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Effects of Ca:Mg ratio and pH on soil chemical, physical and microbiological properties and grass N yield in drained peat soil

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ABSTRACT

In three dairy grasslands on peat, minerals were added to manipulate the soil Ca:Mg ratio with or without effect on pH. The responses of soil properties and grass N yield were measured. $CaCO_3$ application led to higher soil Ca:Mg ratio and pH_{KCl} compared to the untreated control, decreased N_{total} and C_{total}, and increased P availability. Grass N yield increased in the first year by only 6% of the reduction in soil N_{total}, but not in the second year. A higher pH increased SOM decomposition, especially in soils with high P availability. $MgCO_3$ reduced the Ca:Mg ratio, had little influence on soil parameters and no effect on grass N yield. In contrast, $CaSO_4$ and $MgSO_4$ did not influence pH_{KCl} but reduced grass N yield in most cases. Results suggest stabilisation of organic matter by Ca binding in treatments with added Ca. We conclude that grass N yield was not linked with changes in Ca:Mg ratio but with soil pH. The pH effects on SOM decomposition depended on P availability and Ca binding. Hence, to avoid potentially large soil losses of C and N, the current agricultural advice on pH management in peat grasslands should be better adapted to local edaphic characteristics.

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Grassland; histosol; lime; gypsum; kieserite; soil quality; soil organic matter

1. Introduction

Peatlands contain one third of the total global soil carbon reservoir (Parish et al. 2008) and peat soils that are drained to be used for agriculture are major contributors to terrestrial CO_2 emissions (Kasimir-Klemedtsson et al. 1997; Van den Akker et al. 2008; Kluge et al. 2008). The oxic conditions in the top soil of drained peat stimulate decomposition and mineralisation of the soil organic matter (SOM), also in the untilled permanent grasslands that cover 80% of the Dutch peat area. Hence, reducing SOM decomposition while maintaining sufficient grass production for dairy farming is a major challenge in those soils. The organic matter of agricultural peatlands consists

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mainly of old plant residues accumulated during the pre-drainage period, but the top soil contains considerable amounts of young and nutrient-rich organic matter as well, originating from crop residues, organic manures and ditch sludge (Sonneveld and Lantinga 2011). Decomposition of SOM in the top soil is, therefore, an important source of nutrients such as mineralised N. The amount of N uptake by grassland during the first year without N fertilisation may range between 85 and 377 kg N ha^{-1} yr^{-1} (Hassink 1995; Vellinga and André 1999; Van Kekem 2004; Van Beek et al. 2004; Sonneveld and Lantinga 2011; Deru et al. 2019; Pijlman et al. 2020). From analysis of a large data set in 20 dairy grasslands on peat including grass N uptake and the natural variation in soil physical, chemical and biological parameters, Deru et al. (2019) concluded that the Ca:Mg ratio in the top soil was the best single soil parameter correlating positively ($r^2 = 0.49$) with unfertilised grass N yield (i.e. the amount of harvested N, a proxy for soil N supply) in drained peat grasslands. The lack of a correlation with pH suggested that a higher Ca:Mg ratio may increase the uptake of mineralised N by grassland due to improved soil structure, rooting and water availability without increasing the N mineralisation itself, for which pH, via microbial activity, is assumed to be an important factor (Rousk et al. 2011; Deru et al. 2018). Literature provides little evidence for a causal relation between the ratio of cations in soil (i.e. Ca and Mg) and crop yields (Kopittke and Menzies 2007), but the Albrecht Theory of an ‘ideal’ soil Ca:Mg ratio (Albrecht 1975) is still used by commercial advisors and soil laboratories as a measure of soil quality and in their fertilisation advice to farmers. The results presented in Deru et al. (2019) raised the question of whether the soil Ca:Mg ratio in peat grasslands can be manipulated to influence the (unfertilised) grass N uptake without affecting SOM decomposition and N mineralisation, and potentially reduce N losses. Possibly, a higher Ca:Mg ratio would lead to a better soil structure and higher uptake of the mineralised N.

In agriculture, minerals such as calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), magnesite ($MgCO_3$) or gypsum ($CaSO_4$) are applied to counteract negative effects of acidification, compaction and/or Ca or Mg deficiency on crop production (Hoyt 1981; Haynes 1984; Shainberg et al. 1989; Sumner et al. 1990; Bolan et al. 2003; Goulding 2016). Manipulation of soil Ca or Mg with carbonates (e.g. $CaCO_3$, $MgCO_3$) is associated with a rise in pH (Haynes and Naidu 1998). On the other hand, minerals without carbonate, such as gypsum ($CaSO_4$) or kieserite ($MgSO_4$), affect soil Ca or Mg with limited effect on pH (Carter 1986; Bolan et al. 2003; Murphy and Stevens 2010). These minerals, however, have a higher solubility than carbonate minerals and can penetrate deeper into the soil (Reeve and Sumner 1972). Moreover, the sulfate may induce unwanted internal eutrophication in anoxic peat layers (Koerselman et al. 1993; Beltman et al. 2000). Hence, the effects of such minerals are not straightforward as they may vary with soil chemical, physical and biological conditions (Haynes and Naidu 1998; Hamilton et al. 2007; Paradelo et al. 2015; Holland et al. 2018) and can change over time after application (Neale et al. 1997; Grover et al. 2017).

So far, little is known about the effects of liming of peat grasslands on soil N supply and grass production with other minerals than $CaCO_3$. For grasslands on peat soils in the Netherlands, it is advised to reduce soil acidity if pH_{KCl} drops below 4.6 (Commissie Bemesting Grasland en Voedergewassen 2019). This official advice is based on agronomic grounds without explicit reference to effects on peat decomposition and to soil N supply. However, it is important for the farming practice that the present advice to use minerals that influence the soil Ca:Mg ratio is based on experimental knowledge.

Our objective was to investigate whether the positive relationship between unfertilised grass N yield and the natural variation in Ca:Mg ratio in peat soils found by Deru et al. (2019) can be reproduced in a factorial experiment with manipulated Ca:Mg ratio and whether effects can be explained from chemical, physical and microbiological soil properties. A field experiment was carried out during three years on three-peat grasslands with different initial soil Ca:Mg ratios to which four minerals with or without expected effect on pH were added to increase and decrease the Ca:Mg ratio. It was hypothesised that (1) increased Ca:Mg ratio – after addition of Ca containing minerals – would enhance grass N yield and that decreased Ca:Mg ratio – after addition of Mg containing minerals – would reduce grass N yield; (2) these effects would be caused by changes in soil physical properties rather than in N mineralisation; (3) addition of CO_3 containing minerals would increase pH, organic matter decomposition and grass N yield independently of Ca:Mg ratio.

2. Materials and methods

2.1. Study sites and experimental setup

A field experiment was conducted on three permanent grasslands (locations A, B, C) on peat soil (Terric Histosol; FAO 2014) in use by conventional dairy farmers in the west of the Netherlands. The three locations were selected based on their diverging Ca:Mg ratio in the 0–20 cm soil layer: location A had a high (6.9), location B a moderate (4.0) and location C a low Ca:Mg ratio (2.9), which is representative for the range found in this region (Deru et al. 2019). Per location, soil pH_{KCl} was 4.8 (A), 4.9 (B) and 4.5 (C) and SOM was 35% (A), 44% (B) and 34% (C). Location C had a relatively low P availability, probably as a result of reduced application of cattle slurry during the period from c. 1980 to 2006. Field observations in the 0–80 cm soil profile and consultation of the Dutch soil map (www.bodemdata.nl) showed that the profiles of the three-peat soils were comparable. Soil properties of the three locations are presented in Table S1 (supplementary material). The three grasslands were at least ten years old and dominated by *L. perenne*.

At each location, a randomised block experiment with five treatments (including an untreated control) in four blocks was laid out, resulting in 20 experimental plots per location and 60 plots in total (plot size 2.8 × 10 m; sampling and measurements occurred at minimum 0.5 m from the plot borders). The four minerals used were: $CaCO_3$, $CaSO_4$, $MgCO_3$, and $MgSO_4$. With these treatments, changes in Ca:Mg ratio were to be expected with increased pH (minerals with CO_3) or without increased pH (minerals with SO_4). Amounts used in the experiment (Table 1) were aimed at a strong effect on the Ca:Mg ratio in the soil, as a prerequisite to test the first hypothesis and were therefore higher

Table 1. Amounts of Ca, Mg, CO_3 and SO_4 applied per treatment (kg ha^{-1}). Total application was divided in three equal gifts in February, September and December 2014.

Treatment	Ca	Mg	CO_3	SO_4
Control	–	–	–	–
$CaCO_3$	2400	–	3590	–
$CaSO_4$	2600	–	–	6230
$MgCO_3$	–	760	1870	–
$MgSO_4$	–	760	–	3000

than common doses for grassland soils but not uncommon in research (Rashid et al. 2014; Ahmad et al. 2020). The treatments with $CaCO_3$ or $CaSO_4$ were similar in Ca application, whereas those with $MgCO_3$ or $MgSO_4$ were similar in Mg application. Ca application was higher than the Mg application, in line with the differences in CEC saturation in soil. Total amounts were applied in three equal parts during 2014: 33% in February, 33% in September and 33% in December.

During the application year (2014), the farmers continued their normal grassland management, which included fertilisation (a combination of cattle slurry manure and mineral N fertiliser, in line with the fertilisation advice (Commissie Bemesting Grasland en Voedergewassen 2019)), mowing and grazing by cattle. During the monitoring years (2015 and 2016), the plots were not fertilised or grazed. Monthly temperature and precipitation data for 2015 and 2016 are provided in Fig. S1 (supplementary material). Briefly, both years were characterised with a late start of the growing season, but with warmer summers than the long-term average. The year 2016 started wetter than normal but had a particularly dry summer until October.

2.2. Measurements

2.2.1. Soil chemical parameters

Before the start of the growing season of 2015 (February), soil measurements were carried out to determine whether treatments had the desired effects on the soil Ca:Mg ratio. At each location and per experimental plot, a soil sample consisting of 15 ± 1 randomly taken soil cores in the 0–20 cm layer was collected (auger diameter 1.2 cm; Eijkelkamp, Giesbeek, the Netherlands) (completely filled cores) and analysed with the same methods as used by Deru et al. (2019). Fresh samples were first sieved through a 1 cm mesh to remove plant remains and debris, then air-dried and ground to pass a 2 mm sieve, and subsequently analysed for Ca, Mg, K, Na and Al with a Mehlich 3 extractant (Mehlich 1984) (soil:extractant ratio 1:10). From these data, CEC and Ca:Mg ratio were calculated (Ross and Ketterings 1995).

In October 2015, another soil sample per experimental plot at the three locations consisting of 40 ± 2 randomly taken soil cores was collected 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 homogenised. A sub-sample was taken for determination of hot water extractable carbon (HWC). HWC was measured according to the method of Ghani et al. (2003). A second sub-sample 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}), ammonium-lactate extractable P (P_{AL}) and $CaCO_3$ content 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 the 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 potential P availability of grassland soils (Reijneveld et al. 2014), was determined according to Egnér et al. (1960) (NEN 5793). $CaCO_3$ content was analysed with near-infrared spectroscopy (Malley et al. 1999). Concentrations were expressed per soil dry weight.

Plant Root Simulator (PRS™) probes (Western Ag Innovations, Inc., Saskatoon, Canada) with ion exchange membranes were used in 2016 to measure plant-available mineral N at location B, as possible treatment effects on SOM mineralisation would release mineral N. PRS probes were used in the control and $CaCO_3$ treatments during weeks 1, 3 and 5 after the first cut. In each plot, four anion and four cation exchange PRS probes were placed at randomly chosen spots. The probes were carefully pressed 12 cm into the soil, ensuring good contact with the soil for measurement in the 2–8 cm soil layer, and removed after one week. After removal, the probes were washed free of adhering soil with deionised water, and were eluted with 0.5N HCl solution for 1 h; the eluate was analysed for NO_3^- and NH_4^+ by colorimetry using an automated flow injection analysis (FIA) system (Western Ag. Innovations Inc. PRS^{TM}-Probe Operations Manual, 2010).

2.2.2. Soil physical parameters

Soil physical measurements were carried out in situ in November 2015 as described below.

Penetration resistance was measured in each experimental plot at the three locations using an electronic penetrologger (Eijkelkamp, Giesbeek, the Netherlands) with a cone diameter of 2.0 cm^2 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). Load-bearing capacity was measured at 10 randomly chosen spots per plot with a penetrometer (5.0 cm^2 penetration surface, 60° apex angle), and was expressed as the average value of the maximum force (N) needed to push the cone through the sod.

Soil structure was determined in two blocks per experimental plot: one collected at 0–10 cm and one at 10–25 cm depth. Both blocks were collected from the same 20×20 cm area. In each block, the percentage of crumbs, sub-angular blocky elements and angular blocky elements was visually estimated as described by Peerlkamp (1959) and Shepherd (2000).

Water infiltration rate was measured at locations A and B at three randomly chosen spots per experimental plot, as described by Van Eekeren et al. (2010). Briefly, a PVC pipe of 15 cm high and 15 cm in diameter was put into the soil to a depth of 10 cm. To determine infiltration rate, 500 ml water was poured into each ring and the infiltration time was recorded. When the infiltration time exceeded 15 min, the remaining water volume was estimated and the total infiltration time was calculated by linear extrapolation. From these data, the infiltration rate (mm min^{-1}) was calculated.

2.2.3. Soil PLFA and tea bag index

In all experimental plots at location B (the site with median Ca:Mg ratio), a field-moist soil sample of c. 200 g (0–10 cm, auger diameter 2.3 cm, 10 cores) was collected in October 2015 and used for phospholipid fatty acid (PLFA) analyses. 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 analysed 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.

In 2016, organic matter decomposition potential was measured in all experimental plots at location B (the site with median Ca:Mg ratio) using the tea bag index (TBI) method (Keuskamp et al. 2013). This method provides an indication of treatment effects on decomposition processes in soil, as effects of pH were expected. This simplified litter bag experiment was carried out with commercially available tetrahedron-shaped synthetic tea bags with sides of 5 cm containing c. 2 g of green tea or rooibos tea, as indicated by the manufacturer (Lipton, Unilever). The green tea consisted of 89% green tea, and the rooibos tea consisted of 93% rooibos. The mesh size of 0.25 mm allowed microorganisms and mesofauna to enter the bags, but excluded macrofauna (Keuskamp et al. 2013). The teabags were weighed (mg) and the weight of the labels, string and net material was determined for 10 bags per type of tea and averaged to calculate net weight loss after the incubation. On the 11th of April 2016, the bags were incubated in all experimental plots at location B. In each plot, a 10 × 10 × 8 cm hole was dug and 4 tea bags of each type were placed at 8 cm depth. The sod was replaced on top of the teabags and the site was marked with a metal plate. Eighty-five days after incubation, the teabags were dug up, placed in plastic bags and transported to the laboratory. The teabags were air-dried (48 h) before gently removing all adhering soil and root material. Dry weight was determined after drying at 70°C for 24 h. The stabilisation factor (S) and the decomposition rate constant (k) of the incubated organic matter were calculated as outlined by Keuskamp et al. (2013). S is indicative of inhibiting effects of environmental conditions on the breakdown of the added labile organic matter. k represents short-term decomposition of new labile organic matter.

2.2.4. Grass dry matter and N yield

Grass dry matter (DM) and N yield were measured in four cuts per year during 2015 and 2016 at all locations with a Haldrup plot harvester (J. Haldrup a/s, Løgstør, Denmark). Harvest dates in 2015 were 19–22 May, 7–9 July, 25–27 August and 7–9 October; harvest dates in 2016 were 19 May, 18 July, 31 August and 12 October. For each harvest, fresh biomass, DM content after drying at 70°C for 24 h and total N content (Kjeldahl) were determined. From these data, DM yield (kg DM ha^{-1}) and N yield (kg N ha^{-1}) were calculated per harvest (cut) and summed per year. The yields of the second cut at location C in 2015 were excluded as by mistake cows had entered the experimental field.

2.3. Statistical analyses

The Shapiro-Wilk test of normality (Royston 1982) was used in R (version 4.0.3) to test parameter residuals for normal distribution. Parameters that did not meet the assumption of normality were log-transformed. Further statistical analyses included analysis of variance (ANOVA), principal component analysis (PCA) and redundancy analysis (RDA).

ANOVA (Genstat 19th edition, VSN international) was carried out on parameters to test for location and treatment effects and their interactions, including block effects per location. In cases of significant effects ($P \leq 0.05$), differences between means were determined with the least significant differences (l.s.d.; $\alpha = 5\%$). For soil parameters that were measured at location B only (PRS probes, TBI, PLFA), one-way ANOVA was used to test for treatment effects.

PCA and RDA were carried out in R with packages vegan 2.5-4 and pack for 0.0-8. Analyses including data of the three locations together were carried out with the residuals of the parameter values, calculated by fitting a linear model for location and block effects (location + block). This was done to exclude main location effects, because we were interested in the variation induced by treatments or by treatment × location interactions only. When analyses included data of one location only, the raw data were used. PCA was performed on the correlation matrix (of residuals) to visualise the variation of the soil parameters across treatments and provide an overview of correlations between parameters as projected on the principal component planes. RDA was performed to identify relationships between soil parameters and grass N yield parameters in four separate data sets: one including the three locations and three including one location each. Per separate data set, the number of explanatory soil parameters was first reduced by withdrawing parameters showing strong collinearity one by one, as indicated by a variance inflation factor (VIF) > 10 (Borcard et al. 2011). For RDA including all locations, and for locations A and C, the data sets comprised soil chemical and physical parameters. For location B, the data set also included PLFA and TBI parameters, as these were measured at this location. A list of parameters in the reduced data sets for RDA is presented in Table S2 (supplementary material). Within the RDA analysis, an ordination value for N yield values was generated and used in subsequent regression. This procedure was followed by permutation tests and a forward selection to identify the most relevant soil parameters explaining grass N yield, which was only run on significant ($P \leq 0.05$) models. Next, for each significant model, alternative models were computed using the same procedure, but after withdrawing the first selected explanatory parameter from the input data set. This was done to explore which other subsequent significant model parameters would be selected. For models including more than one explanatory parameter, partitioning of variance was carried out to calculate adjusted r^2 per parameter and assess the contribution of each parameter to grass N yield.

3. Results

3.1. Soil properties

The PCA shows that the treatments created gradients in soil properties (Figure 1). The plots with $CaCO_3$ had clearly different soil properties compared to the other treatments, especially along the first principal component. The control and the $CaSO_4$, $MgCO_3$ and $MgSO_4$ treatments differed only in the second principal component. The two treatments with added Mg showed little mutual differences in soil parameters. Treatments with added Ca had higher Ca:Mg ratio and CEC, and lower HWC and less crumb structures compared to those with added Mg. Treatments with added CO_3 had, or tended to have, higher pH_{KCl} and lower SOM, N_{total}, C_{total} and P_{total} compared to those with added SO_4.

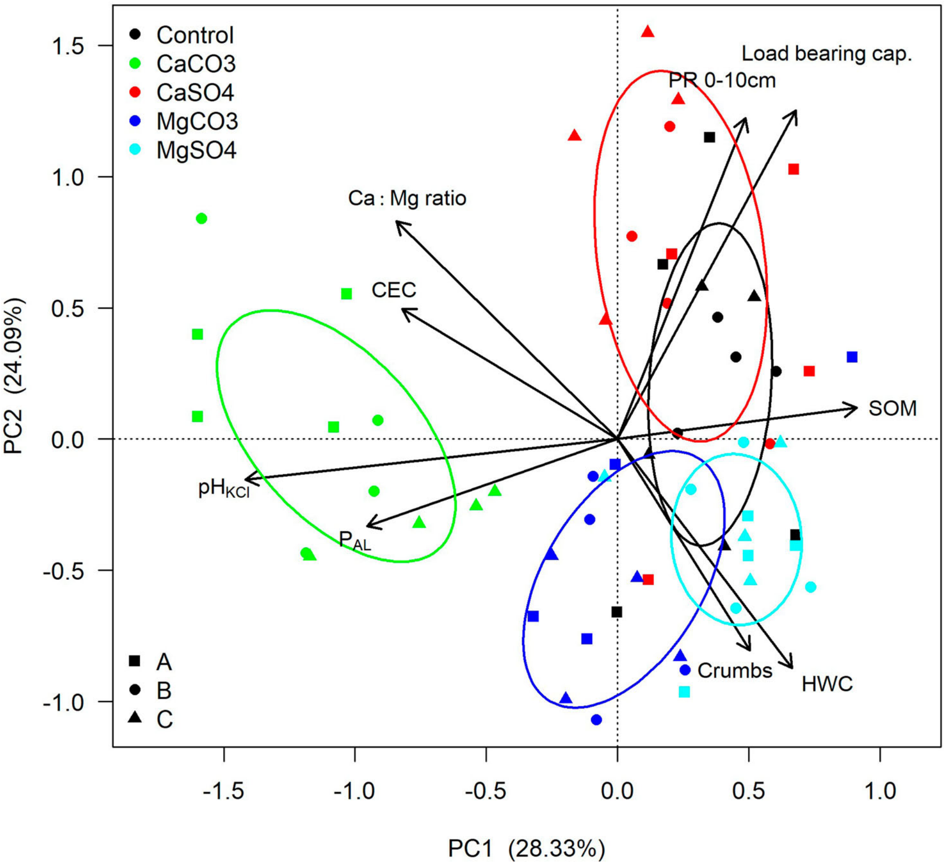

Figure 1. Axes 1 and 2 of a principal component analysis (PCA) of the residuals of soil chemical and physical parameters after conditioning for location and block. Treatments: Control, $CaCO_3$, $CaSO_4$, $MgCO_3$, $MgSO_4$; indicated with different colours. Locations: A, B, C; indicated with different symbols. Ellipses per treatment with 50% confidence. PR: penetration resistance. Parameters were omitted from the figure when their arrows closely overlapped other arrows, indicating a strong mutual correlation in the two-dimensional plane of the two first principal components. This was the case for N_{total}, C_{total} and P_{total} overlapping SOM, and for PR 10–20 cm and PR 20–30 cm overlapping PR 0–10 cm.

Many soil parameters differed between the three locations (Table S1 in supplementary material) but showed no interaction between location and treatment (Table 2), meaning that for those parameters the location differences were not influencing the treatment effect. Soil pH_{KCl} was significantly increased in treatments including CO_3, compared to the control (Table 2), with a larger effect of $CaCO_3$ than of $MgCO_3$, due to the higher rate of CO_3 in the $CaCO_3$ treatment (Table 1), whereas treatments with SO_4 did not affect pH_{KCl}. C_{total} was decreased by the CO_3 containing minerals, compared to the control, and the SO_4 containing minerals had no effect. The amount of soil $CaCO_3$ was negligible. N_{total} and P_{AL} were only influenced by $CaCO_3$, N_{total} negatively and P_{AL} positively. Load-bearing capacity and penetration resistance in the 0–10 cm layer were increased by $CaSO_4$. With CO_3 containing minerals, the load-bearing capacity and penetration resistance in the 0–10 cm layer were reduced. The percentage of crumbly soil elements and water infiltration rate were unaffected by treatments.

Table 2. Treatment effects for the soil chemical and physical properties not showing treatment × location interaction*. Means with the same superscript are not significantly different ($\alpha = 0.05$).

Parameter	Unit	*P*-value	Control	$CaCO_3$	$CaSO_4$	$MgCO_3$	$MgSO_4$
Soil chemical							
CEC	meq 100 g^{-1}	0.037	20.9^a	27.3^b	23.1^{ab}	21.8^a	22.1^a
pH_{KCl}	–	<0.001	4.7^a	6.1^c	4.7^a	5.0^b	4.8^a
C_{total}	g 100 g^{-1}	0.008	20.9^b	20.5^a	21.1^b	20.4^a	21.1^b
N_{total}	g 100 g^{-1}	0.006	1.70^b	1.63^a	1.68^b	1.67^b	1.69^b
P_{AL}	mg P_2O_5 100 g^{-1}	0.002	45.1^a	50.0^b	43.6^a	46.0^a	45.1^a
Soil physical							
Soil moisture	g 100 g^{-1}	0.006	52.1^a	52.5^{ab}	53.1^{bc}	52.3^a	53.2^c
Load-bearing capacity	*N*	<0.001	235^{bc}	211^a	243^c	207^a	217^{ab}
Pen.res.[†] 0–10 cm	*N*	0.006	112^{bc}	105^{ab}	118^c	104^a	110^{ab}
Crumbs in 0–10 cm	g 100 g^{-1}	0.457	66.7	60.8	64.2	73.3	69.2
Water infiltration	mm min^{-1}	0.615	3.5	2.5	3.1	2.9	2.4

[*] *P*-values of the treatment × location interaction were higher than 0.10 for all parameters in this table. Soil parameters with significant treatment × location interaction are presented in Figure 2 and Table S2 (supplementary material).

[†] Penetration resistance.

The treatments showed a significant interaction with location for Ca:Mg, SOM, HWC, P_{total} and penetration resistance in the 10–20 and 20–30 cm layers ($P < 0.001$, $P = 0.009$, 0.003, 0.034, 0.011 and 0.007, respectively: Figure 2 and Table S3 in supplementary material). At all sites Ca:Mg ratio was increased by Ca containing minerals (with little difference between $CaCO_3$ and $CaSO_4$), decreased by Mg containing minerals, and the effect of $MgCO_3$ was smaller than that of $MgSO_4$. The directions of the Ca:Mg effects were similar in the three locations, but the magnitudes were not: the effect of Mg containing minerals on the Ca:Mg ratio, as compared to the control, decreased with decreasing initial Ca:Mg ratio, i.e. the Ca:Mg ratio in the control plots. Differences in SOM were observed at location A between the two Ca-containing minerals (lowest in $CaCO_3$) and at location C between the two Mg-containing minerals (lowest in $MgCO_3$), whereas at location B no effect was measured. For HWC, the treatments with Ca had dissimilar effects depending on location: $CaCO_3$ at location A led to a reduction compared to the control, no change at location B and an increase at location C. The other minerals did not affect HWC, except for $CaSO_4$, which increased HWC at location B. P_{total} was affected at location A only: in the $CaCO_3$ and $MgSO_4$ treatments a decrease was observed compared to the control.

$MgCO_3$ showed marginally suppressive treatment effects on mycorrhizal NLFA (Table 3). Other PLFA parameters were also lowest with $MgCO_3$, but the differences were not significant. The stabilisation of decomposition (*S*) measured at location B with the tea bags index method was higher with $CaSO_4$, indicating inhibition of the breakdown of organic matter, and lower with $MgCO_3$ (Table 3). There was no significant effect on *k*.

Although the soil mineral N measured with PRS probes at location B was higher with $CaCO_3$ application compared to the control during the three measuring periods, differences were not statistically significant. There was a significant gradual decrease in soil mineral N from week 1 to week 5 in both the control and the treated plots (Table 4). This effect was determined by nitrate, as no effects were observed for ammonium.

3.2. Grass N yield

Grass N yield of the control plots, cumulated over 4 cuts per year, varied between 180 kg N ha^{-1} (location C) and 221 kg N ha^{-1} (B) in 2015 and between 198 kg N ha^{-1} (B) and

Table 3. Treatment effects on soil microbiological (PLFA) and tea bag decomposition parameters measured at location B. Means with the same superscript are not significantly different ($\alpha = 0.05$).

Parameter	Unit	*P*-value	Control	$CaCO_3$	$CaSO_4$	$MgCO_3$	$MgSO_4$
Soil microbiological							
Microbial PLFA	nmol g^{-1}	0.170	72.2	78.7	67.2	54.1	75.7
Bacterial PLFA	nmol g^{-1}	0.176	39.2	43.4	37.1	30.0	41.2
Saprotrophic fungal PLFA	nmol g^{-1}	0.563	0.91	0.99	0.93	0.66	0.92
AMF NLFA	nmol g^{-1}	0.058	0.65	0.57	0.79	0.32	0.57
Actinomycetes PLFA	nmol g^{-1}	0.171	4.05	4.24	3.72	2.53	3.99
Gram-positive PLFA	nmol g^{-1}	0.178	24.2	26.8	24.6	19.0	26.2
Gram-negative PLFA	nmol g^{-1}	0.224	5.50	5.57	4.82	3.83	5.52
Tea bag indices							
Stabilization factor (*S*)	–	0.003	0.25^b	0.24^{ab}	0.27^c	0.23^a	0.25^b
Decomposition rate (*k*)	–	0.137	0.014	0.013	0.016	0.014	0.017

246 kg N ha^{-1} (A) and was influenced by treatments. There was a location × treatment interaction in 2015 ($P = 0.016$) and a main treatment effect in 2016 ($P < 0.001$) without interaction with location ($P = 0.130$) (Figure 3). In 2015, location A had a similar N yield with both CO_3 minerals compared to the control but a lower N yield with both SO_4 minerals. In locations B and C, positive effects of $CaCO_3$ but no effects of the other minerals were found in 2015. In 2016, in all locations, differences between treatments were similar to those found in 2015 at location A: similar N yield with $CaCO_3$ and $MgCO_3$ compared to the control but lower N yield with $CaSO_4$ or $MgSO_4$.

Grass N yield per individual cut over the years 2015 and 2016 showed significant treatment effects from the second half of 2015 onwards (Figure 4). The plots treated with $CaCO_3$ had higher N yields than the control in the last two cuts of 2015, but no such effect was observed in 2016. In the plots treated with $CaSO_4$, a negative effect appeared in the last cut of 2015, continued in the first cuts of 2016 but recovered to the level of the control at the end of 2016.

3.3. Regression analysis with grass N yield and soil properties

The regression analysis linking grass N yield in 2015 and 2016 with soil properties measured in October 2015 resulted in a number of explanatory models (Table 5). Cumulative N yield per year was explained in 2015 but not in 2016. Per grass cut, significant models were found in 2015 for cuts 3 and 4 and in 2016 for cuts 1 and 2. In most models for the three locations together, pH_{KCl} was the first selected explanatory parameter, followed by P_{AL} or CEC. For cut 1 of 2016, C_{total} was the first selected explanatory parameter. Only in one specific model (cut 2 in 2016) a marginal contribution of Ca:Mg

Table 4. Effects of $CaCO_3$ application and week of measurement after the first cut in 2016 on the availability of mineral N in the soil (mg m^{-2} wk^{-1}) measured with PRS probes at location B.

	Week 1		Week 3		Week 5		*P*-value[a]		
Parameter	Control	$CaCO_3$	Control	$CaCO_3$	Control	$CaCO_3$	T	W	T×W
Total mineral N	41.4	50.8	20.7	36.5	11.8	13.4	0.275	0.032	0.610
NO_3-N	38.6	49.5	19.2	35.0	7.5	11.2	0.206	0.024	0.659
NH_4-N	2.8	1.4	1.5	1.4	4.3	2.2	0.198	0.202	0.479

[a] T: treatment, W: week.

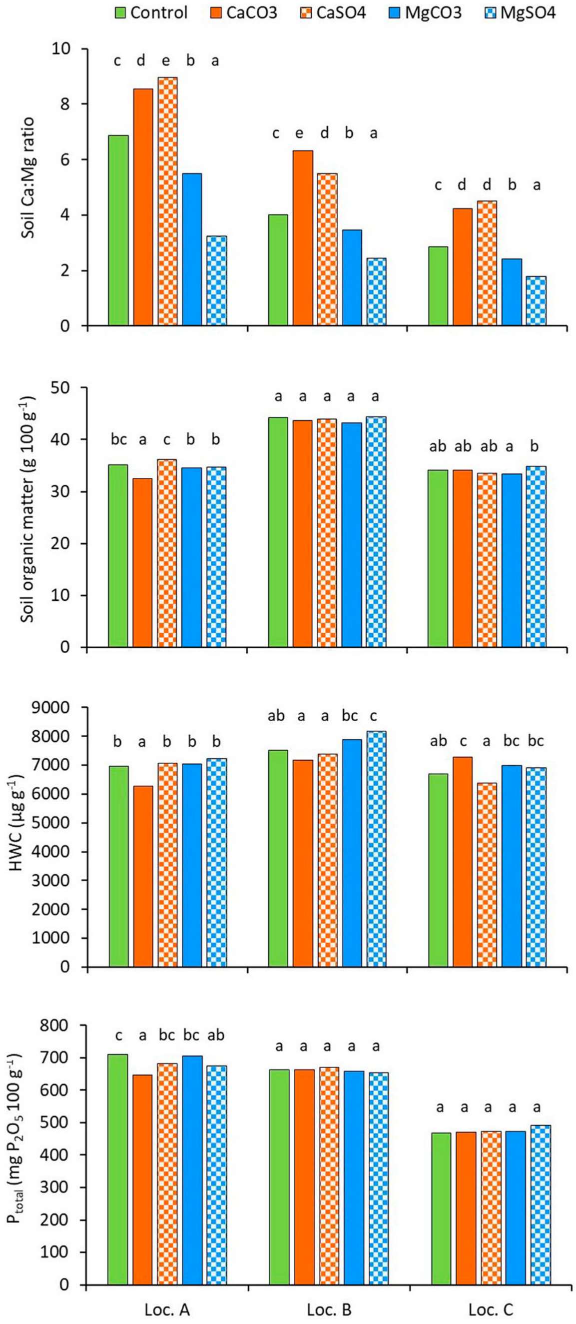

Figure 2. Ca:Mg ratio, Soil organic matter, hot water extractable carbon (HWC) and P_{total} at the three locations, each showing significant location × treatment effects ($P < 0.001$, $P = 0.009$, 0.003 and 0.034, respectively). Within a location, bars with the same letter are not significantly different ($\alpha = 5\%$).

ratio to grass N yield was found. In location A, P_{total} best explained the variation in grass N yield for the first cut of 2016. No significant models were found for locations B or C in either 2015 or 2016.

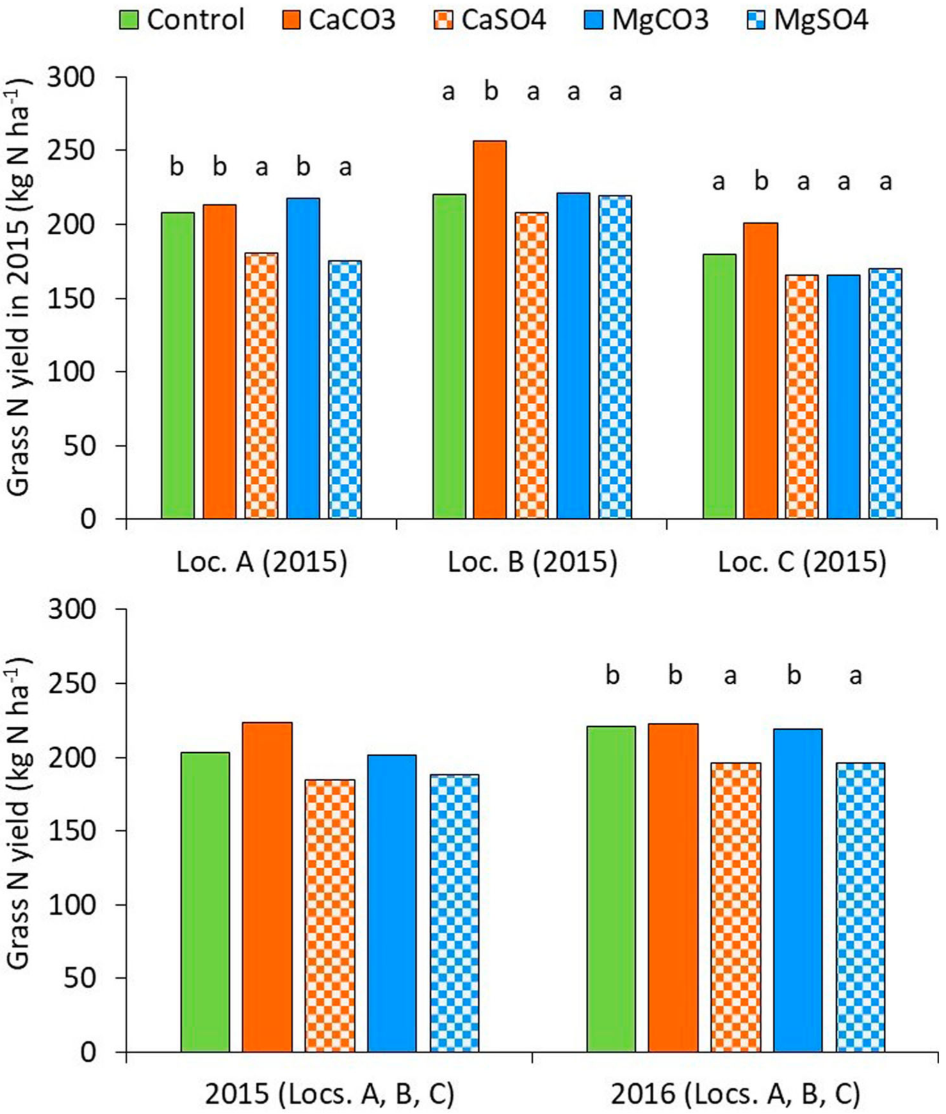

Figure 3. Grass N yield (kg N ha^{-1}) in 2015 per location (above; significant location × treatment effect, $P = 0.016$) and in 2015 and 2016 over the three locations (below; for 2016: $P = .130$ for location × treatment effect and $P < 0.001$ for treatment effect). In location C, 2015, grass N yield does not include the 2nd cut (see Methods). Within a location (above – 2015) or year (below – 2016), bars with the same letter are not significantly different ($\alpha = 5\%$).

4. Discussion

4.1. Effects of treatments on grass N yield

A considerable range of soil Ca:Mg ratios was created by the treatments and grass N yield was increased or reduced depending on treatment, location and year (Figures 3 and 4). However, changes in Ca:Mg ratio (Figure 2) did not correspond to changes in grass N yield (Figure 3). Instead, pH_{KCl} and to a lesser extent P_{AL} and CEC were selected

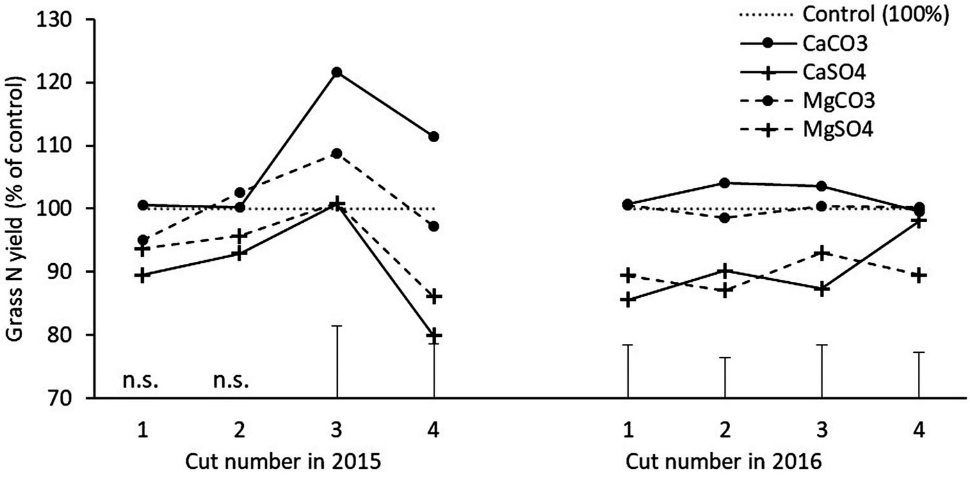

Figure 4. Grass N yield (kg N ha^{-1}) per cut in 2015 and 2016, expressed as a percentage of the control plots. Means of three locations and four replicates. Vertical bars on the x-axis represent the l.s.d. per cut in case of significant ($P \leq 0.05$) treatment effect. Mean values of the control plots for each consecutive cut (1 to 4) are 72, 44, 62 and 40 kg N ha^{-1} in 2015 and 76, 57, 45 and 43 kg N ha^{-1} in 2016.

in the regression procedure as explanatory variables for the variation in grass N yield (Table 5).

Our first hypothesis, that grass N yield would be positively correlated with soil Ca:Mg ratio, was based on a correlative field study (Deru et al. 2019) but did not stand the factorial experimental test: grass N showed clear treatment effects that were primarily grouped per

Table 5. Soil parameters that significantly ($P \leq 0.05$) explain the variation in grass N yield after addition of $CaCO_3$, $CaSO_4$, $MgCO_3$, $MgSO_4$ or no mineral.

Location (s)	Dependent parameter (N yield)	Removed from explanatory data	r^2_{adj}	Explanatory parameter(s) (partial r^2_{adj} in brackets)
A, B, C	2015: year total	–	0.55	pH_{KCl} (0.33), P_{total} (0.04), C_{total} (0.12), N_{total} (0.06)
		pH_{KCl}	0.23	P_{AL} (0.22), C_{total} (0.09)
A, B, C	2015: cut 1	–	–	n.s.
A, B[a]	2015: cut 2	–	–	n.s.
A, B, C	2015: cut 3	–	0.32	pH_{KCl} (0.16), Crumbs (0.11), C_{total} (0.05)
		pH_{KCl}	0.27	CEC (0.07), P_{AL} (0.07), C_{total} (0.05), Crumbs (0.05), Pen.res. 0-10cm
A, B, C	2015: cut 4	–	0.18	pH_{KCl} (0.28), P_{total} (-0.01), Pen.res. 10-20 cm (-0.01)
A, B, C	2016: year total	–	–	n.s.
A, B, C	2016: cut 1	–	0.25	C_{total} (0.07), Pen.res. 0–10 cm (0.06)
		C_{total}	0.13	pH_{KCl}
A, B, C	2016: cut 2	–	0.55	pH_{KCl} (0.32), N_{total} (0.11)
		pH_{KCl}	0.08	P_{AL} (0.10), Ca:Mg ratio (0.07), Pen.res. 20–30 cm (0.05)
A, B, C	2016: cut 3	–	–	n.s.
A, B, C	2016: cut 4	–	–	n.s.
A	2016: cut 1	–	0.75	P_{total} (0.82), HWC (0.32), CEC (0.18)
B	(none significant)			
C	(none significant)			

Notes: The explanatory parameters considered are mentioned in Table S1 (supplementary material). Explanatory parameters from a regression model were included with a forward selection procedure, which was only run on significant ($P \leq 0.05$) models. Alternative simpler models are presented if also significant (see Methods for details).

[a] N yield data missing for location C.

anion (CO_3 or SO_4) and less per cation (Ca or Mg) (Figure 3). Interestingly, the effects tended to decrease during the course of the experiment (Figure 4). Haynes and Naidu (1998) described influences of $CaCO_3$ on soil properties as a short-term effect (≈1 yr) of higher pH on soil microbiological activity, SOM decomposition and N mineralisation, and of higher Ca concentration on soil structure *versus* long-term indirect effects (i.e. >10 yrs) on SOM via increased crop growth. Indeed, pH was the most important predictor of N yield in our experiment (Table 5). In acid soils, a positive effect of liming and increased pH on grass yield is assumed to be caused by increased N mineralisation and P availability as a result of increased microbial activity (although effects may be temporary as discussed above), combined with reduced aluminium toxicity (Haynes 1984; Haynes and Naidu 1998; Bolan et al. 2003; Murphy 2007). In our soils, aluminium concentrations were very low (< 0.5% of CEC and no treatment effect), but in line with the above literature, the higher P_{AL} concentration in soil after addition of $CaCO_3$ (Table 2), the selection of both P_{AL} and pH_{KCl} for the models explaining grass N yield (Table 5) and the higher N content in the grass in 2015 (Table S4 in supplementary material) indicate a positive effect of pH, due to raised N and P mineralisation at higher pH. Moreover, this effect tended to decrease from 2015 to 2016 (Figures 3 and 4; Table S4) or was not shown by the soil mineral N contents in 2016 (Table 4). Hence, these observations may explain the different outcome of the present study compared to Deru et al. (2019), indicating mainly short-term soil chemical effects on pasture yield that may have masked possible longer term Ca and Mg effects via soil physical properties (Dontsova and Norton 2002). In contrast, the study of Deru et al. (2019) was based on grasslands with a stable management and no liming for at least five years, thus where differences in soil Ca:Mg ratio were likely to originate from soil type and longer term management history. So, within the time-span of the present study, no evidence was found for our second hypothesis, that Ca:Mg effects would be caused by changes in soil physical properties rather than in N mineralisation.

In 2015 a higher N yield and pH were measured with the addition of $CaCO_3$, whereas a reduced N yield was found with minerals containing SO_4 in 2015 (location A) and 2016 (all locations). Our measurements do not provide a clear explanation for these effects of SO_4 (Table 5). Increased exchangeable Ca concentration and higher ionic strength of the soil solution in acid mineral soils treated with gypsum ($CaSO_4$) is normally found to increase clay flocculation and reduce penetration resistance and aluminium toxicity, generally leading to better water infiltration, deeper rooting and higher crop yield (Shainberg et al. 1989; Sumner et al. 1990). The higher penetration resistance and generally lower grass yields (Figure 3; Fig. S2 in supplementary material) in our experiment are therefore unexpected. Peat soils have very different physical properties than mineral soils (Schwärzel et al. 2002; Sognnes et al. 2006) and the addition of gypsum ($CaSO_4$) has been found to increase peat physical stability (Kolay and Pui 2010; Saberian and Rahgozar 2016). In our experiment, this effect may possibly have resulted in modified soil physical properties with higher penetration resistance negatively influencing grass growth.

4.2. Effects of treatments on soil properties

C_{total} and N_{total} were similarly influenced per treatment at each location, whereas SOM and HWC showed a significant interaction with location. This indicates that mineralisation was affected by the added minerals but also by local edaphic properties.

The changes in C_{total} observed in the top 10 cm soil layer after the addition of $CaCO_3$ or $MgCO_3$ in October 2015 are equivalent to a loss of 2.8 Mg C ha^{-1} in comparison with the control (with a bulk density of 0.54 g cm^{-3} measured in the same soil type and region in dairy grasslands (Deru et al. 2018)). Estimates of the background CO_2 emission from drained peat grasslands in the Netherlands range between 2.2 and 5.8 Mg C ha^{-1} yr^{-1} from the whole peat layer (Jacobs et al. 2007; Van den Akker et al. 2008). Thus, CO_3 liming increased the peat decomposition rate substantially, at least during the first two years.

C_{total} was similarly reduced by the application of $CaCO_3$ and $MgCO_3$ despite the higher application rate of CO_3 in the $CaCO_3$ treatment and the stronger pH increase (Tables 1 and 2). An assumed effect of enhanced microbial respiration by increased pH does therefore not provide a sufficient explanation for the similar effects on C_{total}. In soil, Ca ions can stabilise organic matter by Ca binding (Rowley et al. 2018), thereby reducing microbial respiration and mineralisation. This may have occurred in the $CaCO_3$ treatment, in contrast to the treatment with $MgCO_3$, which possibly explains the similar reduction of C_{total} in both treatments despite the higher application rate of CO_3 in the $CaCO_3$ treatment and the stronger pH increase.

C_{total} was not influenced by SO_4 containing minerals, which confirms that it was the addition of the CO_3 in the $CaCO_3$ and $MgCO_3$ treatments that primarily caused its reduction. However, SO_4 may leach to the deeper anoxic peat layers and act as alternative electron acceptor, enhancing the decomposition of organic matter and initiating internal eutrophication in deeper layers (Smolders et al. 2006). Although the microbial decomposition processes involved are likely to be much slower than those in the oxic peat layer, addition of SO_4 to drained peat grasslands is not advisable and should at least be evaluated in the longer term and in the whole peat layer.

The amount of soil C is determined by the balance between decomposition and inputs, which complicates the interpretation of results simply based on differences in C concentration between treatments. In our experiment, differences in N_{total}, however, can be primarily attributed to N mineralisation, as N deposition (c. 30 kg ha^{-1} per year in the Netherlands) was the same in all treatments and no further substantial N input source such as legumes was present. Assuming no changes in soil bulk density, our results show that addition of $CaCO_3$ increased N mineralisation (as indicated by lower N_{total}) whereas the other treatments had no effects. The lack of effect by $MgCO_3$ may be due to the lower amount of CO_3 applied in this treatment compared to the amount of CO_3 applied in $CaCO_3$ (Table 1). Compared to the control, a decrease in N_{total} equivalent to 380 kg N ha^{-1} was measured in the $CaCO_3$ treatment. Of this amount, only 21 kg was recovered as additional grass N yield in the $CaCO_3$ treatment compared to the control during that year. This would imply that nearly all N mineralised during the 1.5 years following the first addition of $CaCO_3$ was either emitted as N_2O or N_2, or leached as NO_3 to deeper layers. Nitrate in anoxic peat layers can act as an electron acceptor similarly to SO_4, as discussed above.

Literature shows positive effects on bacterial PLFA following $CaCO_3$ addition and raised pH (Frostegård et al. 1993; Söderberg et al. 2004; Kramer and Gleixner 2008; Börjesson et al. 2012); the lack of significant treatment effects on PLFA parameters in our experiment (Table 3) is therefore unexpected. However, PLFAs were measured in location B, where no significant effects in SOM and HWC were found (Figure 2). Still, pH, C_{total} and N_{total} were affected at the three locations, including B, which indicates effects on microbial decomposition. Apparently, the treatments did not affect the microbial abundance (as indicated by PLFA), but possibly only its activity.

SOM and HWC, being both organic matter fractions, showed a significant interaction of treatment with location, suggesting that organic matter breakdown or build-up processes were different per location. The three experimental locations differed not only in Ca:Mg ratio (A > B > C), but also in soil P availability (A > B > C; Table S1 in supplementary material). For treatment effects in SOM and HWC (Figure 2) we propose tentative explanations based on literature showing that decomposition of organic matter (in our case SOM and HWC) is pH dependent (Ghani et al. 2003; Fernández-Calviño and Bååth 2010), but may also be hampered under P limitation (Amador and Jones 1993; Cheshire and Chapman 1996; Brouns et al. 2016) and reduced by Ca binding (Rowley et al. 2018), and finally that the concentration of soluble C (such as HWC) is increased at higher pH due to microbial and chemical dissolution of SOM, and reduced by addition of Ca (Andersson and Nilsson 2001; Oste et al. 2002). Firstly, in location A, lower SOM and HWC in the treatment with $CaCO_3$ is explained by the higher pH resulting in faster decomposition of SOM and HWC, outweighing binding effects of added Ca. The proportionally similar decrease in P_{total} (Figure 2) points in the same direction. Secondly, in location C, the low soil P availability may have limited the effect of higher pH (by addition of $CaCO_3$) on increasing the organic matter decomposition, resulting in no change in SOM but an increase in HWC due to increased dissolution of SOM at higher pH. Lastly, in locations B and C, Ca binding after addition of $CaSO_4$ may have reduced the solubility of organic compounds, without interference of changed pH, which was reflected by a lower HWC when compared to $MgSO_4$ (Figure 2). In location B, the changes in tea bag stabilisation index (S) (lower with $CaCO_3$, higher with $CaSO_4$; Table 3) also indicate that an increased soil Ca concentration without change in pH (by application of $CaSO_4$) reduces the (potential) breakdown of organic matter. The above supports our third hypothesis that CO_3 addition increases pH, SOM decomposition, and grass N yield independent of the Ca:Mg ratio. However, we show that location differences in soil P availability partly determine the effects of added minerals and that Ca binding may reduce the concentration of soluble SOM compounds and decomposition. We also demonstrate that increased pH leads to undesirable SOM mineralisation with associated C and N losses and marginally higher pasture yields. Therefore, the agricultural advice for an optimal pH and use of liming minerals in peat grasslands should be revised based on field experiments in a larger number of grasslands to better represent the variation in edaphic conditions within this soil type.

5. Conclusions and recommendations

Addition of Ca and Mg containing minerals in peat grasslands influenced the SOM dynamics and the soil chemical and physical properties, and this was especially true for the treatment with the highest CO_3 input, in this case, $CaCO_3$. Contrary to our first hypothesis that was based on a previous correlative study in 20 dairy grasslands on peat, grass N yield was primarily linked with changes in soil pH and not with changes in Ca:Mg ratio.

Of the applied minerals, $CaCO_3$ had the largest influence on grass N yield, and increased also pH and (indicators of) SOM decomposition without evidence for our second hypothesis that grass N yield would correlate with soil physical properties. Grass N yield increased (+21 kg N ha^{-1}) one year after applying the $CaCO_3$ mineral, but the coinciding strong decrease in the soil N stock (−380 kg N ha^{-1}) indicated low utilisation of the (extra) mineralised N and a disproportional environmental risk of increasing the pH of peat soils. The additional decrease in soil C_{total} was of the same order of magnitude as the background CO_2-C emission from drained peat grasslands. The minerals containing SO_4 reduced grass N yield in most cuts and $CaSO_4$ application increased soil penetration resistance below the 10 cm top soil. Thus, during the first two years after the experimental manipulation of the soil Ca:Mg ratio, effects of added minerals were more determined by the anions (CO_3 or SO_4) than by the cations (Ca or Mg). Based on these results, we hypothesise that a mineral that contains both Ca and Mg, like dolomite ($CaMg(CO_3)_2$), will also predominantly influence soil and grass properties via the anion. But as cation effects may appear in the longer term, we recommend measurements over a longer period. Our results further show that accelerated SOM decomposition after $CaCO_3$ application was mainly related to increased pH, as hypothesised. However, in soils with low P availability, SOM was not decreased even at higher pH, possibly due to P limited microbial activity. Moreover, in the treatment with added Ca but without pH-effect ($CaSO_4$), lower HWC and a higher teabag stabilisation factor suggested a stabilisation of organic matter by Ca binding.

The results of our experiment do not support Ca:Mg ratio as a useful measure of soil quality for increased herbage production in peat grasslands without extra SOM mineralisation and losses of C and N. To avoid those losses, the agricultural pH advice for peat grasslands should be better adapted to the local soil properties that influence SOM decomposition, such as initial pH and P availability. Moreover, advice should be specific in the type and quantity of mineral to use based on the cation and anion composition and expected effect on pH and SOM mineralisation.

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