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# Why does mineral fertilization increase soil carbon stocks in temperate grasslands?



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### ABSTRACT

Globally, grasslands are a major land cover type and store significant amounts of soil organic carbon (SOC). Fertilization with the major plant nutrients nitrogen (N), phosphorus (P), and potassium (K) may affect SOC stocks, e.g., by altering aboveground and belowground plant productivity, species composition, litter composition and decomposition, and microbial metabolism. However, belowground responses to fertilization in grasslands are not fully understood, hampering accurate predictions of SOC dynamics. In this study, seven different long-term grassland fertilization experiments (16-58 years) in Germany and the Netherlands were sampled to determine the effects of mineral fertilization (i.e., N, P, K, PK, and NPK) compared with unfertilized plots (Control) on SOC stocks and root C stocks. Soils were sampled to a depth of 100 cm or to the maximum depth possible. Potential litter decomposition was assessed using Lipton Rooibos and Lipton Green teas as standardized and well-characterized litter materials. In the topsoil (0-30 cm depth), PK, NPK, and NPK+ (increased NPK) fertilization had significant positive effects on SOC stocks, with annual sequestration rates of 0.28, 0.13, and 0.37 Mg ha<sup>-1</sup> yr<sup>-1</sup>, respectively, within an average time span of 34 (PK, NPK) or 20 (NPK +) years. For NPK fertilization, 1.15 kg of N was needed to sequester 1 kg SOC. Increased SOC stocks could not be explained by altered belowground C inputs or decomposition rates, since root C stocks were not affected by fertilization and potential litter decomposition was unchanged. The highly significant increases in dry matter yield with PK and NPK fertilization and resulting higher aboveground C inputs were also unlikely to explain the observed SOC stock changes, since increases were only observed at soil depths > 10 cm. However, significantly narrower root C:N ratios were observed for the N (26.9), PK (36.5), NPK (36.8), and NPK+ (33.2) treatments than for the Control (41.8), which may have caused increased microbial C use efficiency, positively affecting SOC storage. The narrower C:N ratios in PK treatments were explained by significantly increased abundance of legumes. We concluded that the carbon footprint of fertilization-induced SOC sequestration needs to be considered when the latter should be accounted for as a climate mitigation measure. Thereby, PK fertilization has much lower CO<sub>2</sub> costs than NPK fertilization due to N input via legumes.

#### 1. Introduction

Globally, grasslands are a major land use type, covering 68% of the total agricultural area, and soils under grass vegetation store large amounts of organic carbon (C), which is sensitive to land use and land management (Bolin et al., 2000; Soussana et al., 2004). These management-induced changes in soil organic C (SOC) dynamics might have significant effects on global biogeochemical cycles and therefore need

to be understood (Rumpel et al., 2015). At the UNFCCC COP21 in Paris in 2015, the '4 per mille Initiative - Soil for Food Security and Climate' was launched, which targets a global annual increase in SOC of 4‰ (www.4p1000.org). This goal is based on the fact that total annual anthropogenic  $CO_2$  emissions are equal to 4‰ of total global SOC stocks. It is important to understand how grassland management can contribute to achieving this goal. The major management interventions potentially influencing SOC dynamics in grasslands are fertilization,

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grazing, cutting, mulching, and species choice (McSherry and Ritchie, 2013; Rumpel et al., 2015; Poeplau et al., 2016b). However, for all those interventions, results across studies are contradictory and the processes involved are not fully understood, which hampers successful modeling of grassland management effects. Therefore, more studies are needed to identify whether aboveground manipulations in grasslands have a significant impact on belowground processes that affect SOC dynamics and stocks.

The availability of macro-nutrients such as nitrogen (N) and phosphorus (P) has been identified as the major driver for the C cycle in soils (Christopher and Lal, 2007; Kirkby et al., 2014). This is due to effects on both C inputs and effluxes, but potentially also on direct and indirect C stabilization mechanisms, such as altered microbial C use efficiency (growth over uptake) (Spohn et al., 2016) and aggregate formation by arbuscular mycorrhiza fungi (AMF) (Rillig et al., 2001; Sochorová et al., 2016). In agricultural systems, nutrients are applied in organic or mineral form to maximize plant productivity and maintain soil fertility. For croplands, the fertilization-induced increase in biomass is acknowledged to have a positive effect on SOC stocks (Ladha et al., 2011; Kätterer et al., 2012; Leifeld et al., 2013). For several Swedish longterm cropland fertilization experiments, Kätterer et al. (2012) found an annual increase in SOC of  $1 \text{ kg ha}^{-1}$  for each kg mineral nitrogen (N) applied. However, in a global meta-analysis of synthetic N fertilizer application on SOC, Alvarez (2005) detected significant increases in SOC stocks only when crop residues were returned to the soil. This underlines the importance of C input to soils for maintaining and building up SOC stocks.

Nutrient availability has a strong influence on plant C allocation. Under nutrient deficiency, plants have to invest more in belowground organs to acquire nutrients from the soil, while when belowground resources are non-limiting, plants invest more in aboveground biomass (Marschner et al., 1996). Thus, an increase in total net primary production might not lead to higher soil C inputs when the majority of aboveground biomass is exported, which is usually the case in managed grasslands. Even though roots have been acknowledged to be a key component for SOC dynamics (Rasse et al., 2005), very few studies have examined root biomass in relation to SOC in grasslands (Sochorová et al., 2016). This may be due to the high workload in determining root C, but also to the damage to long-term field experiments caused by root sampling.

Nutrient availability also affects microbial metabolism, and thus C outputs (Manzoni et al., 2012; Kirkby et al., 2014; Murphy et al., 2015; Spohn et al., 2016). Such findings are often explained by stoichiometric constraints on microbial biosynthesis, with soil microbial biomass having an average C:N:P ratio of 60:7:1 (Cleveland and Liptzin, 2007; Spohn, 2015). In this case, increased nutrient availability could potentially have a double-positive effect on SOC stocks, i.e., increased C inputs and a higher degree of biosynthesis and thus lower respiratory C losses per unit C metabolized (Leifeld, 2012). This has recently been confirmed by Sanderman et al. (2017), who found increasing SOC stocks and increased turnover rates accompanying increasing

agricultural productivity. However, Poeplau et al. (2015) found a negative effect of PK fertilization on SOC stocks in Swedish arable soils, despite higher net primary production compared with long-term unfertilized soils. In a subsequent incubation experiment, they were able to link such losses to stimulated microbial activity after PK addition, although the reasons for that remained unclear (Poeplau et al., 2016a).

In contrast to croplands, in which most fertilization studies are performed (Debreczeni and Körschens, 2003), grasslands respond to contrasting nutrient additions with alterations in plant community composition, whereby nutrient inputs have the potential to reduce species richness (Stevens et al., 2004). Altered composition of plant species and functional traits has been found to affect SOC dynamics (De Devn et al., 2008; Steinbeiss et al., 2008). In the course of a 12-year grassland biodiversity experiment under N-limited conditions, Fornara and Tilman (2008) found five-fold higher SOC accumulation in plots with 16 species than in monoculture. They attributed this mainly to the joint presence of legumes and C4-grasses and the resulting increase in belowground biomass. Furthermore, a shift in plant community composition might be associated with changed litter quality and properties such as C:N ratio (Berendse, 1998), which has an influence on its decomposability and thus on SOC dynamics (Janssen, 1996). Even within the same species, contrasting nutrient availability can lead to strong contrasts in plant stoichiometry (Poeplau and Kätterer, 2017).

Studies of fertilization effects on SOC in grassland ecosystems are limited. In a global meta-analysis of grassland management effects on SOC, Conant et al. (2001) found a total of 40 studies reporting SOC responses to any kind of fertilization, including organic fertilization. They found an average annual increase in SOC stocks of  $0.3 \text{ Mg ha}^{-1}$  $yr^{-1}$  with fertilization. In contrast, in a recent study Sochorová et al. (2016) found significant negative effects (-20%) of NP fertilization on SOC stocks in a German grassland experiment and explained this mainly by decreased mycorrhiza abundance. A detailed understanding of nutrient effects on C dynamics and resulting total SOC stocks is therefore lacking. In this study, seven long-term grassland fertilization experiments were sampled to investigate how varying addition of the macronutrients N, P, and K influences total SOC stocks and how this might be related to: i) above- and belowground biomass responses to fertilization, ii) root litter stoichiometry, and iii) potential litter decomposition.

## 2. Materials and methods

#### 2.1. Long-term experiments

Seven different long-term grassland experiments with gradients in mineral fertilization in Germany (six) and the Netherlands (one) were identified and sampled (Table 1). All of these experiments are cut once or several times a year and not grazed. They thus represent meadowtype grasslands and were set up to study intensification effects on biomass production and quality and on species composition. Experiments with organic fertilization, and thus external C inputs, were

Table 1

List of sites with site code, latitude and longitude, mean annual temperature (MAT,  $^{\circ}$ C), mean annual precipitation (MAP, mm), clay, silt, and sand content [%], soil pH measured in water (pH<sub>H2O</sub>), the average dry matter yield [Mg DM ha<sup>-1</sup>] of the unfertilized plots since the start of the experiment as an indicator of site productivity as well as annual cutting frequency (Cutting).

Site code	Site	Latitude	Longitude	MAT	MAP	Clay	Silt	Sand	pH <sub>H2O</sub>	Yield	Cutting
HA	Hayn	51°34'01"	11°04'22"	6.5	618	24	53	23	5.4	6	3
ID	Iden	52°46'51"	11°54'07"	9	524	17	26	57	6.5	3.4	4
MO	Mordfleck	50°38'11"	10°46'36"	4.9	1345	21	76	3	4.7	2.3	2
LI	Lichtenhain	50°35'18"	11°07'54"	6.4	902	19	54	27	5.6	2.8	2
OB	Oberweißbach	50°34'47"	11'08'49"	6.4	902	13	53	34	5.8	4.7	1/2
НО	Hohenheim	48°44'33"	8°54'55	8.1	693	21	75	4	6.8	4.3	2
WA	Wageningen	51°58'16"	5°38'17"	9.7	818	58	38	4	5.4	4.9	2

Cutting frequency at OB: one cut in the Control, two cuts in the NPK treatment.

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