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**Land use intensity, rather than plant species richness, affects the leaching risk of multiple nutrients from permanent grasslands**

Running head: Risk of nutrient leaching from grasslands

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## **Keywords**

Drought, Fertilization, Grassland biodiversity, Grazing, Ion-exchange resin bags, Mowing, Nitrate leaching, Phosphorus

## **Primary Research Paper**

### **Abstract**

The intensification of land use constitutes one of the main drivers of global change and alters nutrient fluxes on all spatial scales, causing landscape-level eutrophication and contamination of natural resources. Changes in soil nutrient concentrations are thus indicative for crucial environmental issues associated with intensive land use. We measured concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N, P, K, Mg and Ca by using 1326 ion-exchange resin bags buried in 20 cm depth beneath the main root zone in 150 temperate grasslands. Nutrient concentrations were related to land use intensity i.e., fertilization, mowing, grazing intensities, and plant diversity by structural equation modelling. Furthermore, we assessed the response of soil nutrients to mechanical sward disturbance and subsequent reseeding, a common practice for grassland renewal.

Land use intensity, especially fertilization, significantly increased concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N, K, P and also Mg. Besides fertilization (and tightly correlated mowing) intensity, grazing strongly increased NO<sub>3</sub>-N and K concentrations. Plant species richness decreased P and NO<sub>3</sub>-N concentrations in soil when grassland productivity of the actual year was statistically taken into account, but not when long-term averages of productivity were used. Thus, we assume that in the actual study year a distinct drought period might have caused the observed decoupling of productivity from fertilization and soil nutrients. Breaking-up the

grassland sward drastically increased  $\text{NO}_3^-$ -N concentrations (+146%) but reduced  $\text{NH}_4$ -N, P and K concentrations, unbalancing soil nutrient stoichiometry and boosting the risk of N leaching. Reseeding the sward after disturbance did not have a short-term effect on nutrient concentrations. We conclude that renewal of permanent grassland should be avoided as far as possible and future grassland management has to strongly rise the effectiveness of fertilization. Additionally, grassland management might have to increasingly taking care of periods of drought, in which nutrient additions might not increase plant growth but potentially only facilitate leaching.

## Introduction

Agricultural intensification constitutes one of the main drivers of global environmental change, as it has led to wide-reaching changes in nutrient fluxes from local to continental scales (Erb *et al.*, 2016; Smith *et al.*, 2016). Given the worldwide growing demand for food, this intensification is likely to strengthen within the following decades (Godfray *et al.*, 2010). Intensification also affects the management of grassland by increasing fertilization, cutting rates and/or increasing stocking densities and recurrent sward renewal (Blüthgen *et al.*, 2012; Buchen *et al.*, 2017). Today's nutrient inputs in agricultural land are often associated with unwanted eutrophication or contamination, which have destabilizing and detrimental side-effects on ecosystems and the services they provide (Firbank *et al.*, 2008; Binzer *et al.*, 2016). Losses of nitrate ( $\text{NO}_3^-$ ) from the topsoil to the groundwater are particularly adverse as they cause serious health risks when contaminating drinking water (Cameron *et al.*, 2013). Besides, phosphorus (P), which is strongly enriched in many agriculturally used soils due to periods of intensive fertilization (Schärer *et al.*, 2007), also provoke severe environmental problems especially by facilitating the eutrophication of surface waters. However, losses and pathways of P are still notably poorly understood (Jensen *et al.*, 2000; Stuart & Lapworth,

2016). The same is true for losses of potassium (K), the third most intensively applied nutrient (Kayser & Isselstein, 2005). As agriculture has generally been identified as the most prominent cause of these environmental problems (Di & Cameron, 2016), a thorough assessment of land use effects on soil nutrient fluxes is urgently needed for all (potentially) intensively managed land use types including also permanent grasslands (Blüthgen *et al.*, 2012).

Nutrient dynamics in grassland soils are mostly driven by fertilization and defoliation i.e., mowing and grazing. Especially the overly use of organic and inorganic fertilizers and intensive livestock grazing are associated with enrichment and subsequent potential losses of different nutrients to the environment (Robson *et al.*, 2007; Alt *et al.*, 2011; Vaieretti *et al.*, 2013). Intensive grassland management is also associated with recurring sward renewal by reseeding after breaking-up the old sward. Despite the detection of significant environmental problems caused by losses of N in multiple pathways (Whitmore *et al.*, 1992; Kayser *et al.*, 2008; Velthof *et al.*, 2010; Buchen *et al.*, 2017), a broad and well replicated assessment of multiple soil nutrients in response to sward destruction is still lacking, limiting our knowledge on environmental effects of intensive grassland management on nutrient dynamics (Krol *et al.*, 2016).

Plant community characteristics have often been related to soil nutrient concentrations in grassland ecosystems. In agriculturally used grasslands with a sufficient nutrient supply, increasing soil P concentrations were shown to negatively affect plant diversity (e.g. Janssens *et al.*, 1998; Rudolph *et al.*, 2017). Or, the other way round, higher plant diversity of experimental grasslands was related to lower  $\text{NO}_3^-$  concentrations (Niklaus *et al.*, 2001; Mueller *et al.*, 2013) or leaching (Leimer *et al.*, 2016), assumingly due to a more complete N

use at higher diversity (Kleinebecker *et al.*, 2014). Furthermore, changes in the functional composition, e.g. the presence and cover of legume species, can considerably affect soil  $\text{NO}_3^-$  concentrations (Mallarino & Wedin, 1990; Leimer *et al.*, 2016). Thus, a higher number of soil nutrients might be more closely related to the composition and diversity of the grassland vegetation than hitherto assumed.

Measurements of soil nutrients are often restricted to a certain point of time, when a soil sample is taken, ignoring the temporal variability of major drivers of nutrient dynamics such as the timing of land use, varying weather conditions and changes in soil biological activity (Kleinebecker *et al.*, 2011; Regan *et al.*, 2014; Klaus *et al.*, 2016). To representatively assess soil nutrients it is important to integrate over longer time spans. Therefore, ion exchange resin (IER) bags were designed (e.g. Binkley & Matson, 1983; Robson *et al.*, 2007; Salmon *et al.*, 2016; Dawes *et al.*, 2017) and already successfully used to assess concentrations and losses of e.g.  $\text{NO}_3^-$  (Pampolino *et al.*, 2000; Qian & Schoenau, 2002). However, up to now, studies using IER bags to assess land use effects on soil nutrients in grasslands failed to take all relevant macro-nutrients into account and lacked an appropriate and realistic assessment of land use intensity by involving unrealistic high fertilization rates or by ignoring low-intensity sites.

In this work, we used 1326 IER bags to assess effects of land use intensity on concentrations of  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ ,  $\text{PO}_4\text{-P}$ , K, Mg, and Ca in 20 cm depth beneath the main root zone from March to August in 150 grasslands in three study regions in Germany (Table 1). Nutrient concentrations were related to fertilization, mowing and grazing intensities, environmental site factors and different vegetation characteristics. Additionally, we measured the responses of nutrient concentrations to mechanically breaking-up (down to 10 cm) and reseeding the grassland sward. We hypothesized that

- (1) intensification of grassland management i.e., fertilization, mowing and grazing intensities, significantly increases soil nutrient concentrations beneath the main root zone.
- (2) plant diversity is negatively related to soil nutrient concentrations.
- (3) due to the decomposition of the remnants of the old sward, sward destruction drastically increases concentrations of all nutrients.
- (4) immediate reseeding after sward destruction buffers the increase in soil nutrient concentrations due to fast regrowth of the plant community.

## Materials and Methods

### *Study regions and experimental design*

Our study was performed in the framework of the Biodiversity Exploratories for long-term functional biodiversity research (Fischer *et al.*, 2010). The three German study regions are (Fig. S1): the biosphere reserve Schorfheide-Chorin in Brandenburg (NE Germany), a geologically young, post-glacial landscape, the National Park Hainich and surroundings in Thuringia (Central Germany), consisting mainly of an undulating calcareous low mountain range covered with loess depositions, and the biosphere area Schwäbische Alb in Baden-Württemberg (SW Germany), a calcareous mid mountain range. In each of the three regions, 50 permanent grasslands were selected on the most common soil types along a gradient of land use intensity (Table 1). Mean and range of land use intensities are representative for large parts of Central Europe (Blüthgen *et al.*, 2012). Grassland management ranged from unfertilized sheep pastures and single-cut meadows up to highly fertilized meadows and mown pastures with three cuts per year or intensive grazing. Plant species richness varied strongly among grasslands with an overall mean of 29 species per 16 m<sup>2</sup> (min/max = 13/69).

For more details on study regions see Table 1. In 2015, the early summer months were remarkably dry in two out of our three study regions (Fig. S2).

All 150 grassland plots were used for an observational study relating land use and plant diversity to soil nutrient concentrations, while nearly half of the plots were also used to assess effects of mechanically breaking up and reseeding the sward ( $n = 73$  in total). Therefore, a full-factorial experiment was established on four  $7\text{ m} \times 7\text{ m}$  subplots on each of the experimental sites containing one *disturbance only*, one *seed addition only*, one *disturbance and seed addition combination* and one *control* treatment. For the sward disturbance, the top 10 cm of the grassland were treated by intensive rotovation tilling or rotary harrowing in October 2014. The sward was broken up and turned over, creating open patches with bare soil while fragments of the former sward, such as grass tussocks, were left on the subplots and could (partly) regrow. Reseeding was done by adding a highly diverse, region-specific seed mixture of native plant species to each subplot. Species of all three functional groups i.e., grasses, legumes and non-legume herbs, were included into the mixtures, except for the Schorfheide region where the mixture did not contain any legumes. Seeds were added twice, with two thirds of the mixtures added after sward disturbance in November 2014 and one third in March 2015 to facilitate establishment success. The total amount of seeds sown was  $5.37\text{ g} \times \text{m}^{-2}$  in Schwäbische Alb,  $4.11\text{ g} \times \text{m}^{-2}$  in Hainich and  $3.47\text{ g} \times \text{m}^{-2}$  in Schorfheide. For further details on the experimental design see Klaus *et al.* (2017).

#### *IER bags, soil sampling and lab analyses*

Nutrient concentrations were measured in situ using 1326 ion-exchange resin bags (IER bags; Sibbeson, 1977; Binkley & Matson, 1983; Lajtha, 1988, Skogley & Dobermann, 1996), made out of nylon fabric containing anion/cation mixed-bed resin beads plus specific resin beads

for anionic heavy metals and phosphate (Firma TerrAquat, Nürtingen, Germany). Bags contained in total 19.5 g (dry weight) resin and were of a round shape with a diameter of 5 cm. Three bags per plot subplot were installed in all grasslands for approx. 145 days from March to early August 2015. Thus, in experimental subplots, IER bags were put into the soil four months after sward disturbance. As the vast majority of (both mineral and organic) agricultural fertilizers is applied from mid-March to June, our measurements will have covered almost the whole fertilization season in 2015. Resin bags have been buried at 20 cm depth accessed from above by temporarily taking out a soil core with a corer sampler (diameter 5 cm) and thoroughly placing back the previously extracted soil column into the hole. In the study regions Schwäbische Alb and Hainich, at some very shallow soils, bags could be installed in 10-15 cm depth only. Statistical analyses will account for differences in installation depth among plots (see below). Due to the exponential decline of root biomass and root densities with soil depth (Frank *et al.*, 2010; Ravenek *et al.*, 2014), concentrations of nutrients in soil solution at 20 cm depth can be seen as indication for an increased risk of nutrient leaching (Pampolino *et al.*, 2000).

After removal, IER bags were stored in a fridge at 4°C. Extraction was done for each bag separately. 15 g resin was extracted with 100 ml 1M NaCl, in two steps of two parallels of 50 ml each, shaked for 30 min and filtered. The measurement of NH<sub>4</sub>-N and NO<sub>3</sub>-N was performed with a Continuous Flow Auto Analyser (Skalar Analytic B.V., Breda, The Netherlands). Calcium (Ca<sup>2+</sup>, called Ca hereafter), magnesium (Mg<sup>2+</sup>, called Mg hereafter), and potassium (K<sup>+</sup>, called K hereafter) concentrations were analysed using a Spectro ARCOS ICP-OES (Spectro Analytical Instruments, Kleve, Germany). A standard Fassel-type torch (inner diameter: 1.8 mm) in axial position was applied for the elemental determination. The sample introduction was carried out by a cross flow nebulizer and a Scott spray chamber.

Argon was used as nebulizer gas with a flow rate of 0.8 L/min, as auxiliary plasma gas with a flow rate of 0.85 L/min and as cooling gas with a flow rate of 12.0 L/min. The radio frequency power was 1400 W and wavelengths were selected on their highest sensitivity. The samples were either diluted 1:10 in ultra-pure water (Millipore Milli-Q system, Schwalbach, Germany) or measured directly. To determine PO<sub>4</sub>-P (called resin-P or just P hereafter) another aliquot of the resin beads (15 g) was extracted with 100 ml 0.5M H<sub>2</sub>SO<sub>4</sub> following the same protocol as described above. All concentrations are given as mean values per plot in mg\*g<sup>-1</sup> (dry weight) resin. For plots where all three replicates were below detection limit we set 10% of the lowest measured value, since total absence of the respective nutrient is unlikely. However, this procedure had just been applied to one Ca and 11 Mg values. One observational and two experimentally treated grasslands had to be excluded from the study for technical reasons.

For general soil characterisation, composite soil samples based on 14 sampling points along two transects per plot were collected in May 2011 and 2014 in the upper 10 cm of the soil. Samples were dried at 40°C and sieved to <2mm. In 2011, particle size distribution was determined by a combination of wet sieving with sedimentation following DIN-ISO 11277. In 2014, pH was measured as well as total C and N concentrations. The pH was measured as a mean of two replicates using a glass-electrode in 0.01 M CaCl<sub>2</sub> with a soil solution ratio of 1:2.5. A subsample of soil was ground with a ball mill and used to analyse total soil carbon and nitrogen contents by dry combustion in an elemental analyser (VarioMax, Hanau, Germany). Carbonate content was determined after organic material was combusted at 450°C for 16 hours using the same elemental analyser and organic carbon content (C<sub>org</sub>) was calculated as difference between total and inorganic carbon. Soil depth was evaluated based on a soil core taken at the centre of the plot with a motor driven soil corer (Cobra TT, Atlas

Copco AB, Nacka, Sweden) with 8.3 cm inner diameter. Genetic soil horizons were identified and depth to rock or C horizon was measured. Soil moisture was measured continuously every 10 minutes in 10 cm depth using a DeltaT ML2X soil moisture probe (Delta-T Devices, Cambridge, GB) on every grassland. We used averaged data from April to July 2015, when IER bags were in soil. Due to sensor errors, seven soil moisture values had to be replaced by the mean value of the neighbouring plots on the same soil type within the same region.

#### *Vegetation sampling*

The number and estimated cover of all vascular plant species and the cover of bare soil were recorded annually from mid-May to early June 2009 to 2015 on a 4 m × 4 m area of all 150 grasslands, simultaneously in the three study regions. In 2015, seven months after disturbance and six months after the first, and two months after the second seed addition (reseeding), this procedure was also done on 2 m × 2m areas in all four subplots on the 73 experimental grasslands. Temporary fences ensured that plots where neither mown nor grazed prior vegetation and biomass sampling. At the same time as the vegetation records and in close proximity to them, aboveground plant community biomass was harvested as a proxy of productivity (peak standing crop) in eight quadrats of 0.25 m<sup>2</sup> adjacent to each vegetation record. Harvested plant material was dried for 48 h at 80 °C and weighed to the nearest gram.

#### *Land use information*

To quantify land use intensity, questionnaires were used to gather information from farmers on the annual amount of fertilizer applied (kg nitrogen × ha<sup>-1</sup>), the frequency of mowing (number of cuts) and the grazing intensity (number of livestock units × days × ha<sup>-1</sup>) on each grassland in 2014 and 2015 separately, as described in Blüthgen *et al.* (2012). Values of both

years were averaged and standardized for further analysis by dividing raw values by the specific mean across all plots.

### Statistics

Measurements of the three resin bags per plot/subplot were averaged to gain a stable mean. Multiple linear regressions were used to relate nutrient concentrations of the 150 grasslands to management, environmental characteristics and vegetation. We included fertilization and grazing intensities, cover sums of legumes and grasses, soil moisture, soil pH, clay and silt contents, soil C<sub>org</sub> content, final installation depth of IER bags and study region. Highly correlated variables of  $r_S \geq 0.7$  were excluded in model selection: soil depth and sand content were highly (negatively) correlated with clay contents, mowing was highly correlated with fertilization (positive) and grazing (negative). Summary outputs ensure that all other variables are taken into account before significance of a single variable is calculated (ANOVA type II). Full models were reduced according to the AIC employing the step-function in R. The installation depth of the IER bags was never significantly related to any nutrient concentration, indicating that the small differences in deep burial of the bags on shallow soils did not skew our results.

Multiple linear regression models were repeated with plant species richness (average of 2009–2015) as additional explanatory factor. When this turned out to be significant, structural equation models (SEMs) were calculated to test whether relationships between plant species richness, management and soil nutrient concentrations are direct or indirectly i.e. depending on each other and/or aboveground productivity. This is important as all these variables are known to be inter-correlated to some degree in real-world grasslands (Klaus *et al.*, 2011). For the calculation of the SEMs, we used residuals of the previous models which were re-run

with all significant factors but without plant species richness, fertilization and grazing. We started with a SEM full model (Fig. S3) and stepwise removed all paths with  $P > 0.05$  to save degrees of freedom. Due to the annual variability of aboveground productivity, in 2015 potentially influenced by the relatively dry period in summer (Fig. S2), we decided to calculate two comparable SEMs with either the actual (2015) or a long-term average of aboveground productivity (2009-2015).

To account for the split-plot design of the sward disturbance and reseeding experiment, we calculated linear mixed-effects models (LMEs) including plot as random factor to test for separate and combined effects of both experimental factors. Study region was added as an (further) interacting factor accounting for potentially relevant differences at the regional scale, such as climate, geology or land use history (Klaus *et al.*, 2013). For all regression analyses, variables were square root or log transformed where necessary to achieve normal distribution. Model assumptions were tested using diagnostic plots. All statistical analyses were performed with the program package R (R Development Core Team, 2016). For the SEMs, we used the package *lavaan* (Rosseel, 2012) and for the linear mixed effects models the *nlme* package (Pinheiro *et al.*, 2016).

## Results

### *Effects of land use intensity on soil nutrient concentrations*

Regression models predicting nutrient concentrations by management, vegetation characteristics and additional environmental factors explained 17-44% of the variation in the respective nutrient, with the lowest explanatory power for  $\text{NO}_3\text{-N}$  and the highest for K and Ca (Table 2). Except Ca, all nutrient concentrations increased with fertilization intensity, particularly strong the concentrations of K,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and P. Grazing intensity likewise

increased especially K and NO<sub>3</sub>-N concentrations. Soil moisture was just weakly related to Mg and NH<sub>4</sub>-N, showing that the rather dry summer did not considerably influence nutrient concentrations measured with IER bags.

Site and regional factors explained a certain proportion of variability, although in some nutrient concentrations a relatively high proportion of the variability remained widely unexplained. Soil pH particularly affected Ca and Mg positively and P negatively, while C<sub>org</sub> in soils was strongly associated with low K and P concentrations. Clay contents increased especially Ca, P, and NO<sub>3</sub>-N concentrations, while silt was only significantly related to Ca (positive) and K (negative). The functional composition of the plant community seemed to be less important for soil nutrient concentrations, as of both plant functional groups just legume cover was significant related to one of the nutrients i.e., weakly and negatively to Ca concentrations (Table 2).

#### *Effects of plant diversity on soil nutrient concentrations*

When residuals of the previously calculated models were further explained by plant species richness, only P and NO<sub>3</sub>-N concentrations were significantly affected (P: t = -1.99,  $P < 0.05$ ,  $R^2 = 0.03$ ; NO<sub>3</sub>-N: t = -2.16,  $P < 0.01$ ,  $R^2 = 0.04$ ). In both cases, plant species richness was negatively related to soil nutrient concentrations but explained only a rather small additional proportion of variance. When further exploring plant species richness and management effects on P and NO<sub>3</sub>-N concentrations, structural equation modelling yielded different results when either actual or long-term values of productivity were used (Figs 1 and 2). Although in all models, fertilization and/or grazing intensities consistently increased the P and NO<sub>3</sub>-N concentrations, plant species richness was only significantly directly related to concentrations in the actual models, when productivity of 2015 was used (Figs 1a and 2a). When the long-

term average of productivity was used, this path lost its significance and was substituted by a positive correlation of productivity and nutrient concentrations (Figs 1b and 2b), which was not significant in the actual models. Thus, in the long-term model, soil nutrient concentrations were only indirectly (negatively) related to plant species richness via productivity. Additionally, while in the actual model, fertilization had no significant effect on productivity, this was true for long-term productivity, suggesting that in 2015, the relatively dry summer months (Fig. S2) could have changed mechanistic interactions by decoupling fertilization and productivity as well as productivity and soil nutrient concentrations. Productivity in 2015 was not strongly but significantly related to the long-term average of productivity (2009 to 2014;  $R^2 = 0.38$ ;  $p < 0.001$ ).

Management effects were absolutely stable among all SEMs in that fertilization (and for NO<sub>3</sub>-N concentrations also grazing) had greater direct effects increasing soil nutrient concentrations than increasing (actual and long-term average) productivity, pointing at generally low effectiveness of fertilization. Findings of direct effects of plant species richness on P and NO<sub>3</sub>-N concentrations in the actual model did not change, when species richness of 2015 was used (data not shown), which is why we show only models with the more robust long-term average of plant species richness (2009-2015).

#### *Effects of sward disturbance and reseeding*

Experimental sward disturbance down to 10 cm depth, simulating effects of breaking-up the old sward for grassland renewal, strongly affected all nutrient concentrations across all plots (Table 3; Fig. 3). The only exceptions were Ca and Mg concentrations, for which just significant interactions of disturbance with study region were found. While P (-42%), K (-32%) and also NH<sub>4</sub>-N (-32%), concentrations decreased due to sward disturbance, NO<sub>3</sub>-N

strongly increased (+146%). Seed addition (reseeding) did not significantly affect any nutrient concentration, neither in general nor in interaction with study region or with sward disturbance (Table 3; Fig. 3). Regional differences in concentrations and treatment effects are given in Fig. S4 in the supporting material. The vegetation survey revealed that the vegetation had not recovered from sward disturbance in May 2015, seven month after disturbance, in that the proportion of bare ground was considerably higher and productivity still much lower on both disturbance compared to control plots, independently from reseeding (Fig. S5).

## Discussion

We measured concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N, P, K, Mg and Ca in 20 cm soil depth in agricultural grasslands to assess effects of land use intensity, plant species richness and grassland renewal on soil nutrients. Due to the exponential decline of root biomass with soil depth especially beginning at 20 cm (Frank *et al.*, 2010), high nutrient concentrations indicate enhanced risk of increased nutrient leaching and relocation (Haygarth *et al.*, 1998; Pampolino *et al.*, 2000), particularly as resin bags predominantly capture highly mobile fractions of the respective nutrient. The major aim of this study was to analyse different effects of real-world intensive grassland management and plant diversity on potential nutrient leaching by using for example realistic fertilization levels of up to 300 kg N per ha per year, overcoming limitations of previous work where often extreme and unrealistic amounts of especially N were applied to very few sites (e.g. Barraclough *et al.*, 1992; Scholefield *et al.*, 1993; Di & Cameron, 2002).

### *Effects of land use intensity on soil nutrient concentrations*

Our study revealed NO<sub>3</sub>-N, NH<sub>4</sub>-N, K, P and even Mg concentrations to be significantly affected by land use intensity (Table 2). Especially fertilization intensity increased all these nutrient concentrations beneath the main root zone, extending findings of previous studies to a wide range of different nutrients measured across a broad gradient of land use in temperate grasslands (e.g. Jensen *et al.*, 2000; Kayser & Isselstein, 2005; Schröder *et al.*, 2010; Alt *et al.*, 2011). In contrast, Ca concentrations were almost unaffected by land use and only related to soil pH, clay and silt contents and weakly also to the proportion of legumes in the vegetation.

Increased concentrations of NO<sub>3</sub>-N, NH<sub>4</sub>-N and P are particularly problematic, potentially threatening ground water quality and causing eutrophication of adjacent habitats. High NO<sub>3</sub>-N concentrations due to intensive fertilization put surface and ground water at risk, as NO<sub>3</sub><sup>-</sup> is very mobile and likely to be leached or translocated (Cameron *et al.*, 2013). Fertilization and grazing intensities increased the risk of NO<sub>3</sub><sup>-</sup> leaching to almost the same extent (Table 2). All other soil and vegetation characteristics failed to explain NO<sub>3</sub>-N concentrations, except a weak positive effect of the clay content, resulting in rather low explained variance for this important nutrient.

Strong effects of grazing on NO<sub>3</sub>-N are in line with previous work emphasizing that grazing and associated dung and urine patches are highly important for explaining raised NO<sub>3</sub>-N in soil (e.g. Barraclough *et al.*, 1992; Scholefield *et al.*, 1993; Ledgard *et al.*, 2011). However, in contrast to previous studies, where mowing was barely related to NO<sub>3</sub>-N leaching (Di & Cameron, 2002; Cameron *et al.*, 2013), we have to stress that in intensively used real-world grasslands, mowing intensity is strongly correlated with fertilization intensity (Blüthgen *et*

*al.*, 2012; Socher *et al.*, 2012), pointing at the risk of  $\text{NO}_3^-$  leaching in grasslands which are not grazed but mown. This is supported by Schröder *et al.* (2010), who found considerable  $\text{NO}_3^-$  leaching under cut grasslands. Thus, our results indicate that the assumption of  $\text{NO}_3^-$ -N leaching from grasslands being almost entirely restricted to grazing should definitively be changed. Although mowing removes nutrients more effectively than grazing, the removal of nutrients from agriculturally managed grasslands by mowing is, nevertheless, easily compensated by fertilization and a combination of atmospheric N depositions, N turnover in soil and fixation of N by legumes (Olde Venterink *et al.*, 2002).

Fertilization also strongly affected  $\text{NH}_4^+$ -N concentrations. Although  $\text{NH}_4^+$  is mostly bound to clay minerals and organic matter and is thus less likely leached from the soil, it can easily be oxidized to  $\text{NO}_3^-$  and subsequently leached (Cameron *et al.*, 2013), additionally increasing the potential of  $\text{NO}_3^-$  leaching due to intensive fertilization (Table 2).

K concentrations were also increased by both fertilization and grazing. Beside direct inputs by fertilizers, dung and especially urine depositions cause a high risk of K leaching (Kayser *et al.*, 2007), although this leaching is not detrimental to e.g. groundwater quality. Additionally, K availability strongly depends on soil properties (the parent soil material) and is low in sandy and C<sub>org</sub>-rich soils, but generally higher in those rich in clay minerals (Kayser & Isselstein, 2005). One further important factor explaining high K concentrations is the leaching of corresponding anions such as  $\text{NO}_3^-$  (Kayser *et al.*, 2007). Differences in K leaching between the studied regions might be explained at least partly by different amounts of competing cations, e.g. Ca and Mg (Fig. S4).

We also found resin P concentrations, which characterize a mobile P fraction and widely ignore P bound to particles, to be strongly increased by fertilization intensity (Table 2). Mobile P is particularly problematic as it can be translocated by surface discharge and base flow as well as in drainage water from grasslands to adjacent habitats leading to eutrophication especially of surface waters (Haygarth *et al.*, 1998; Stamm *et al.*, 1998; Preedy *et al.*, 2001; McDowell *et al.*, 2004). Today, leaching of P is also getting into the focus of ground water quality (Stuart & Lapworth, 2016), for example caused by increased stocking densities (Jensen *et al.*, 2000). However, in our study P was not significantly related to grazing intensity, potentially as we pooled different types of livestock (sheep, cattle, horses) for the analyses. As it could be assumed, concentrations of available P were also driven by soil pH due to fixation of P especially by Ca. Yet, our study shows that P availability is considerably driven by grassland management not only in the uppermost centimetres of a soil (e.g. 0-5 cm; Schärer *et al.*, 2007), but also deeper in soil, where leaching is most likely.

The functional composition of the grassland vegetation was hardly related to nutrient concentrations. The cover of legumes, although proven to foster NO<sub>3</sub>-N leaching of unfertilized experimental grasslands (Scherer-Lorenzen *et al.*, 2003; Leimer *et al.*, 2016), was not related to NO<sub>3</sub>-N concentrations, assumedly because nutrient-poor, low intensity grasslands often exhibit rather high proportions of legumes (Klaus *et al.*, 2011). This once more shows the persistent importance of real-world studies to evaluate findings from strongly controlled experiments, especially if these do not take effects of fertilization into account. Generally, our study proved IER bag measurements to be a very useful and promising approach to integrate temporally highly variable management effects on soil nutrient concentrations. This relatively simple approach strongly improves the prediction of changes in nutrient dynamics driven by land use intensity.

### *Effects of plant diversity on soil nutrient concentrations*

We found significant direct negative effects of plant species richness on NO<sub>3</sub>-N and P concentrations in soil (Figs 1a and 2b). However, these negative effects depended on the particular situation in 2015 as they became insignificant when long-term mean values of productivity were used (Figs 1b and 2b). In 2015, the distinct summer drought might have caused a decoupling of productivity and fertilization as well as of soil nutrients and productivity, resulting in a direct negative effect of plant species richness on NO<sub>3</sub>-N and P concentrations. However, in our study, the contribution of the drought to this effect cannot be analysed in further detail. Thus, future research has to clarify whether the observed effect is mechanistic, in that at least in dry years plant diversity insures more complete nutrient uptake, or whether it appeared just due to the variability in environmental conditions and annual productivity under water shortage, temporarily disconnecting productivity and nutrient fluxes. In 2015, not nutrients but rather water availability might have been predominantly limiting plant growth, although except a weak effect on NH<sub>4</sub>-N, no resin nutrient concentration was significantly related to soil moisture measurements (Table 2).

Our study puts findings of generally decreased NO<sub>3</sub><sup>-</sup> concentrations (and leaching) in experimental swards of higher species richness into perspective (Niklaus *et al.*, 2001; Mueller *et al.*, 2013; Leimer *et al.*, 2016), as in real-world grasslands, plant diversity is mostly controlled (and overruled) by fertilization and productivity, and is no independent factor (Klaus *et al.*, 2013). Furthermore, the statistically significant effect of plant species richness found in experimental approaches might strongly depend on artificial plant assemblages especially on monocultures, which do not exist in real-world permanent grasslands (Scherer-Lorenzen *et al.*, 2003; Lepš, 2004). Therefore, real-world grasslands are often much richer in plant species compared to experimental grassland communities. On the other hand, under

global climate change, when phases of drought are likely to occur more frequently (IPCC, 2013) altering causal networks of major drivers, potential effects of (ineffective) fertilization and plant species richness on soil nutrient concentrations might become increasingly relevant and should thus be studied in more detail.

#### *Effects of grassland renewal*

Destroying the old grassland sward and reseeding the vegetation is a frequently applied technique in intensive grassland management to maximise or maintain sward productivity. The experimental sward disturbance down to 10 cm depth, which is quite similar to the techniques used during grassland renewal, significantly affected all nutrient concentrations in 20 cm depth (Fig. 3; Table 3). Just Ca and Mg concentrations were only regionally affected (Fig. S4). While disturbance decreased NH<sub>4</sub>-N, P and K concentrations, indicating fixation or chemical transformation of mobile fractions, NO<sub>3</sub>-N strongly increased assumedly due to the mineralization of the remnants of the destroyed sward and reduced plant uptake. Reseeding, although done twice to increase establishment success, could not mitigate disturbance-driven changes in soil nutrient concentrations. Assumedly, as all seeded plants have still been in seedling stages, they might not have been able to take up the released nutrients, although our experiment did not use herbicide to kill the old sward before mechanical destruction, leaving (regrowing) tussocks on the soil surface (Klaus *et al.*, 2017). Our results put findings into perspective that a quick reseeding might be able to (partly) compensate nutrient losses from sward destruction (Buchen *et al.*, 2017). As our measurements were placed in 20 cm depth, beneath the main root zone, the drastically increased concentrations strongly indicate enhanced leaching of NO<sub>3</sub><sup>-</sup> to the ground water, potentially causing severe problems for drinking water quality. This is in line with studies finding increased gaseous N fluxes from ploughed grasslands (e.g. Whitmore *et al.*, 1992; Di & Cameron, 2002; Kayser *et al.*, 2008;

Buchen *et al.*, 2017), highlighting the importance to account for the risk of losing N in different forms during grasslands renewal, especially when done in autumn (Velthof *et al.*, 2010).

Reductions in NH<sub>4</sub>-N and K concentrations might be partly attributable to (temporal) physical and chemical changes due to the mechanical sward disturbance, leading to increased fixation of these cations. Additionally, NH<sub>4</sub>-N concentrations were most likely also reduced due to increased aeration of the soil, fostering nitrification. Nevertheless, even after accounting for the mean reduction in NH<sub>4</sub>-N (-32%), sward disturbance resulted in more than twofold total resin N concentrations (+110%) indicating a doubling of potential N losses via leaching.

Decreasing concentrations of P and K after disturbance could also be explained by reduced activity of plants i.e., decreased release of root exudates and other substances locally affecting soil pH and diminishing especially P availability (Hinsinger, 2001). These decreasing mechanisms were more important than increasing factors such as reduced uptake of P and K from fertilizers due to lower levels of biomass production at disturbed but regularly managed plots. Furthermore, increased aeration in combination with easily decomposable organic matter and increased N availability might also have enhanced soil microbial activity and consequently P and K uptake by microorganisms. Despite uncertainty on certain processes triggered by sward disturbance, we showed that sward disturbance significantly altered soil nutrient stoichiometry with unknown subsequent effects on the ecosystem.

## *Implications*

Our study shows that intensification of grassland management is a severe risk to the environment by increasing nutrient leaching potential and unbalancing soil nutrient stoichiometry, especially when management practices include regularly breaking-up the sward for grassland renewal. This eutrophication potential is a major current and future problem of habitat and species conservation, accounting for enormous losses in biodiversity (Firbank *et al.*, 2008; Isbell *et al.*, 2013) and threatens the supply of clear drinking water, especially by leaching of NO<sub>3</sub>-N, jeopardizing ground water quality. This is especially important as our results may have even underestimated the risk of leaching for specific regions where the average landscape level fertilization intensity is much higher than in our study regions, e.g. in Northwest Europe (Smit *et al.*, 2008). Thus, we recommend to avoid breaking-up the grassland sward, especially in autumn (Buchen *et al.*, 2017), and to strongly increase the effectiveness of fertilization, which appeared to be markedly low. Especially during phases of drought, nutrient additions might not be used to increase plant growth and are thus additionally provoking nutrient leaching.

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## Tables

**Table 1.** Environmental conditions and land use intensity of the three study regions according to Fischer *et al.* (2010) and Blüthgen *et al.* (2012). Land use intensities were calculated as annual mean values of 2014 and 2015. Each regions contains 50 observational grasslands of which 23 to 25 were additionally experimentally treated. For more details see methods section. Soil types are given according to IUSS Working Group (2007).

	Study region		
Toponym	Hainich-Dün	Schorfheide-Chorin	Schwäbische Alb
Location in Germany	Central	Northeast	Southwest
Geology	Calcareous bedrock with loess layer	Rather flat post-glacial landscape with sandy ridges and fens	Calcareous bedrock with loam layer and karst phenomenon
Dominant soil types	Cambisols, Stagnosols and Vertisols	Histsols, Gleysols, Cambisols, Luvisols and Albeluvisols	Leptosols and Cambisols
pH CaCl <sub>2</sub> as mean (min/max)	6.8 (5.0/7.3)	6.4 (4.6/7.4)	6.2 (5.2/7.3)
Silt (g·kg <sup>-1</sup> ) as mean (min/max)	520 (335/871)	372 (72/713)	407 (213/757)
Clay (g·kg <sup>-1</sup> ) as mean (min/max)	423 (60/646)	174 (41/427)	536 (146/708)
Sand (g·kg <sup>-1</sup> ) as mean (min/max)	58 (17/113)	454 (110/846)	57 (8/199)
Soil organic carbon (g·kg <sup>-1</sup> ) as mean (min/max)	37.7 (19.1/71.2)	103.8 (10.3/353.4)	64.3 (40.2/91.3)
Elevation (m a.s.l.)	285–550	3–140	460–860
Annual temperature (°C)	6.5–8.0	8.0–8.5	6.0–7.0
Annual precipitation (mm)	500–800	500–600	700–1000
Plant species richness (16m <sup>2</sup> )	33 (15/69)	22 (13/30)	32 (18/56)
<b>Land use intensities:</b>			
Fertilization (kg N·ha <sup>-1</sup> )	34.5 (0/205.1)	5.7 (0/73.6)	38.5 (0/341.5)
Mowing (number cuts)	1.1 (0/3)	0.7 (0/2)	1.4 (0/3.5)
Grazing (livestock units·grazing days·ha <sup>-1</sup> )	108 (0/994)	197 (0/825)	79 (0/638)

**Table 2.** Linear regression models of IER bag nutrient concentrations with land use, vegetation and environmental parameters (according to ANOVA type II output). Note that full models were stepwise reduced. Significance and effect of study regions is automatically given relative to the study region Schwäbische Alb. Effect refers to a positive or negative estimate of the respective factor. Significant factors are given in bold. The factor "installation depth of IER bags" was included in the first model, but has never been selected for a final model.

	Ca	Adjusted R <sup>2</sup>	Model P	Mg	Adjusted R <sup>2</sup>	Model P	K	Adjusted R <sup>2</sup>	Model P
		0.42	<0.001		0.24	<0.001		0.44	<0.001
	Effect	t	P	Effect	t	P	Effect	t	P
(Intercept)	↓	-3.17	<b>0.002</b>	↓	-0.70	0.486	↑	<b>10.75</b>	<b>&lt;0.001</b>
Region Hainich	-	-	-	↑	<b>2.37</b>	<b>0.019</b>	↑	<b>2.52</b>	<b>0.013</b>
Region Schorfheide	-	-	-	↓	-0.70	0.483	↓	<b>-2.25</b>	<b>0.026</b>
Grazing	-	-	-	-	-	-	↑	<b>3.02</b>	<b>0.003</b>
Fertilization	-	-	-	↑	<b>2.04</b>	<b>0.043</b>	↑	<b>2.97</b>	<b>0.003</b>
Soil pH	↑	<b>3.80</b>	<b>&lt;0.001</b>	↑	<b>2.67</b>	<b>0.008</b>	-	-	-
Cover grasses	-	-	-	-	-	-	-	-	-
Cover legumes	↓	<b>-2.07</b>	<b>0.041</b>	↓	-1.41	0.162	↑	1.65	0.101
Soil moisture	-	-	-	↑	1.78	0.077	-	-	-
C <sub>org</sub>	↓	-1.78	0.077	-	-	-	↓	<b>-4.52</b>	<b>&lt;0.001</b>
Clay	↑	<b>5.97</b>	<b>&lt;0.001</b>	↓	-1.69	0.094	-	-	-
Silt	↑	<b>2.58</b>	<b>0.011</b>	-	-	-	↓	<b>-2.60</b>	<b>0.010</b>
	<i>n</i> = 143			<i>n</i> = 141			<i>n</i> = 143		
	P	Adjusted R <sup>2</sup>	Model P	NO <sub>3</sub> -N	Adjusted R <sup>2</sup>	Model P	NH <sub>4</sub> -N	Adjusted R <sup>2</sup>	Model P
		0.36	<0.001		0.17	<0.001		0.31	<0.001
	Effect	t	P	Effect	t	P	Effect	t	P
(Intercept)	↑	<b>6.58</b>	<b>&lt;0.001</b>	↑	<b>5.45</b>	<b>&lt;0.001</b>	↑	<b>7.04</b>	<b>&lt;0.001</b>
Region Hainich	-	-	-	-	-	-	↑	<b>2.86</b>	<b>0.005</b>
Region Schorfheide	-	-	-	-	-	-	↓	<b>-2.24</b>	<b>0.026</b>
Grazing	↑	1.43	0.154	↑	<b>3.92</b>	<b>&lt;0.001</b>	-	-	-
Fertilization	↑	<b>5.32</b>	<b>&lt;0.001</b>	↑	<b>3.93</b>	<b>&lt;0.001</b>	↑	<b>3.58</b>	<b>&lt;0.001</b>
Soil pH	↓	<b>-3.48</b>	<b>&lt;0.001</b>	-	-	-	-	-	-
Cover grasses	-	-	-	↑	1.50	0.136	-	-	-
Cover legumes	-	-	-	-	-	-	-	-	-
Soil moisture	-	-	-	-	-	-	↑	<b>2.03</b>	<b>0.044</b>
C <sub>org</sub>	↓	<b>-4.37</b>	<b>&lt;0.001</b>	-	-	-	↓	-1.46	0.147
Clay	↑	<b>2.76</b>	<b>0.007</b>	↑	<b>2.27</b>	<b>0.025</b>	-	-	-
Silt	-	-	-	-	-	-	-	-	-
	<i>n</i> = 142			<i>n</i> = 144			<i>n</i> = 143		

**Table 3.** Results of linear mixed effects models testing significant effects of the two experimental treatments (reseeding = seed addition, sward destruction = disturbance) and their combination including study regions and all possible interactions on resin bag nutrient concentrations. Significance values shown were derived from ANOVA Type I outputs. Significant factors and interactions are given in bold ( $P < 0.05$ ;  $n = 71$  grasslands with a total of 284 subplots).

	DF	Ca		Mg		K	
		F	P	F	P	F	P
(Intercept)	1	<b>2123.93</b>	<b>&lt;0.001</b>	<b>208.98</b>	<b>&lt;0.001</b>	<b>488.32</b>	<b>&lt;0.001</b>
<b>Seed addition</b>	1	0.48	0.491	1.51	0.221	0.03	0.871
<b>Sward disturbance</b>	1	0.35	0.557	0.01	0.933	<b>41.00</b>	<b>&lt;0.001</b>
Study region	2	<b>224.78</b>	<b>&lt;0.001</b>	<b>20.36</b>	<b>&lt;0.001</b>	<b>3.95</b>	<b>0.024</b>
<b>Seed addition*Disturbance</b>	1	0.01	0.925	0.08	0.778	1.26	0.262
<b>Seed addition*Study region</b>	2	0.69	0.500	0.13	0.874	0.15	0.861
<b>Disturbance*Study region</b>	2	<b>10.41</b>	<b>&lt;0.001</b>	<b>3.12</b>	<b>0.047</b>	<b>7.16</b>	<b>0.001</b>
<b>Seed addition*Disturbance*Study region</b>	2	0.57	0.567	0.41	0.666	0.25	0.776
	DF	P		NO <sub>3</sub> -N		NH <sub>4</sub> -N	
		F	P	F	P	F	P
(Intercept)	1	<b>503.86</b>	<b>&lt;0.001</b>	<b>1348.30</b>	<b>&lt;0.001</b>	<b>2469.76</b>	<b>&lt;0.001</b>
<b>Seed addition</b>	1	0.01	0.908	0.16	0.689	0.23	0.630
<b>Sward disturbance</b>	1	<b>65.00</b>	<b>&lt;0.001</b>	<b>312.61</b>	<b>&lt;0.001</b>	<b>21.25</b>	<b>&lt;0.001</b>
Study region	2	2.20	0.118	0.59	0.557	<b>10.22</b>	<b>&lt;0.001</b>
<b>Seed addition*Disturbance</b>	1	0.08	0.778	0.83	0.362	0.00	0.966
<b>Seed addition*Study region</b>	2	0.05	0.952	0.22	0.800	0.26	0.774
<b>Disturbance*Study region</b>	2	<b>3.37</b>	<b>0.037</b>	<b>4.48</b>	<b>0.013</b>	<b>3.76</b>	<b>0.025</b>
<b>Seed addition*Disturbance*Study region</b>	2	0.51	0.600	0.40	0.672	0.02	0.977

## Figure captions

**Fig. 1** Structural equation models testing direct and indirect effects of plant species richness, management and a) actual and b) long-term mean values of productivity on NO<sub>3</sub>-N concentrations in soils of permanent grasslands. Actual productivity and NO<sub>3</sub>-N concentrations originate from 2015, fertilization and grazing intensities are averages of 2014 and 2015, and vascular plant species richness and long-term productivity are long-term averages from 2009 to 2015. All non-significant paths were stepwise removed. Bold lines indicate significant positive effects while broken lines indicate negative effects. Standardized path coefficients are given. Significance levels: \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$

**Fig. 2** Structural equation models testing direct and indirect effects of plant species richness, management and a) actual and b) long-term mean values of productivity on P concentrations in soils of permanent grasslands. Actual productivity and P concentrations originate from 2015, fertilization and grazing intensities are averages of 2014 and 2015, and vascular plant species richness and long-term productivity are long-term averages from 2009 to 2015. All non-significant paths were stepwise removed. Bold lines indicate significant positive effects while broken lines indicate negative effects. Standardized path coefficients are given. Significance levels: \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$

**Fig. 3** Mean resin bag concentrations in control and sward disturbance subplots in the first season after experimental set up in 71 grasslands ( $n = 284$  subplots). For significant treatment effects see Table 2 and for regional effects see Fig. S4 in supporting information.

## Accepted Article

### Supporting Information

Additional Supporting Information can be found in the online version of this article:

**Fig. S1** Study regions located across Germany

**Fig. S2** Monthly precipitation as mean values from 1953 to 2014 and actual values from 2015 of the three study regions.

**Fig. S3** Full model used for SEM analyses.

**Fig. S4** Resin bag nutrient concentrations as affected by sward disturbance and reseeding (separately and in combination) in the first season after experimental treatments were applied to 71 grasslands in three study regions.

**Fig. S5** Number of vascular plant species (on 2 m × 2 m), cover of bare soil and aboveground plant biomass production (as peak standing crop) recorded in May/June 2015 in the 71 grasslands of the sward disturbance and reseeding experiment.





