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Effects of organic and inorganic fertilizers on soil properties related to the regeneration of ecosystem services in peat grasslands

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ABSTRACT

Worldwide, agricultural grasslands on drained peat contribute to CO_2 emission but provide also provisioning and supporting ecosystem services that are managed by farmers. This paper evaluates the performance of organic and inorganic fertilizers in relation to soil biotic and abiotic properties linked to soil ecosystem services of peat grasslands with biodiversity goals. Effects of cattle slurry, compost, farmyard manure, solid fraction of cattle slurry and inorganic nitrogen (N) fertilizer with and without added sawdust were compared to those of an unfertilized control in a three-year field experiment. Total N input was targeted at 120 kg N ha^{-1} $year^{-1}$; total carbon (C) input was variable due to different C:N ratios of source materials. The abundance of earthworms in spring was increased with solid fraction (+35 % as compared to the control), which had the largest C input, and was reduced with inorganic N (−24 %). Combining inorganic N with sawdust did not affect earthworm abundance. Bacteria (determined by phospholipid fatty acid analysis) were increased following inorganic N fertilizer application with (+65 %) or without (+52 %) sawdust. Arbuscular mycorrhizal fungi were reduced by all fertilizers (−40 to −88 %), but less so by those with large C input (solid fraction) or with lignin-rich C input (sawdust + inorganic N fertilizer). Physical and chemical soil properties related to water infiltration and soil organic matter (SOM) decomposition were marginally influenced by fertilizers. The measured changes in soil pH, P availability and (micro)biology may affect SOM dynamics in the longer term. Grass dry matter yield was similarly increased by all fertilizers (+7 to 11 %) whereas grass N yield was increased by fertilizers only when the applied N was in mineral form (+16 to 20 %). From our results, we propose that the moderate use of organic fertilizers with a high and non-humified organic matter content such as solid fraction of cattle slurry can be part of a regeneration strategy in peat grasslands with biodiversity goals.

1. Introduction

Grasslands on drained peat soils in temperate regions are traditionally managed to maximize the herbage production. However, key issues in present and future farming practice on these soils are to reduce nutrient losses by optimizing fertilization (Deru et al., 2019; Pijlman et al., 2020; Vellinga and André, 1999), and increasingly to deliver supporting and regulating ecosystem services (MEA, 2005; Schröder et al., 2020). These services are related to the support of biodiversity like meadow birds (Beintema, 1986), to the regulation of water quantity (Deru et al., 2018; Van den Born et al., 2016) and to the mitigation of climate change by minimizing peat decomposition and associated nitrogen (N) mineralization, N and carbon (C) emission and land subsidence (Bobbink et al., 1998; Kasimir-Klemedtsson et al., 1997; Parish et al., 2008; Schothorst, 1977). Integration of ecosystem services in future farming is being promoted as 'regenerative agriculture', an approach to farming including reduced application of external inputs and increasing the use of organic fertilizers (Erisman et al., 2016; Schreefel et al., 2020; Strootman et al., 2020).

Grasslands for dairy production on peat in the Netherlands are commonly fertilized with a combination of slurry manure and inorganic N fertilizer. Grasslands with biodiversity goals, for example under agri-environment schemes (Verhulst et al., 2007), are often moderately fertilized with straw-rich farm yard manure (Deru et al., 2018). The organic matter and minerals added by organic fertilizers feed detritivorous soil biota like earthworms (Bünemann et al., 2006; De Goede

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et al., 2003; Onrust and Piersma, 2019), which affect the soil physical properties that are important for the regulating ecosystem services of climate adaptation and water regulation (Lavelle et al., 2006; Van Eekeren et al., 2009). Earthworms play a key role in the ecosystem service of support of biodiversity, because they are a food source for predators in higher trophic levels such as badgers and (meadow) birds (Macdonald, 1983; Muldowney et al., 2003; Onrust and Piersma, 2017; Vickery et al., 2001) and also benefit lower trophic level organisms through their contribution to soil structure formation, pore distribution and food resources availability (Brown, 1995; Loranger et al., 1998). Moreover, organic matter input via fertilizers may compensate for soil organic matter (SOM) decomposition of drained peat. Therefore, the use of fertilizers rich in organic matter is seen as a practical measure to support the delivery of multiple ecosystem services in agricultural grasslands. However, the common use of barns with slatted floors (producing slurry manure) in dairy farms has reduced the availability of straw-rich farmyard manure. Hence, for the wide use of agri-environment schemes in the peat regions a search for alternatives is necessary. Other C-rich fertilizers can be the solid fraction produced by slurry separating techniques used in dairy farming (Gebrezgabher et al., 2015; Hjorth et al., 2010), or other widely available products like composted organic wastes or sawdust.

The objective of this paper is to assess the effects of a range of fertilizers on soil properties and grass parameters related to the delivery of the main ecosystem services in peat grassland: support of biodiversity, water regulation, climate regulation and herbage production (Deru et al., 2018). The fertilizers tested represent the current practice in peat grasslands (cattle slurry manure, inorganic N fertilizer, cattle farmyard manure) and alternative fertilizers that could be used in future regenerative grassland management on drained peat (compost, solid fraction of cattle slurry manure, and sawdust combined with inorganic N fertilizer). Fertilizers were applied to grassland plots in a field experiment during three consecutive growing seasons. In relation to the ecosystem services support of biodiversity, water and climate regulation, and herbage production we hypothesized that: (1) C-rich fertilizers increase the abundance of earthworms, especially the detritivorous species, and the microbial biomass; (2) C-rich fertilizers with positive effects on soil biota increase soil porosity, water infiltration and rooting density, and SOM; and (3) C-rich fertilizers with low mineral N contents and high C:N ratio reduce herbage dry matter yield and N yield.

2. Materials and methods

2.1. Experimental setup

A field experiment was conducted from 2013 till 2015 in the west of the Netherlands on a permanent grassland on peat soil (Terric Histosol; FAO, 2014) at the experimental dairy farm at Zegveld. The top 0–10 cm soil had a SOM content of 56 g 100 g^{-1} and a pH_{KCl} of 4.5. A randomized block experiment was laid out in March 2013 with six fertilizer treatments and a control treatment (no fertilizer; named “Contr” in this paper) in six blocks, resulting in 42 experimental plots. The six fertilizer types were: cattle slurry manure from the experimental farm with conventional management (“Slurry”), mature compost of kitchen and garden waste from a municipal composting plant (“Comp”), cattle farmyard manure from a conventional dairy farm (“FYM”), solid fraction of the cattle slurry manure (“SFrac”, obtained by pressurized filtration (Hjorth et al., 2010)), inorganic N fertilizer (“IF”; calcium ammonium nitrate, 27 % N) and a combination of inorganic N fertilizer and sawdust (“IF+SD”). Plot size was 4 × 10 m, except for the slurry plots that were 5.2 × 10 m due to the width of the application device. Sampling and measurements occurred at minimum 0.5 m from the plot borders. Slurry was applied by slit injection (Huijsmans et al., 2001) and all other fertilizers were applied by hand. Fertilizer application rates were targeted at 120 kg total N ha^{-1} $year^{-1}$ (divided in two applications per year: one in February/March and one in May; Supplementary Table S1). However, due to fluctuating fertilizer N contents (especially FYM and Comp) and delayed fertilizer analyses, realized N application rates were not exactly 120 kg ha^{-1} $year^{-1}$ ($N_{min} + N_{org}$). A rate of 120 kg total N ha^{-1} $year^{-1}$ is relatively low for conventional dairy production grasslands (Commissie Bemesting Grasland en Voedergewassen, 2019) but usual in grasslands under agri-environment schemes (Kleijn et al., 2004). Organic matter input was different per fertilizer due to the differences in C:N ratio. The application rate of sawdust was adjusted to the amount of C_{total} applied in the Comp treatment. All plots, including control plots, were additionally fertilized with 200 kg K_2O ha^{-1} $year^{-1}$, divided in two applications per year (March and May) (Commissie Bemesting Grasland en Voedergewassen, 2019). Realized fertilizer application quantities and organic matter and nutrient inputs are presented in Table 1.

Before the experiment, the grassland had been managed conventionally with mainly cutting, winter grazing with sheep and a normal fertilization regime with both slurry manure and inorganic fertilizer (Commissie Bemesting Grasland en Voedergewassen, 2019). During the experiment, no fertilization was carried out in addition to the treatment amounts, but the normal grassland management was continued in the first two years. During the monitoring year of 2015, the plots were only cut for herbage measurements (see below) and not grazed.

2.2. Measurements

Soil and aboveground measurements were carried out from April to October 2015, and were selected to provide an overview of fertilizer effects of potential importance for the ecosystem services support of biodiversity, water regulation, climate regulation and herbage production. An overview of all measurements with the sampling dates is presented in Table 2. Most soil parameters were measured in October. As earthworms and larvae of crane flies and of click beetles living in grassland soils are an important food source for meadow birds during the pre-breeding period in spring (Galbraith, 1989), sampling of these organisms was carried out in April. The soil temperature under grassland (−10 cm) from the closest weather station was 9 °C (average in April 2015; www.knmi.nl). Soil moisture and penetration resistance were measured both in April and October.

2.2.1. Soil biological parameters

Earthworms and insect larvae were sampled in April in two soil cubes (20 × 20 × 20 cm) per plot in the top soil layer. Earthworms were hand-sorted, counted, weighed (without emptying their stomachs) and fixed in alcohol prior to identification. Abundance and biomass were expressed per m^2. Adults and juveniles were identified to species (Sims and Gerard, 1985; Stöp-Bowitz, 1969) and classified into functional groups (epigeic, endogeic and anecic species) (Bouché, 1977). Larvae of crane flies (Tipulidae; leatherjackets) or click beetles (Elateridae; wireworms) were counted and abundance was expressed per m^2.

Phospholipid fatty acids (PLFA) were analyzed in a field-moist soil sub-sample of the soil sampled in October for the soil chemical analyses (see Section 2.2.2). PLFA were measured as a proxy of microbial biomass and to examine microbial community structure. PLFA were extracted from 4 g of fresh soil using the procedure described by Palojärvi (2006), and analyzed by gas chromatography (Hewlett-Packard, Palto Alto, CA, USA). PLFA are the primary lipids composing the membranes of living cells. Phospholipid fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0, cy17:0, 18:1ω7 and cy19:0 were chosen to represent bacteria and PLFA 18:2ω6 was used as a marker of saprotrophic fungi (Hedlund, 2002). The neutral lipid fatty acid (NLFA) 16:1ω5, which occurs in storage lipids (such as spores) of arbuscular mycorrhizal fungi (AMF), was used as marker of AMF (Vestberg et al., 2012). The sum of PLFA i15:0, a15:0, i16:0, i17:0 and a17:0 was used as a measure of Gram-positive bacteria, and cy17:0 and cy19:0 as representing Gram-negative bacteria. PLFA 10Me16:0, 10Me17:0 and 10Me18:0 were used for measures of actinomycetes.

Table 1

Application quantities of fertilizers (fresh and dry matter (DM) rates) and ash, organic matter (OM), C and mineral inputs, and fertilizer C:N ratio. Total N input is the sum of mineral N (N_{min}) and organic N (N_{org}). Average values per hectare and per year over the years 2013–2015.

Treatment	Fertilizer		Ash[a]	OM	C	N_{min}	N_{org}	P_2O_5	C:N
	Mg ha^{-1} (fresh)	Mg ha^{-1} (DM)	kg ha^{-1}						
Contr	–	–	–	–	–	–	–	–	–
Slurry	43.2	2.39	615	1778	796	58	61	43	6.7
Comp	10.4	7.38	4106	3272	1472	4	109	50	12.9
FYM	22.9	5.10	802	4284	1922	15	118	73	14.3
SFrac	24.5	6.89	644	6240	2802	26	97	69	22.8
IF	0.45	0.45	0	0	0	120	0	0	0
IF+SD	3.77	3.17[b]	27	2679	1443	120	3	0	11.7

Treatment codes: Contr: control, Slurry: cattle slurry manure, Comp: compost, FYM: farmyard manure, SFrac: solid fraction of cattle slurry manure, IF: inorganic N fertilizer, IF+SD: inorganic N fertilizer and sawdust.

[a] Mineral fraction.

[b] 2.72 Mg sawdust + 0.45 Mg N fertilizer.

Table 2

Overview of aboveground and belowground measurements and sampling dates. See Materials and methods for details.

Set	Measurement(s)	Sampling date in 2015
Soil biological	Earthworms; leatherjackets; wireworms microbial phospholipid fatty acids (PLFA)	April 13th October 13th
Soil chemical	pH_{KCl}; N_{total}; P_{total}; P_{AL}; C_{total}; soil organic matter (SOM); hot water extractable carbon (HWC)	October 13th
Soil physical	Penetration resistance; soil moisture; soil structure; root density; water infiltration	April 13th and October 13th October 13th
Herbage yield	Dry matter yield, N content, N yield	May 15th, June 29th, August 19th, September 30th
Botanical	Plant soil cover and species	June 19th

2.2.2. *Soil chemical parameters*

A soil sample from each experimental plot consisting of c. 50 randomly taken soil cores was collected in October from the 0–10 cm layer (auger diameter 2.3 cm; Eijkelkamp grass plot sampler, Giesbeek, the Netherlands), sieved through a 1 cm mesh to remove plant remains and debris, and homogenized. Sub-samples were taken for determination of hot water extractable carbon (HWC) according to the method of Ghani et al. (2003), PLFA (see Section 2.2.1.) and chemical analysis. The sub-sample for chemical analysis was dried at 40 °C prior to analysis of soil acidity (pH_{KCl}), soil organic matter (SOM), total carbon (C_{total}), total nitrogen (N_{total}), total phosphorus (P_{total}) and ammonium-lactate extractable P (P_{AL}) by Eurofins Agro (Wageningen, the Netherlands). Soil pH_{KCl} was measured in 1 M KCl (NEN-ISO 10390 2005). SOM was determined by loss-on-ignition at 550 °C during 3 h after drying at 105 °C during 6 h (NEN 5754 2005). C_{total} was measured by incineration of dry material at 1150 °C, after which the CO_2 produced was determined by an infrared detector (LECO Corporation, St. Joseph, Mich., USA). For determination of N_{total}, evolved gasses after incineration were reduced to N_2 and measured with a thermal-conductivity detector (LECO Corporation, St. Joseph, Mich., USA). P_{total} was measured with Fleishmann acid (Houba et al., 1997) and P_{AL}, commonly used in the Netherlands to assess the P supply capacity of grassland soils (Reijneveld et al., 2014), was determined according to Egnér et al. (1960) (NEN 5793).

2.2.3. *Soil physical parameters*

Soil moisture was determined in April and October in a homogenized 0–10 cm soil sample (see Section 2.2.2). Soil was dried at 105 °C for 24 h and moisture content was expressed as percentage of fresh soil weight.

Penetration resistance was measured in April and October in each plot using an electronic penetrologger (Eijkelkamp, Giesbeek, the Netherlands) with a cone with 2.0 cm^2 penetration surface and a 60° apex angle. Cone resistance was recorded per cm of soil depth and expressed as an average of 7 penetrations per layer (0–10, 10–20, and 20–30 cm).

Soil structure and rooting were determined in October in two soil cubicles (20 × 20 cm) per experimental plot between the soil depth of 0–10 cm and of 10–25 cm. In each cubicle the percentage of crumbs, subangular blocky elements and angular blocky elements was visually estimated by one experienced person as described by Peerlkamp (1959) and Shepherd (2000), and root density was assessed by scoring visible roots (score 1–10; 1 for no roots and 10 for above average) (De Boer et al., 2018).

Water infiltration rate was measured in October at three randomly chosen spots per experimental plot as described by Van Eekeren et al. (2010). Measurements were carried out in 5 of the 6 blocks (35 plots). A PVC pipe of 15 cm high and 15 cm in diameter was pushed into the soil to a depth of 10 cm. To determine infiltration rate, 500 ml water was poured into each pipe and the infiltration time was recorded. When the infiltration time exceeded 15 min, the remaining water volume was estimated and the infiltration time for 500 ml was calculated by linear extrapolation. From this data, the infiltration rate (mm min^{-1}) was calculated.

2.2.4. *Grass yield and botanical composition*

Grass dry matter (DM) and N yield were measured in four harvests during 2015 with a Haldrup plot harvester (J. Haldrup a/s, Løgstør, Denmark). Harvest dates were May 15, June 29, August 19 and September 30. For each harvest, fresh biomass, DM content after drying at 70 °C for 24 h and total N content (Kjeldahl) were determined. From this data, herbage DM yield (Mg DM ha^{-1}) and herbage N yield (kg N ha^{-1}) were calculated per harvest and summed per year. Apparent N recovery (kg N·kg N^{-1}) was calculated according to Vellinga and André (1999) as (N yield$_{(fertilized)}$ − N yield$_{(non\text{-}fertilized)}$) / (N fertilization rate).

Measurement of botanical composition took place on the 19th of June following the method of Sikkema (1997) and consisted of visually estimating the relative soil cover of the sward and the proportion of each species therein (relative abundance).

2.3. *Statistical analyses*

The Shapiro-Wilk test of normality (Royston, 1982) was used in R (version 3.4.4) to test for normal distribution of the residuals of treatments and blocks. Parameters that did not meet the assumption of normality of residuals were log-transformed prior to further statistical analysis. The significance of the treatment effect was calculated for each parameter by analysis of variance (ANOVA; Genstat 19th edition, VSN international) using one-way ANOVA (randomized block design). In cases of significant effects ($P \leq 0.05$), least significant differences between means were determined (l.s.d.; $\alpha = 5$ %). In the text, a number following a mean and "±" refers to the standard error of the mean. For the parameters showing significant treatment effects, principal component analysis (PCA) was performed in R (packages vegan 2.5-4 and packfor 0.0-8) to visualize the variation of these parameters across treatments and provide an overview of their correlations as projected on the first two principal components (PC1 and PC2). All data are archived in Deru et al. (2022).

3. Results

3.1. Soil properties

3.1.1. Earthworms and insect larvae

The abundance of earthworms was highest in SFrac (480 ± 66 earthworms m^{-2}) and lowest in IF (271 ± 38 earthworms m^{-2}), and higher in SFrac than all treatments but Contr and IF+SD (Fig. 1A; Supplementary Table S2). The abundance of epigeic earthworms (Fig. 1B; 96 ± 13 to 230 ± 31 earthworms m^{-2}) and total earthworm biomass (Fig. 1C; 99 ± 11 to 175 ± 27 g fresh weight m^{-2}) were also highest in SFrac; only IF+SD was not significantly lower than SFrac ($P = 0.048$). Of all juveniles, the percentage of epigeic ones was lowest in Slurry and Comp and highest in IF, IF+SD and SFrac (Fig. 1D). Endogeic earthworms (130 ± 21 to 250 ± 54 worms m^{-2}) were not significantly influenced by treatments ($P = 0.267$).

Earthworm parameters correlated positively, but weakly, with the fertilizer input of organic matter and of mineral N (Table 3).

The combined leatherjacket and wireworm abundance ranged between 88 ± 16 and 233 ± 72 m^{-2}. The abundance of wireworms ($P = 0.039$), but not of leatherjackets ($P = 0.247$), was significantly influenced by fertilizer type. Wireworm abundance was lowest in Contr and Comp, and highest in Slurry, FYM and SFrac (Supplementary Table S2).

3.1.2. Microbial phospholipid fatty acids (PLFA)

Slurry and Comp reduced PLFA concentrations compared to Contr (Fig. 2A). Inorganic N fertilizer (IF and IF+SD) resulted in the highest PLFA concentrations, a significant increase compared to the treatments with organic fertilizers ($P < 0.001$). Broadly the same patterns were observed for bacterial, fungal, Gram-positive and Gram-negative PLFA (Supplementary Table S3). Arbuscular mycorrhizal fungi (AMF; as indicated by NLFA) were reduced in all treatments compared to Contr, especially in FYM and Comp (Fig. 2B). SFrac and IF+SD showed the smallest reductions. The fungal:bacterial PLFA ratio was highest in

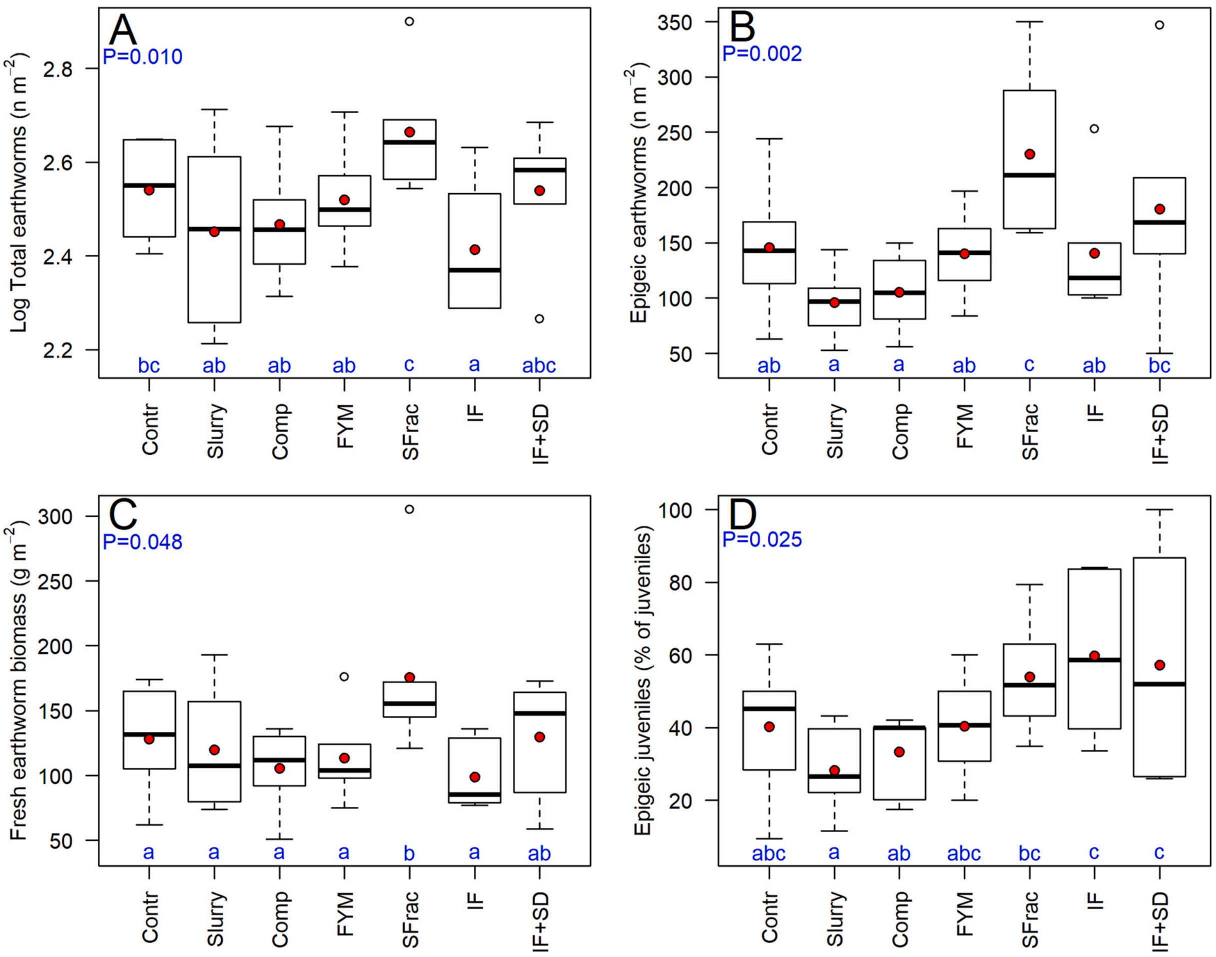

Fig. 1. Effects of fertilizers on earthworm abundance (A: total; B: epigeic), fresh biomass (C) and proportion of juvenile epigec earthworms (D) in April of the third fertilization year. Panel A is based on log transformed data. Untransformed data are available in Supplementary Table S2. Treatment codes: Contr: control, Slurry: cattle slurry manure, Comp: compost, FYM: farmyard manure, SFrac: solid fraction of cattle slurry manure, IF: inorganic N fertilizer, IF+SD: inorganic N fertilizer with sawdust. Red dot: mean; thick horizontal line: median; vertical size of the box: interquartile range; whisker: variability outside the quartile; open dot: outliers (if any). Overall significance of treatment effect is indicated in each panel. Based on the least significant difference (l.s.d.; $\alpha = 5$ %), means marked with a similar letter do not significantly differ.

Table 3

Pearson correlations (r; n = 42) of earthworm and PLFA parameters with fertilizer input of organic matter, total N, mineral N and organic N (average of 2013–2015). Only significant correlations are presented: standard font: $P \leq 0.05$; **bold**: $P \leq 0.001$.

Fertilizer input	Earthworm				PLFA[a]		
	Total abundance	Epigeic	Juveniles	% of juv. epigeic	Total microbial	AMF NLFA	Fung.:bact. ratio
Organic matter	0.35	0.32	0.40				
Total N						**−0.56**	
Mineral N				0.35	**0.53**		−0.41
Organic N					**−0.54**	**−0.52**	0.35
P					**−0.50**	−0.44	

[a] Correlation coefficients and significances for bacterial, fungal, gram-positive and gram-negative PLFA with soil and fertilizer parameters were similar to those of total microbial PLFA.

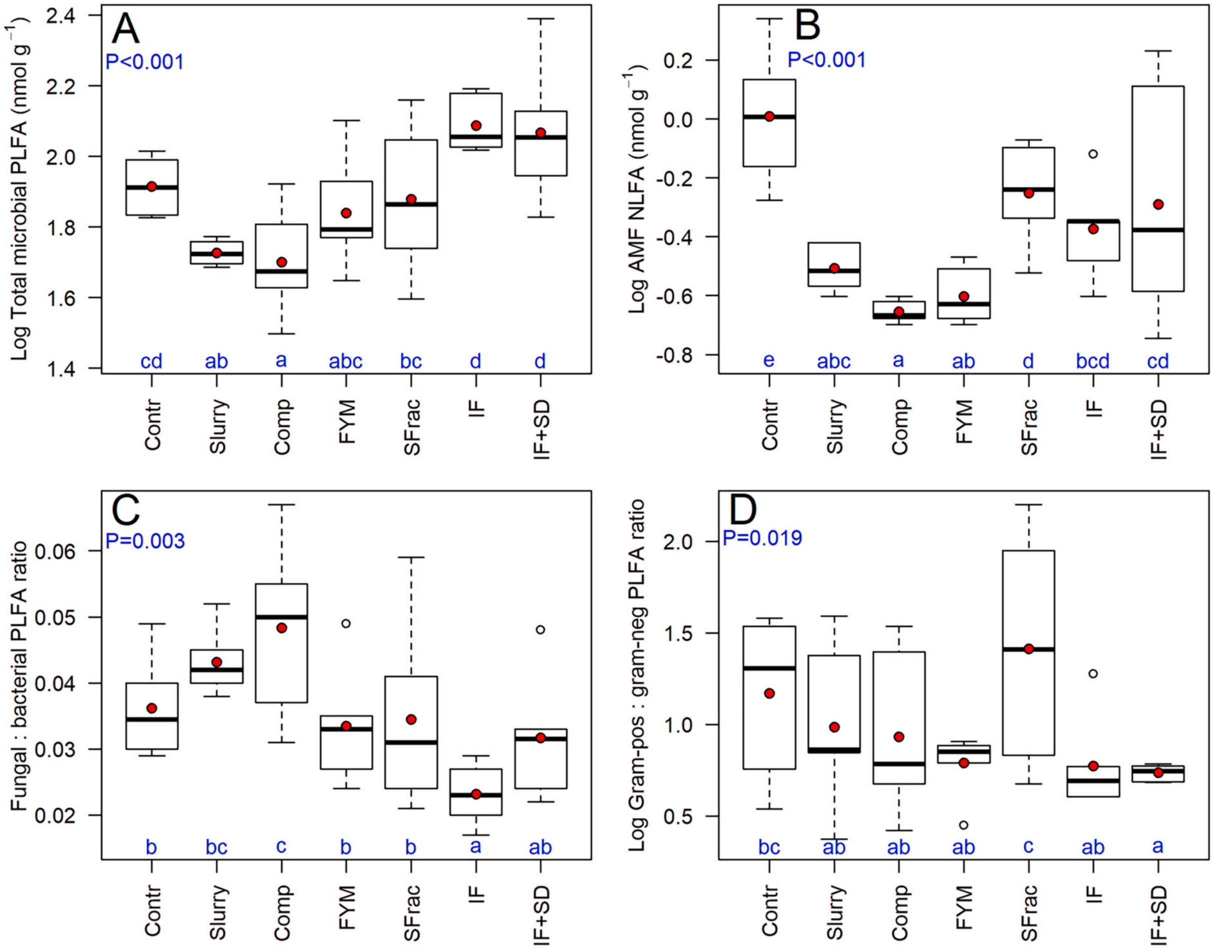

Fig. 2. Effects of fertilizers on soil microbial community structure as indicated by PLFA and NLFA: total microbial PLFA (A), arbuscular mycorrhizal fungi (AMF) NLFA (B), fungal:bacterial PLFA ratio (C) and gram positive to gram negative PLFA ratio (D) in October of the third fertilization year. Panels A, B and D are based on log transformed data. Untransformed data are available in Supplementary Table S3. Treatment codes and figure explanation: see Fig. 1.

Comp and lowest in IF (Fig. 2C). The Gram-positive to Gram-negative ratio was highest in SFrac and lower in IF+SD compared to Contr (Fig. 2D).

PLFA parameters correlated with fertilizer N and P input (Table 3). Total microbial PLFA, and also bacterial, fungal, gram-positive and gram-negative PLFA, correlated positively with N_{min} input but negatively with N_{org} and P input. AMF NLFA was negatively correlated with N and P input.

3.1.3. Chemical and physical soil properties

SOM, pH_{KCl} and P_{AL} were significantly influenced by treatments (Fig. 3), but not C_{total}, HWC, N_{total}, C:N ratio or P_{total} (Supplementary Table S4). SOM was lowest in Slurry and Comp; in Comp (55.0 ± 0.3 %) this was significantly lower than in Contr (56.0 ± 0.3 %). The highest pH_{KCl} was found in FYM (4.62 ± 0.02) and Comp (4.62 ± 0.03) and the lowest value in IF (4.47 ± 0.02). FYM and Comp had the highest P_{AL}.

Effects on penetration resistance were found in October only: in 0–10 cm, values were lowest with the organic fertilizers and highest in IF (Fig. 3D). Soil moisture was highest in FYM and SFrac in April as well as in October (Supplementary Table S5). Other differences in soil physical aspects were observed in the 10–25 cm layer, where Slurry and Comp had a higher percentage of crumb structures and a high root density. Compared to the control, fertilizers tended to reduce the water infiltration ($P = 0.070$).

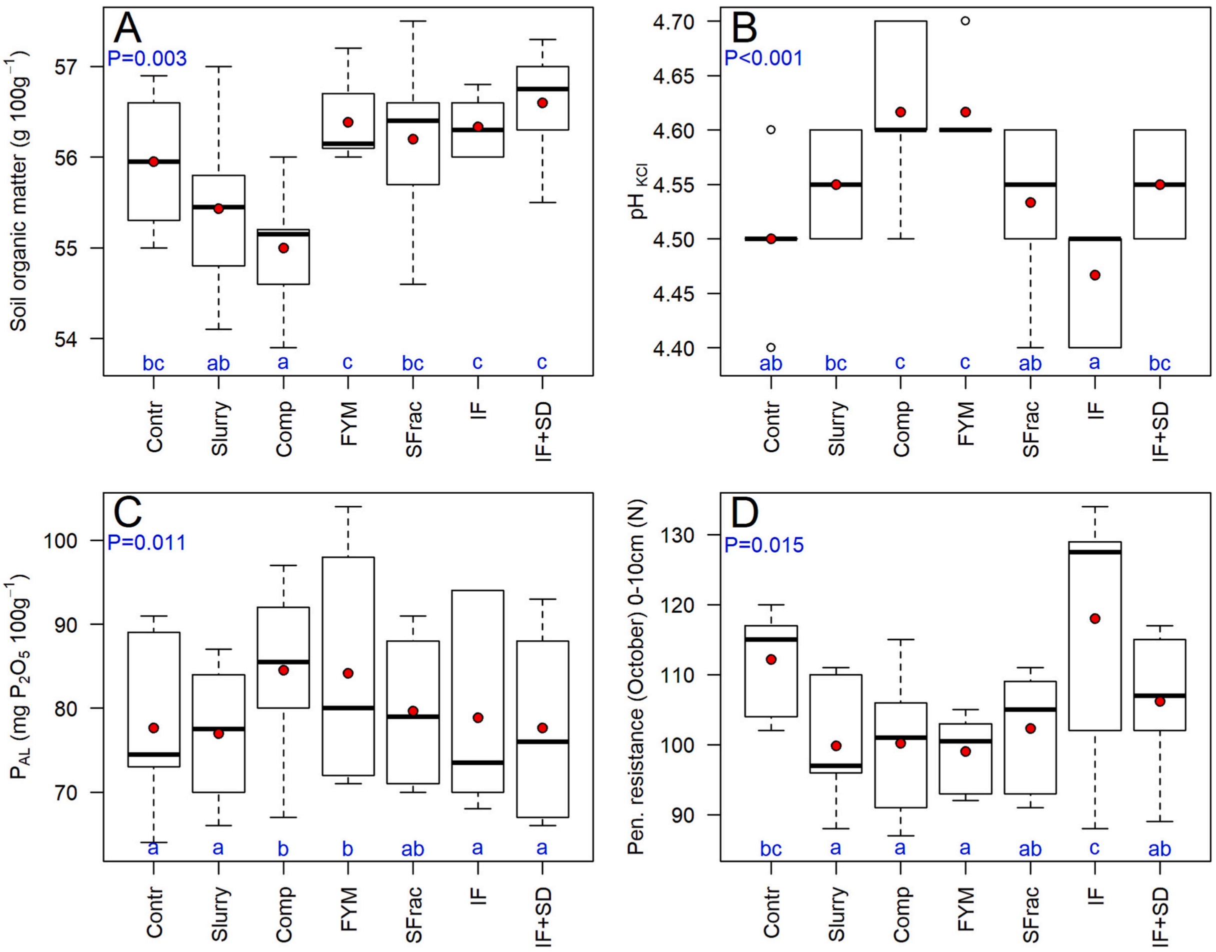

Fig. 3. Effects of fertilizers on soil chemical and physical parameters: soil organic matter content (SOM; A), pH_{KCl} (B), P_{AL} (C) and penetration resistance in 0–10 cm (October) (D) in the third fertilization year. More soil chemical and physical data in Supplementary Tables S4 and S5. Treatment codes and figure explanation: see Fig. 1.

3.2. Herbage production and botanical composition

The herbage yield was affected by the treatments (Fig. 4A). Compared to the unfertilized control, the fertilizers increased herbage DM yield by c. 10 % but the type of fertilizer did not matter. This was different for the herbage N yield which was 302 ± 11 kg N ha^{-1} year^{-1} in the unfertilized plots, was not significantly higher in any of the four organic fertilizer treatments (Slurry, Comp, FYM and SFrac), but increased by 16–20 % in IF and IF+SD as compared to Contr (Fig. 4B; $P < 0.001$). Treatment effects on herbage DM and N yields were different in the two first harvests compared to the two last harvests (Supplementary Table S6); DM and N yields correlated positively with the fertilizer mineral N input for the first two harvests, but negatively for last two harvests (Table 4). The opposite correlations were found with the fertilizer organic N input and fertilizer P input. The average herbage N content was lower in all organic fertilizers as compared to Contr and higher in IF+SD (Fig. 4C). Apparent N recovery ranged between 0.0 ± 0.1 and 0.5 ± 0.1 kg N·kg N^{-1} (with a large variation within treatments) and was intermediate in Slurry, lowest for the remaining organic fertilizers and highest in IF and IF+SD (Fig. 4D). There was no treatment effect on the soil cover percentage of productive grasses, total grasses or dicotyledons (Supplementary Table S7), nor on soil cover of individual plant species (data not shown).

3.3. PCA with soil and herbage parameters

The PCA including soil properties and herbage yield shows a grouping of treatments along the first axis consisting of Slurry, Comp and FYM with higher pH_{KCl} at the one side, and of Contr, IF and IF+SD with higher penetration resistance, microbial PLFA and herbage N and DM yield at the other side (Fig. 5). Along the second axis, SFrac and FYM were positively correlated with soil moisture, SOM and the abundance of earthworms and contrasted with Slurry, Comp and IF.

4. Discussion

4.1. Support of biodiversity

4.1.1. Earthworms

In line with the first hypothesis, the earthworm biomass was largest in the grasslands fertilized with SFrac, which was the fertilizer with the highest organic matter input (Table 1).

We sampled earthworms in April, close to the first of the two yearly moments of fertilizer application (Supplementary Table S1). The rapid reproduction of epigeic earthworms (Bouché, 1977) may explain the increase in SFrac. Apparently, SFrac was of added nutritional value for the epigeic earthworms even at high SOM content (as potential food source). This is in line with experimental evidence that for earthworms C availability is limiting, irrespective of the SOM content (Tiunov and Scheu, 2004) and that earthworms are mostly resource limited (Salamon et al., 2006). Leroy et al. (2008) proposed that C availability for earthworms also depends on the quality of the added organic material: humification and stabilization in compost reduces its nutritional value compared to farmyard manure. In our experiment, both the total C input

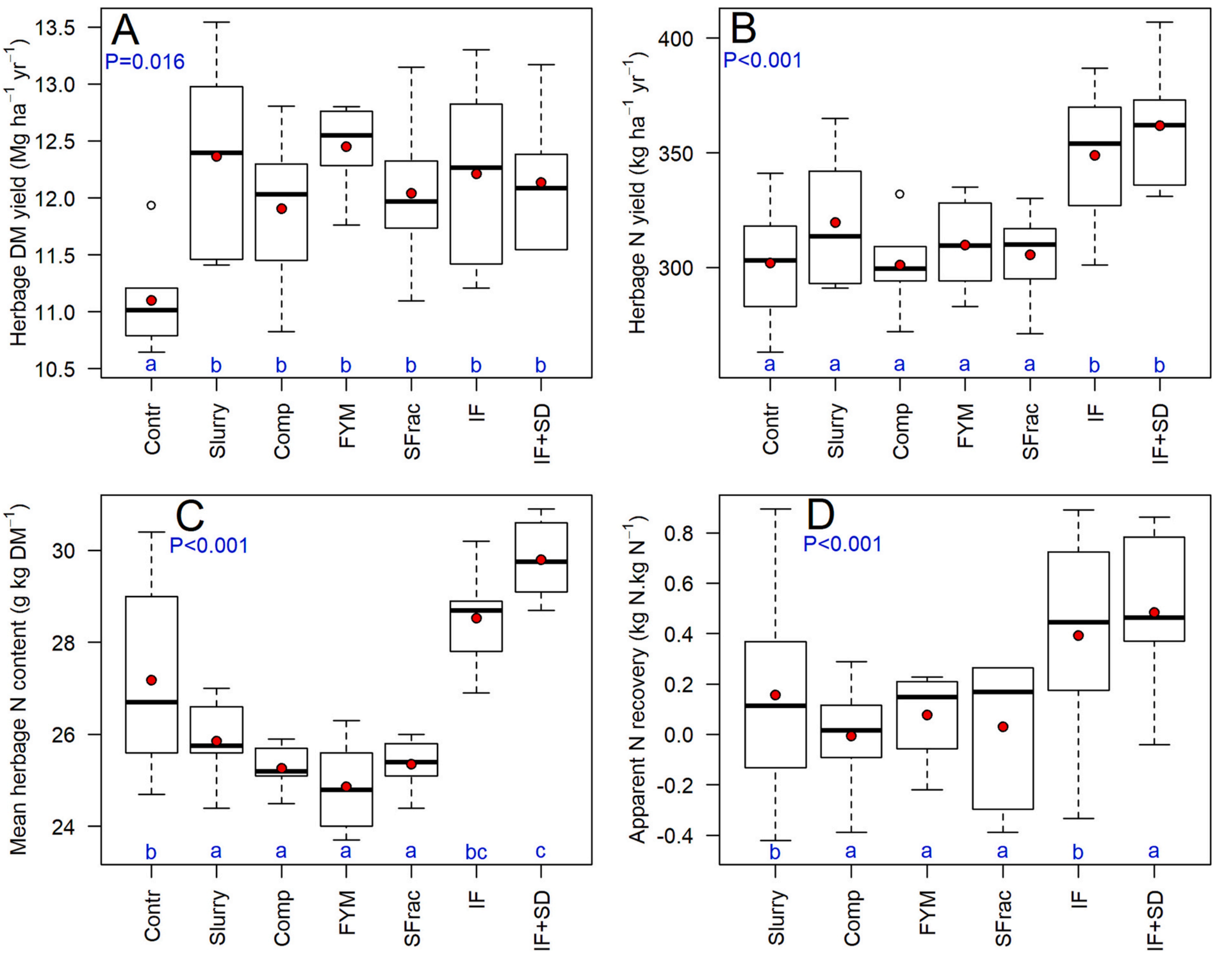

Fig. 4. Effects of three years of fertilizer treatments on herbage dry matter (DM) yield (A), N yield (B), year mean N content (C) and apparent N recovery (D) during the third fertilization year. Effects per individual harvests and on DM contents, see Supplementary Table S6. Treatment codes and figure explanation: see Fig. 1.

Table 4
Pearson correlations (r; n = 42) of fertilizer input of C, N_{total}, $N_{mineral}$, $N_{organic}$ and P (average of 2013–2015) with herbage dry matter (DM) or nitrogen (N) yields in 2015 per harvest (H1 − H4) and year total across all treatments. Only significant correlations are presented: standard font: $P \leq 0.05$; **bold**: $P \leq 0.001$.

Fertilizer input	Herbage DM yield					Herbage N yield				
	H1	H2	H3	H4	Total	H1	H2	H3	H4	Total
C		−0.35	0.31	**0.54**			−0.42		0.47	
Total N	**0.53**				**0.53**				0.31	
Mineral N	0.48	**0.75**	**−0.58**	**−0.52**		**0.70**	**0.79**	−0.38	−0.38	**0.67**
Organic N		**−0.52**	**0.52**	**0.77**		**−0.51**	**−0.64**	0.34	**0.63**	−0.44
P		**−0.53**	**0.55**	**0.75**		**−0.49**	**−0.65**	0.37	**0.60**	−0.43

and its quality differed between treatments. This may explain the lack of differences in earthworm abundance and biomass between Slurry (low C input with high nutritional value), Comp (moderate C input with low nutritional value) and FYM (high C input with moderate nutritional value). Similarly, the same C input of Comp and IF+SD but a higher abundance of epigeic earthworms in the latter (Fig. 1B) indicates that lignin-rich but non-humified material may be of greater nutritional value for those earthworms, at least when combined with mineral N. Inorganic N fertilizer resulted in a high proportion of juvenile epigeic earthworms (Fig. 1D), but the total epigeic population was only higher in SFrac and in IF+SD, thus where (organic) N addition was combined with (large) amounts of C.

4.1.2. Soil microbial abundance and community structure

The increased microbial PLFA after three years of mineral N application compared to the plots with organic fertilizers (Fig. 2A) indicates that in the peat soil the microbial community was limited by the availability of mineral N rather than by C or by organically bound nutrients. This is also shown by the positive correlation between total microbial PLFA and mineral N input (Table 3). Similarly, Song et al. (2017) found positive effects of comparable amounts of mineral N on microbial PLFA in permafrost peat, with regularly added N during the growing season. In our case, the time between N application and PLFA measurements was much longer, which indicates that changes in PLFA were persistent. In mineral soils, that have lower SOM contents than peat soils, PLFA are more responsive to organic matter input than to mineral N input (Börjesson et al., 2012).

In peat grasslands, the microbial community is generally dominated by bacteria (Deru et al., 2018; Kechavarzi et al., 2010; Van Dijk et al., 2004). It is well established that bacteria benefit more from high (mineral) N availability (Bardgett and McAlister, 1999), in our experiment occurring in IF and IF+SD, and that fungi are better adapted to

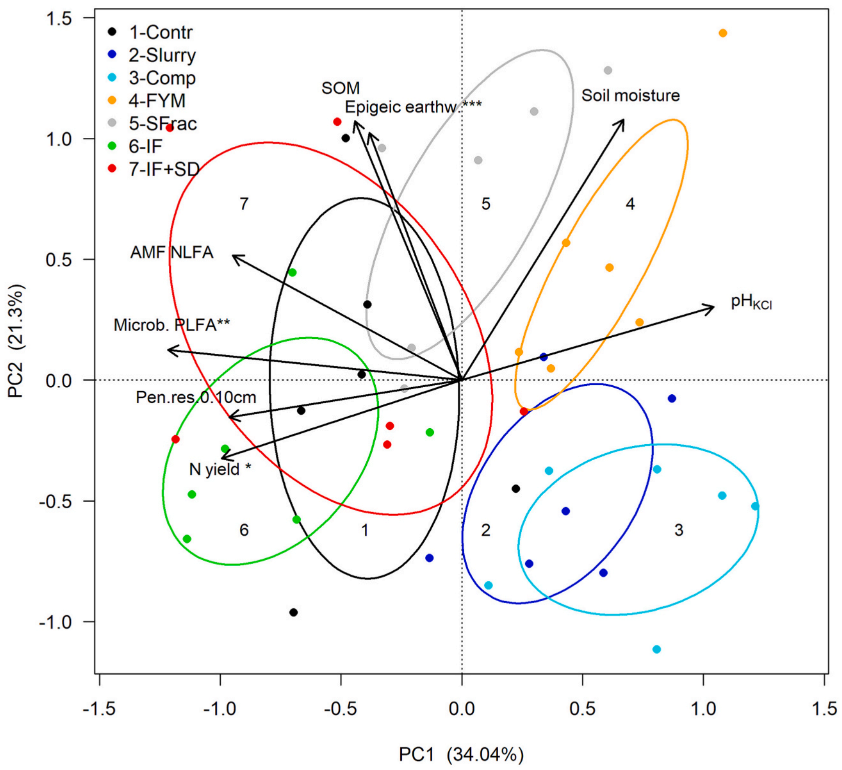

Fig. 5. Axes 1 and 2 of a principal component analysis (PCA) of the measured parameters (soil biological, chemical and physical, and grass N yield) that showed a significant treatment effect. Parameters were omitted from the figure when they had a very short arrow (indicating low correlation with the two first principal components, which was the case for P_{AL}). *similar for herbage DM yield; **similar for other PLFA parameters; ***similar for abundance of earthworms. Ellipses are calculated at 50 % confidence and linked with the treatments with numbers and colors. Treatment codes: see Fig. 1.

environments with more stabilized and recalcitrant organic matter (Paterson et al., 2008), occurring in Comp and IF+SD (Clocchiatti et al., 2020; Senesi and Plaza, 2007). In absolute amounts, the increase of fungal PLFA in the treatment with sawdust (Supplementary Table S3) indicates that fungi benefited from input of lignin-rich C combined with mineral N, which was also reported by Clocchiatti et al. (2020). The high herbage N uptake and ANR in the treatment with sawdust (Fig. 4) indicates that grass yield was not compromised by possible N immobilization due to the high C:N ratio of sawdust (Gad et al., 2015). This may be the result of increased symbiotic nutrient acquisition by the high occurrence of arbuscular mycorrhiza (AMF NLFA, Fig. 2B). Similarly, Contr and SFrac had high AMF NLFA concentrations, but showed no depressive effects on herbage N yields despite potential nutrient limitation either due to lack of N input (Contr) or N immobilization due to high C input (SFrac) (Peters and Jensen, 2011).

Also changes in the amount of Gram-positive and Gram-negative bacteria may be induced by treatments with fertilizers differing in C quality (Börjesson et al., 2012). It has been proposed that Gram-positive bacteria are related to recalcitrant SOM, and Gram-negative bacteria to fresh plant material (Kramer and Gleixner, 2008), and that their ratio can therefore be used as an indicator of C availability for soil microbes (Fanin et al., 2019). However, our results show an increase in Gram-positive to Gram-negative ratio in the treatment with SFrac (Fig. 2D) and no effects on the microbial abundance or fungal:bacterial PLFA ratio. SFrac had the highest input of C, which was composed of young organic material as compared to the peat. Together with a lack of difference in Gram-positive to Gram-negative ratio between IF and IF+SD, these observations suggest that in peat soil with an abundant pool of SOM, the type of bacteria is not strongly influenced by the (recalcitrance of the) added C.

4.2. *Water and climate regulation*

4.2.1. *Water regulation*

We hypothesized that fertilizers with a high C:N ratio and positive effects on soil biota would increase the root density, soil porosity and water infiltration, important for the ecosystem service of water regulation. The range in earthworm abundance created by the fertilizer treatments, the earthworm biomass and proportion of adults were similar to that between dairy and semi-natural grasslands on peat (Deru et al., 2018) where positive effects of earthworm abundance, macropores, root density and soil crumbs on water infiltration were found. However, in the present study the influence of fertilizers on root density, soil crumbliness or water infiltration rate was marginal or not significant (Supplementary Table S5). Deru et al. (2018) investigated grasslands with a constant management for at least five years. We therefore conclude that the type of fertilizer, when applied at the moderate rates used in this study and for a period of three years, influenced the earthworm community but had not enough cumulative effects on soil physical properties to influence the water infiltration.

4.2.2. *Climate regulation*

We hypothesized that fertilizers with high C input and C:N ratio could influence SOM, and with it affect the ecosystem service of climate regulation (Minasny et al., 2017). As our SOM measurements were in a fixed soil layer (top 10 cm), differences do not provide information on effects on the total SOM pool of the peat layer. As an illustration, the decrease of SOM in the case of Comp by 1 %, as compared to Contr, may be explained by a dilution effect (Rühlmann et al., 2006) because the applied compost contained only 44 % organic matter (Table 1: 56 % ash), against 56 % in the peat soil itself.

Besides direct C input and dilution effects, changes in soil chemistry (HWC, pH, P availability) and biology (microbial biomass and community structure, earthworms) provide indirect information on potential changes in SOM decomposition that is discussed hereafter. HWC, a pool of labile C which is seen as a sensitive indicator for SOM decomposition (Deru et al., 2018; Ghani et al., 2003), was not influenced by the treatments. However, in the longer term or at higher fertilization rates, C inputs may be higher and SOM dynamics may be more clearly influenced by changes in soil pH, P availability and (micro)biology (Deru et al., 2021). As compared to organic fertilizers, inorganic N fertilizer tended to increase microbial biomass in favor of bacteria, potentially increasing SOM decomposition, but also to reduce pH, potentially reducing decomposition (Rousk et al., 2011). Organic fertilizers, especially Comp and FYM, added organic matter but also increased pH and P_{AL}, which potentially increase SOM decomposition (Amador and Jones, 1993; Brouns et al., 2016; Rousk et al., 2011). SFrac added large amounts of organic matter without effect on the microbial and soil chemical properties that potentially increase SOM decomposition, but stimulated earthworm biomass which may affect SOM decomposition and greenhouse gas emission (Lubbers et al., 2017).

At the farm, regional or global level, evaluation of the implications of fertilizing strategies for climate regulation should include emissions of greenhouse gases from the total life cycle of the fertilizers. In this regard, alternative uses of the organic fertilizers or of the biomass used to produce these fertilizers should be compared following the total life cycle.

4.3. Herbage production and N uptake

All fertilizers increased the herbage DM yield similarly compared to the control, but the N yield was higher only when inorganic N fertilizer had been applied (IF and IF+SD, Fig. 4). Moreover, the apparent N recovery of inorganic N fertilizer was in the lower range of what has been measured in drained peat grasslands (Deru et al., 2019; Vellinga and André, 1999). These weak effects of both the organic and inorganic fertilizers can be explained by the low application rates (120 kg N ha^{-1} $year^{-1}$) used in this experiment, in combination with the, even for peat soils, high soil N supply (302 ± 11 kg N ha^{-1} $year^{-1}$) (Deru et al., 2019; Pijlman et al., 2020; Vellinga and André, 1999). This is supported by the absence of a decrease in the proportion of productive grasses in the third year after cessation of N fertilization in the control plots.

The clear differences between the organic and the inorganic N fertilizers on herbage N yield among the individual harvests shows that the mineralization of the applied organic N was lower than the grass N demand, especially in the first half of the growing season. This supports our hypothesis that fertilizers with low C:N ratio and high mineral N content would increase herbage yield. Recent studies in drained peat grasslands indicate that the soil N supply depends on the mineralization of organic N from the decomposing SOM, which is mainly temperature-driven (Pijlman et al., 2020) and, in addition, may be reduced by low water availability in summer due to water repellency (Deru et al., 2019). Those studies were carried out by comparing non-fertilized and inorganic N-fertilized plots only. The present experiment including organic fertilizers indicates that the additional N availability from mineralization of fertilizer organic N is low, and that increased abundance of mycorrhizal fungi may have compensated the low N availability (see Section 4.1.2).

Our study in drained peat grassland with a high soil N supply was carried out at low fertilization rate in comparison with mainstream management practice. A practical outcome for agri-environmental grasslands in this soil type is that herbage DM yield is similarly increased by fertilizers applied in the first half of the growing season independent of their chemical composition. From a meadow bird perspective, the sward should not be too dense in the spring to facilitate chick movement and insect picking (Schekkerman and Beintema, 2007). Our results confirm that inorganic N fertilizer is less suitable in these grasslands as it results in the fastest growth in the first harvests due to high N uptake, and that organic fertilizers have moderate effects on grass growth.

5. Conclusions and perspectives

Based on a three-year field experiment, we conclude that some ecosystem services are influenced by fertilizer use within a short period of time. For the ecosystem service ‘support of biodiversity’, we conclude that solid fraction of cattle slurry can be an alternative for farmyard manure in grasslands with biodiversity goals due to its high C:N ratio and its positive effects on the earthworm abundance. Soil physical and chemical properties in the topsoil important for the ecosystem services ‘water regulation’ and ‘climate regulation’ were weakly influenced by fertilizers and effects may become apparent in the longer term. For the ecosystem service ‘herbage production’, we conclude that grass yield was most strongly positively influenced by the amount of added mineral N, which was low in the organic fertilizers. To better understand effects on the ecosystem service of climate regulation, indirect effects of changes in soil pH, P availability and (micro)biology should be investigated on the decomposition of both the added organic matter and the older peat, over a deeper soil layer. As a new hypothesis to be tested we propose that the use of fertilizers high in non-humified organic matter content can be part of a regeneration strategy to deliver multiple ecosystem services in peat grasslands.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The research data are published in Deru et al. (2022) [dataset].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104838.

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