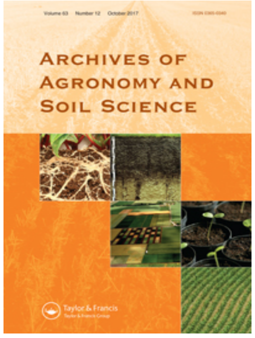

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SHORT COMMUNICATION

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Effect of grazing intensity and soil characteristics on soil organic carbon and nitrogen stocks in a temperate long-term grassland

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ABSTRACT
The effects of different grazing pressures (GPs) on soil properties are not sufficiently understood. The objectives were to analyse the effects of three different extensive GPs on stocks of soil organic C and total N, soil microbial biomass C, basal respiration and mineral N in three different soil depths of a long-term pasture in Central Germany (FORBIOBEN field trial). No significant ($p \leq 0.05$) effects of GP on weighted stocks of soil organic C, total N, soil microbial biomass C, mineral N and basal respiration rate were observed, suggesting that the C and N cycles are coupled in the three grazing treatments. Oxalate soluble Fe contents explained a marked part of the variation of soil organic C (multiple linear regression: $R^2 = 0.64$) and total N contents ($R^2 = 0.64$) in the soils, whereas almost all of the variability of soil microbial biomass C contents and basal respiration was explained by soil organic C contents. Overall, variabilities of soil organic C and N contents were largely explained by oxalate soluble Fe contents, whereas grazing intensity did not affect the C and N dynamics.

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KEYWORDS
Pasture; grazing pressure; microbial biomass; mineral N; basal respiration rate

Introduction

Nearly one third of the German agricultural area is used as grassland (Socher et al. 2013). Intensity of grassland management is varying but often driven by the demand for forage production (Conant et al. 2001). A recommended management practice for low-productivity grasslands in Germany is an extensive herding of cattle for meat production to serve biodiversity and production goals (Isselstein et al. 2007). Type and intensity of grassland management exert strong influence on soil organic carbon (SOC) and total nitrogen (N_t) stocks of pasture soils (Conant et al. 2001). SOC stored in grasslands is climate relevant (Ciais et al. 2010) and is in conjunction with N_t storage important for the productivity of pasture ecosystems (Conant et al. 2001; Haferkamp & MacNeil 2004).

In general, temperate grassland soils are typically rich in SOC because of rhizodeposition (Jones & Donelly 2004) and because of the activity of soil fauna that promote aggregation of soil organic matter and stabilize it for extended periods (Six et al. 2002). C and N cycles are more strongly coupled in grassland soils as compared to permanent cropping because of a more stable C/N ratio of organic matter inputs to soil (Rumpel et al. 2015). Soussana and Lemaire (2014) suggested grazing pressure (GP) in intensively used pastures as the main factor for C return to soil, since the reduced leaf area and the excretal return may affect the C and N cycle markedly. Up to 60% of above-ground dry-matter production is ingested by domestic

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herbivores on intensively used pastures (Lemaire & Chapman 1996). Especially moderate grazing on naturally nutrient poor grasslands could favor nutrient cycling and increase primary production (Klumpp et al. 2007). The leaf area determines the capacity to capture atmospheric CO_2 and may be mainly affected by the GP due to defoliation intensity and frequency and treading by animals (Hodgson 1990). Grazing has an influence on the plant species composition and the development stages of plants (Hodgson 1990; Frame & Laidlaw 2011). Klumpp et al. (2007, 2009) and Klumpp and Soussana (2009) described a shift from less intensively grazed pastures dominated by slow growing plant species producing litter of less quality (high C/N ratio of ~40 and high lignin content) to more intensively grazed pastures dominated by faster growing plants with a higher litter quality. With increasing C/N ratio of plants, the mineralization by microorganisms may decrease, which may result in a longer residence time of organic matter in soil. Moreover, higher digestible plants and plant parts (lower C/N ratio of ~20), dominating on intensively grazed pastures, may induce a decoupling of C and N cycles (Sousanna & Lemaire 2014).

Quantity and quality of the impact of varying GP on SOC and N_t pools may depend on environmental conditions. Previous results show diverse effects of grazing on SOC, whereas many of these differences appear to be the result of variations in climate, soil properties, landscape position, plant community, soil sampling and grazing management practices (Reeder 2002; Haferkamp & MacNeil 2004; Derner et al. 2006; Piñeiro et al. 2010). Consequently, it is not possible to extrapolate from global data sets or from data of arid or semi-arid environments to pasture conditions in the temperate zone. So far, only few studies investigated the effect of varying GP in Germany or Central Europe on SOC stocks using long-term field experiments.

Besides an effect of varying GP on SOC and N_t pools, these pools are also affected by mineral characteristics and pH (Eusterhues et al. 2005; Wiseman & Püttmann 2006). For sites with small variabilities in mineral characteristics and pH, effects of varying GP on SOC and N_t pools may be detectable, whereas for sites with high variabilities, effects of varying GP may be masked.

Based on the results summarized earlier, we hypothesized a lower carbon sequestration under higher GP and a decoupling of C and N cycles with increasing GP. Moreover, we hypothesized changed species composition and substrate quality at increasing GP, which may markedly affect stocks of soil microbial biomass C (C_{mic}) and basal respiration rates. Besides the management effect GP, we hypothesized that SOC and N_t stocks may be described to a marked extent by regression analyses using pH and contents of clay and oxalate soluble Fe (Fe_{ox}) and Al (Al_{ox}) as predictors. The objectives were to analyse the effects of three different GPs on weighted stocks of SOC and N_t in three different soil depths for the extensively grazed FORBIOBEN (Isselstein et al. 2007) field trial in Relliehausen, Germany. Further, the correlation of SOC and N_t concentrations were used to examine coupling of C and N cycles under varying GP. Additional objectives were to analyse the effect of GP on soil microbial C, basal respiration rate and plant available N. Moreover, soil characteristics such as pH, texture and Fe_{ox} and Al_{ox} were analysed that are known as alternative controlling factors of the soil C and N dynamics (Eusterhues et al. 2005; Wiseman & Püttmann 2006).

Materials and methods

Study area

The study site is located near Relliehausen at the Scharfenberg in one of the central German uplands, the Solling (51° 46'N, 9° 42'E), 250 m above the sea level. The mean annual temperature is 8.2°C and the long-term average annual precipitation is 879 mm year^{-1}. The experimental field site is a mesotrophic, moderately species-rich hill grassland on a Vertisol with an average number of plant species of 10.9 m^{-2} (Şahin Demirbağ et al. 2008). The pH is between 4.82 and 7.51, the soil texture ranges between a silt loam and a clay soil. The vegetation type is a Lolio-Cynosuretum

(Scimone et al. 2007) which is dominated by grasses like *Agrostis* spec., *Dactylis glomerata, Lolium perenne, Phleum pratense, Poa* spec., *Taraxacum officinale, Trifolium repens* (Şahin Demirbağ et al. 2008).

Plot description and sampling design

A grazing experiment with cattle (Simmental suckler cows) and no input of fertilizers, pesticides or cutting was established in spring 2005. Grazing management was conducted similar to traditionally extensive grazing. The target compressed pasture height (CPH) classes, which were the result of different GPs, were selected after Isselstein et al. (2007) and Wrage et al. (2012). During winter, the cattle was kept in a barn and during the vegetation period on pasture (without feeding supplements). Start and end of the grazing period were dependent on weather conditions. The study site was subdivided into nine paddocks, each one hectare large, with following GPs each replicated three times (Wrage et al. 2012):

- medium GP, target CPH of 6 cm, designed to utilize herbage growth for optimum livestock production (ca. 3.4 standard livestock units (one standard livestock unit being 500 kg) per ha)
- lenient GP, target CPH of 12 cm, designed to increase biodiversity by not fully utilizing herbage growth (ca. 1.8 standard livestock units per ha)
- very lenient GP, CPH of 18 cm, designed to increase biodiversity by not utilizing herbage growth (ca. 1.3 standard livestock units per ha)

The different GPs were determined by measuring the CPH in the vegetation period at intervals of two weeks using a rising-plate meter (Correll et al. 2003) and subsequently increasing or decreasing stocking rate, if measured CPH deviated from target CPH. The paddocks did not show a homogeneous CPH because of selective grazing of cattle. Therefore, three CPH classes were defined: short (CPH ≤ 5 cm), medium (5 cm < CPH < 12 cm) and tall (CPH ≥ 12 cm). Sample locations within the site were selected from two random points per paddock. Starting from these points, the closest 50 × 50 cm plots of short (CPH ≤ 5 cm), medium (5 cm < CPH < 12 cm) and tall (CPH ≥ 12 cm) CPHs were chosen for soil sampling. In each of these 50 × 50 cm plots, we took two combined samples (in total about 1 kg soil) with an open-sided auger (Edelmann, Eijkelkamp, Giesbeek, the Netherlands; diameter of 6 cm) for basic characterization of soil properties and three combined samples for bulk density with sampling rings (Cylinder Set Model A, Eijkelkamp, Giesbeek, the Netherlands; volume of 100 cm^3). In total, we took soil samples at 18 plots (3 paddocks as field replicates × 2 random points per paddock × 3 plots for each CPH per random point) per GP representing the heterogeneity of sward and pedology of the study location. Soil samples were taken in three depths (0–10, 10–25 and 25–40 cm) in April 2013, resulting in 54 soil samples for basic characterization and bulk density each. The samples for soil characterization were sieved (with 2 mm mesh-size) and stored at 4°C before analysing and the samples for bulk density were dried at 105°C for at least 24 h before weighing. In our study, a focus was put on weighted soil parameters with the proportion of each CPH class as a weighting factor.

Analytics and soil characterization

Basic characterization

Soil bulk density was determined according to DIN ISO 11272 (1998). The pH was analysed by extraction with $CaCl_2$ (25 ml 0.01 M $CaCl_2$, 10 g soil) (ISO 10390 2005). Soil texture was determined by applying the pipet method (DIN ISO 11277 2002). Gravimetric soil moisture content was determined by weighing field-moist soil, drying at 105°C for 24 h and weighing soil again after drying. The determination of Fe_{ox} and Al_{ox} followed DIN 19684-6 (1997). Briefly, a 5 g sample was shaken for 2 h with 50 ml extraction solution (0.1 M ammonium oxalate and 0.1 M oxalic acid) and then filtrated through a fiberglass filter. Afterwards, the filtrates were measured with an atomic absorption spectrometer (SO6AA, GBC, Braeside, Australia) for Fe and Al concentrations.

Determination of total carbon, organic carbon, total nitrogen and carbon stocks

The concentrations of total C (C_t) and N_t in the bulk soil were determined by dry combustion on a CN elemental analyser (Elemental Vario El, Heraeus, Hanau, Germany). The SOC content was calculated as the difference between the contents of C_t and inorganic C. The inorganic C concentration was measured with the Scheibler method following DIN 19682-13 (2009). The SOC stocks of the different soil layers were calculated on an equivalent mass basis as suggested by Ellert and Bettany (1995) to take differences in bulk density of the respective soil layers into account.

Basal respiration and soil microbial biomass

Basal respiration rates were measured using a slightly modified method of Heinze et al. (2010). Thirty grams of soil (adjusted to 50% of their waterholding capacity) were weighed into plastic beakers. That beaker and another one, containing 5 ml 0.5 M NaOH, were placed into 1500 ml Pyrex glass jars containing 20 ml distilled water. The prepared Pyrex glass jars were incubated in a climate cabinet (ICP 800, Memmert, Schwabach, Germany) for 7 days at 22°C in the dark. The evolved CO_2 was determined by titration of the excess NaOH to pH 8.3 with 0.5 M HCl after addition of $BaCl_2$ solution. The incubated soil was used for measuring C_{mic} with the chloroform-fumigation-extraction method. Two subsamples, each 10 g moist soil, were processed per sample. One was fumigated with chloroform and one was not fumigated. The non-fumigated subsample was extracted with 40 ml of 0.5 M K_2SO_4 (30 min by oscillating shaking at 200 rev min^{-1}). To fumigate the other subsamples, they were incubated for 24 h at 25°C with ethanol-free $CHCl_3$ and then extracted like the non-fumigated samples. In the extracts, organic C was determined by dry combustion on a CN elemental analyser (Multi N/C 2100S, Analytik Jena, Germany). Afterwards, C_{mic} was calculated as *Ec*/*k*EC, where *Ec* = (organic C extracted from fumigated soil)–(organic C extracted from non-fumigated soil) and *k*EC = 0.45 (Wu et al. 1990).

Mineral N

Mineral N (N_{min}: sum of NO_3^-–N and NH_4^+–N) was measured using a slightly modified method of Kuderna et al. (1993). The N_{min} was extracted from 10 g of moist soil with 40 ml of 0.5 M K_2SO_4 (30 min by oscillating shaking at 200 rev min^{-1}). Afterwards, the extracts were analysed for NO_3–N and NH_4–N on a continuous flow analyser (Evolution II auto-analyser, Alliance Instruments, Salzburg, Austria).

Calculations and statistical analyses

All statistical analyses were conducted with the statistic software R (Version 3.2.2; R Development Core Team 2014). The two pseudo-replicates per paddock and CPH class were averaged for all analyses. Further, weighted means per paddock and soil depth were calculated for the stocks of SOC, N_t, N_{min} and C_{mic} concentrations and basal respiration rate with the proportion of each CPH class as a weighting factor (for weighted comparisons, see, e.g., Bland & Kerry 1998). The weighted means were analysed with two-factorial analyses of variance (ANOVAs) with the factor grazing intensity (levels: medium, lenient and very lenient GP) and the factor block in case of normality of residuals and homoscedasticity. For stocks of N_{min} in soil depth 0–10 cm and for stocks of C_{mic} in soil depth 25–40 cm, an effect of block was not significant and heteroscedasticity existed; therefore, a Welch ANOVA with the factor grazing intensity was used. Effects were considered significant for $p \leq 0.05$.

Multiple linear regression analyses with stepwise variable selection were carried out for SOC, N_t and C_{mic} concentrations and basal respiration rate in soil depth 0–25 cm with the soil parameters pH and clay, Fe_{ox}, Al_{ox} and SOC concentrations. Only factors with significant contributions were considered and the models with the lowest Akaike information criterion were chosen. Residuals were visually inspected for normality and homoscedasticity.

Spearman's rank correlation was carried out for not normally distributed SOC and N_t concentrations of all treatments and soil depths.

Results and discussion

Weighted stocks of SOC and N_t

For the weighted stocks of SOC and N_t per ha no significant differences between different GPs were observed (Table 1). Further, the unweighted SOC and N_t contents were also not affected by GP or CPH (data not shown). Accordingly, a lower carbon sequestration under higher GP as described by Soussana and Lemaire (2014) was not detected for the study site in Relliehausen. We assume that the extensive cattle herding is adjusted to the location in all three GPs, which has also been reported for some other trials (Haferkamp & MacNeil 2004; Piñeiro et al. 2010). One has to keep in mind that the determination of SOC and N_t stocks is affected by uncertainties in the analytical determination of the respective contents, the determination of bulk densities and the stone contents. In our study, we used the equivalent soil mass approach to compare different treatments. For a critical discussion on factors affecting the determination, which is especially important for repeated sampling in time (which is not a focus of this study), readers may refer to Schrumpf et al. (2011).

The SOC and N_t concentrations were positively correlated ($r = 0.95$, $p < 0.05$; data not shown). Accordingly, the strong correlation of SOC and N_t concentrations suggests that C and N cycles are coupled over all treatments and depths. A decoupling of C and N cycles with an increased GP as described by Soussana and Lemaire (2014) was not observed for the site in Relliehausen.

Weighted stocks of C_{mic}, N_{min} and basal respiration rates

The weighted stocks of C_{mic}, N_{min} and basal respiration rates per ha were not affected by GP (Table 1) or CPH. Differences of plants between the GPs, e.g. varying plant species compositions, varying plant development stages and above-ground biomass, may influence litter and root quality (Frame & Laidlaw 2011). Further, it is well known that litter and root quality of plants (e.g. using C/N ratio and lignin content as simple indicators) may influence the mineralization by microorganisms

Table 1. Weighted soil organic C (SOC) stock, total N (N_t) stock, microbial biomass C (C_{mic}) stock and mineral N (N_{min}) stock and basal respiration among three grazing pressures (GP) and three soil depths; mean values and standard deviations (standard deviations of field replicates are given in parentheses; $n = 3$).

		Medium GP	Lenient GP	Very lenient GP
SOC stock [t ha^{-1}]	0–10 cm	32.8 (7.4)	39.2 (9.7)	31.6 (12.6)
	10–25 cm	35.5 (6.5)	40.6 (2.2)	30.9 (4.2)
	25–40 cm	30.1 (10.0)	31.1 (5.0)	24.3 (12.4)
N_t stock [t ha^{-1}]	0–10 cm	3.24 (0.6)	3.76 (0.9)	3.11 (1.1)
	10–25 cm	3.42 (0.7)	4.00 (0.5)	3.21 (0.3)
	25–40 cm	2.80 (0.7)	2.20 (0.6)	1.68 (0.3)
C_{mic} stock [kg ha^{-1}]	0–10 cm	934 (246)	1010 (250)	834 (210)
	10–25 cm	826 (164)	969 (208)	812 (130)
	25–40 cm	554 (272)	462 (95)	329 (14)
Basal respiration [kg CO2–C ha^{-1} day^{-1}]	0–10 cm	24.8 (9.7)	25.5 (3.8)	19.0 (6.2)
	10–25 cm	25.2 (9.0)	26.4 (2.7)	22.2 (5.0)
	25–40 cm	22.6 (7.6)	28.5 (3.6)	19.9 (10.6)
N_{min} stock [kg ha^{-1}]	0–10 cm	48.9 (4.0)	39.9 (6.9)	46.1 (28.7)
	10–25 cm	9.1 (0.6)	10.5 (2.1)	10.9 (2.1)
	25–40 cm	11.9 (5.0)	11.9 (1.4)	12.5 (5.6)

(Klumpp et al. 2007, 2009; Klumpp & Soussana 2009) and thus the stocks of C_{mic} and also the N mineralization dynamics. However, no effect of the GP on these properties were observed in the trial, presumably because of only small effects of the GPs on the species compositions and substrate quality or because of large spatial heterogeneity of SOC stocks (standard deviations of three field replicates are given in Table 1) which made a detection of treatment effects difficult.

Data variability – influence of mineral soil characteristics and pH

The SOC and N_t contents (data not shown) and also the stocks of SOC, N_t, C_{mic} and basal respiration rate are highly variable for the same GP. We hypothesized that much of this variability is due to the variability of mineral characteristics and pH. The results of the multiple linear regression analysis confirmed this expectation for Fe_{ox}, which is known to stabilize SOC by sorptive mechanisms (Wiseman & Püttmann 2006). A marked part of the variability of SOC and N_t contents in 0–25 cm was explained with Fe_{ox} as predictor for SOC (R^2 = 0.64) and N_t (R^2 = 0.64) (Figure 1). The underlying mechanisms for the stabilization of SOC by different Fe fractions including Fe_{ox} are discussed by Eusterhues et al. (2005).

Almost all of the variability of C_{mic} contents (R^2 = 0.96) and basal respiration rate (R^2 = 0.9) in 0–25 cm was explained by SOC contents, which emphasizes the importance of SOC as nutrient source for the microorganisms.

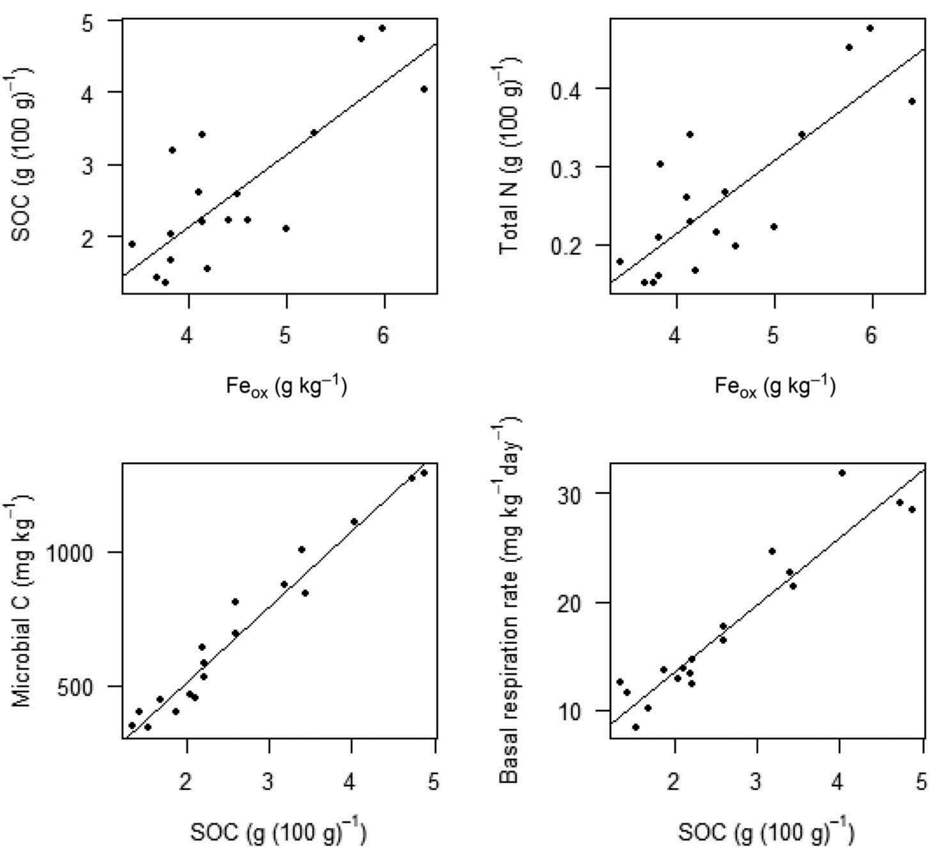

Figure 1. Results of multiple linear regression analyses in soil depth 0–25 cm for the contents of soil organic C (SOC), total N (N_t), microbial biomass C (C_{mic}), and the basal respiration rate. For each regression analysis only one significant explanatory variable ($p \leq 0.05$, top: Fe_{ox}, bottom: SOC) remained in the stepwise variable selection procedure.

Conclusion

The strong correlation between SOC and N_t concentrations in the FORBIOBEN field study over all treatments and soil depths suggests that the C and N cycles are coupled among all GPs. The different CPH classes of each GP did not have any effect on SOC and N_t stocks and no significant effects of the GP on weighted stocks of SOC, N_t, C_{mic}, N_{min} and basal respiration rate were observed, presumably due to a marked spatial variability of soil mineral characteristics (Figure 1 shows the range of Fe_{ox} in 0–25 cm). Particularly, Fe_{ox} contents explained a marked fraction of the variation of SOC (R^2 = 0.64) and N_t contents (R^2 = 0.64) in the soils. SOC in turn is an important nutrient source for microorganisms and positively related to C_{mic} contents and basal respiration.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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