ball BC (2005) Greenhouse gas emissions from a managed grassland. Glob Planet Change 47:201–211. https://doi.org/10.1016/j.gloplacha.2004.10.011

Jones SK, Rees RM, Kosmas D, Ball BC, Skiba UM (2006) Carbon sequestration in a temperate grassland; management and climatic controls. Soil Use Manag 22:132–142. https://doi.org/10.1111/j.1475-2743.2006.00036.x

Keil D, Meyer A, Berner D, Poll C, Schützenmeister A, Piepho HP, 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 SC (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 VH, 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 VH, Whittingham MJ, Báldi A, Eggers S, Francksen RM, Hiron M, Lellei-Kovács E, Rhymer CM, 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 VH, Friedritz L, Hamer U, Kleinebecker T (2020b) Drought boosts risk of nitrate leaching from grassland fertilisation. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137877

Kleinebecker T, Klaus VH, 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

Kleinebecker T, Hölzel N, Prati D, Schmitt B, Fischer M, Klaus VH (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

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 Agroecosyst 103:201–215. https://doi.org/10.1007/s10705-015-9732-3

Lange M, Eisenhauer N, Sierra CA, Bessler H, Engels C, Griffiths RI, Mellado-Vázquez PG, Malik AA, Roy J, Scheu S, Steinbeiss S, Thomson BC, Trumbore SE, Gleixner G (2015) Plant diversity increases soil microbial activity and soil carbon storage. Nat Commun. https://doi.org/10.1038/ncomms7707

Lavorel S, Grigulis K, Lamarque P, Colace MP, 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

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

Luo Q, Gong J, Zhai Z, Pan Y, Liu M, Xu S, Wang Y, Yang L, Baoyin T. T. (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

Ma Z, Shrestha BM, Bork EW, Chang SX, Carlyle CN, Döbert TF, Sobrinho LS, Boyce MS (2021) Soil greenhouse gas emissions and grazing management in northern temperate grasslands. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2021.148975

Malhi SS, Nyborg M, Soon YK (2010) Long-term effects of balanced fertilization on grass forage yield, quality and nutrient uptake, soil organic C and N, and some soil quality characteristics. Nutr Cycl Agroecosystems 86:425–438. https://doi.org/10.1007/s10705-009-9306-3

Midolo G, Alkemade R, Schipper AM, Benítez-López A, Perring MP, 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 GYK, Midwood AJ, Hunt JE, Rumpel C, Millard P, Chabbi A (2019) Grassland management influences the response of soil respiration to drought. Agronomy. https://doi.org/10.3390/agronomy9030124

Näthe K, Levia DF, 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

Oelmann Y, Lange M, Leimer S et al (2021) Above- and belowground biodiversity jointly tighten the P cycle in agricultural grasslands. Nat Commun 12:4431. https://doi.org/10.1038/s41467-021-24714-4

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

Owensby CE, Auen LM (2020) Stocking rate impacts on tallgrass prairie landscape carbon fluxes. Crop Forage Turfgrass Manag 6:1–12. https://doi.org/10.1002/cft2.20048

R Core Team (2020) R: a Language and Environment for Statistical Computing. R Found. Stat. Comput. Vienna, Austria.

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

Rosseel Y (2012) lavaan: An R Package for Structural Equation Modeling. J Stat Softw. https://doi.org/10.18637/jss.v048.i02

Shipley B (2016) Cause and Correlation in Biology. Cambridge

Socher SA, Prati D, Boch S, Müller J, Klaus VH, 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

Solly EF, Schöning I, Boch S, Kandeler E, Marhan S, Michalzik B, Müller J, Zscheischler J, Trumbore SE, 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

Spehn EM, 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

Steinbeiss S, Beßler H, Engels C, Temperton VM, 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

Strecker T, Barnard RL, Niklaus PA, 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

Sun Y, Liao J, Zou X, Xu X, Yang J, Chen HYH, Ruan H (2020) Coherent responses of terrestrial C: N stoichiometry to drought across plants, soil, and microorganisms in forests and grasslands. Agric for Meteorol 292–293:108104. https://doi.org/10.1016/j.agrformet.2020.108104

Tilman D, Isbell F, Cowles JM (2014) Biodiversity and Ecosystem Functioning. Annu Rev Ecol Evol Syst 45:471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Vance ED, Brookes PC, Jenkinson DS (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

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

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

Zechmeister-Boltenstern S, Keiblinger KM, 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 SJ, Wen H, Li X, Fu H, Wan C, Elser JJ (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

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in Ecology with R. Springer, book series: Statistics for biology and health.https://doi.org/10.1007/978-0-387-87458-6