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The effect of soil amendments on the greenhouse gas production in agricultural peat soils

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Soil amendments can improve soil productivity, but they can affect the production and emissions of greenhouse gases (GHG). We studied the effect of gypsum, foundry sand, calcium carbonate and biochar on GHG production rates and microbial community structure in laboratory bottle incubation experiments for peat soils. Four agricultural peatland and two forested peatland soils were selected for the study. Biochar was found to increase nitrous oxide (N_2O) production in the majority of the soil samples by an average of 212% in agricultural soils where the increase was statistically significant. Calcium carbonate ($CaCO_3$) had a similar effect, increasing N_2O production by an average of 319%, but this change was not detected in as many soils. Calcium carbonate and foundry sand amendments also increased carbon dioxide (CO_2) production by an average of 40% and 44%, respectively, in the tested agricultural soils, while biochar and gypsum amendments reduced it by 34% and 28%, respectively. Methane (CH_4) production in all soils was mainly negative, indicating CH_4 uptake, and in agricultural soils, it was mainly unaffected by amendments, except $CaCO_3$, which reduced uptake. In the afforested and forest site soils, however, gypsum and $CaCO_3$ amendments significantly reduced CH_4 uptake by the soil but did not turn the soils into net sources of CH_4. Nitrous oxide production increased with decreasing pH in agricultural soils. The microbial community structure was significantly different between agricultural and forest sites due to a higher abundance of Crenarchaeota phylum in the forest soil, which included mainly the ammonia-oxidizing *Thaumarchaeota*. This, among other differences in the microbial community structure, could explain why the soils reacted differently to the soil amendments. The ordination analysis showed that N_2O production was related to low pH, low sulfate concentration, low soil moisture and low water holding capacity. Conclusively, our results show that the physical and chemical properties of the soil, as well as the structure of the soil microbial community, can determine the way CO_2, CH_4 and N_2O production in agricultural peatland soil changes in response to different soil amendment uses.

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Introduction

Peatlands are a significant component of the global carbon cycle, as they act as the densest storage of soil organic carbon on the planet, with northern peatlands making up 80% of global peat stocks, and holding an estimated 500 Pg of carbon (Hugelius *et al.* 2020). Peatlands drained for agriculture are a significant source of the major agricultural greenhouse gases (GHG): carbon dioxide (CO_2) and nitrous oxide (N_2O), while they are a negligible methane CH_4 source and can even act as a net sink (Maljanen *et al.* 2010, Minkkinen *et al.* 2018, Anthony and Silver 2023). The Global Warming Potentials (GWP) on a 100-year time horizon for N_2O and CH_4 are 273 and 30 times higher than that of CO_2 (Intergovernmental Panel on Climate Change 2023), and they can contribute significantly to the net GHG effect even with smaller fluxes than CO_2. In soils, these gases are mainly produced as a result of microbial degradation of organic matter, but in agricultural peat-based soils their emissions can be significantly influenced by different soil use strategies as well as different soil amendments.

In Finland, agricultural peatlands cover approximately 10% of the total land area used by agriculture, but they are responsible for approximately 50% of the total agricultural GHG emissions (Kekkonen *et al.* 2019, Forsell *et al.* 2023). Restoring agricultural peatlands to their natural state to preserve natural habitats and reduce GHG emissions is a major goal outlined in the European Commission Proposal for a Nature Restoration Law in 2022, but the timeline for restoring 70% of agricultural peatlands in Finland stretches to all the way to the year 2050 (Räsänen *et al.* 2023). Moreover, in some places these peatlands form such a significant portion of the agricultural land, that restoring them in their entirety is unfeasible. Therefore, researching ways to reduce the GHG emissions from these agricultural peatlands, already when they are still in use could potentially help mitigate their environmental impact in matter of years, instead of decades.

Soil amendments are used in agriculture to improve the harvest yield of food crops and silage by changing the soil physical or chemical properties or directly adding plant-available nutrients to the soil, or by otherwise improving nutrient retention by the soil, so that fertilizers are not leached away. There are numerous different types of soil amendments, but in this study, we chose to test the effects of calcium carbonate ($CaCO_3$), gypsum ($CaSO_4 \cdot 2H_2O$), foundry sand, which are aimed at improving plant growth and nutrient availability — and biochar, which is primarily intended to enhance soil carbon sequestration and aeration on the GHG production rate from four different agricultural peatland soils used for growing silage for animals, as well as one afforested agricultural peat field and peatland forest soil.

Calcium carbonate has been one of the most used soil amendments throughout the history of agriculture, which is the reason why it was the first choice for a treatment in this experiment. Its effect is based on increasing the pH of the soil (liming effect), which prevents nutrient leaching by making nutrient cations adhere to soil particles more tightly. Historically the most common way of liming agricultural fields has been through spreading of wood ash, a significant portion of which consists of $CaCO_3$ (Demeyer *et al.* 2001). Previous studies on soil amendments suggests that $CaCO_3$ may reduce N_2O and CO_2 emissions by increasing soil pH and inhibiting denitrification (Xu *et al.* 2023). In acidic soils however, $CaCO_3$ amendment may increase CO_2 emissions in the short term as it first forms carbonic acid (H_2CO_3) when reacting with hydrogen ions in soil water, that then further splits into CO_2 and H_2O (Biasi *et al.* 2008, Rousset *et al.* 2023, Ouerghi *et al.* 2023).

Gypsum is sometimes used to increase soil calcium and sulfate levels, thus providing nutrients to plants and alleviating soil salinity stress (Bello *et al.* 2021). Gypsum has also been reported to decrease N_2O emissions by enhancing soil aeration and reducing denitrification, and the sulfate in $CaSO_4 \cdot 2H_2O$ could decrease CH_4 production (Maljanen *et al.* 2010). Its effects on CO_2 production are less clear, however. In addition to being a direct nutrient additive, gypsum is used in coastal areas in Finland to prevent phosphorus leaching from agricultural soils into Baltic, and as such, it was chosen as the second treatment. (Ekholm *et al.* 2012).

Sand is used as a soil amendment to improve soil texture and carrying capacity, as well as water infiltration and nutrient availability. Foundry sand is a byproduct of metal casting, and after cleaning it of metal residue, it could be used instead of regular sand as an amendment in agricultural peat fields just like regular silica-based sand, instead of being deposited in landfills (Oliveira *et al.* 2011). Foundry sand is expected to have a limited impact on GHG emissions, but its effects on soil texture and water infiltration may indirectly influence GHG production rates (Säurich *et al.* 2019).

Biochar, a carbon-rich material, is produced from the pyrolysis of biomass. It has been shown to enhance nitrogen retention, water holding capacity, and carbon sequestration in soil, while reducing N_2O and CO_2 production by improving soil aeration and enhancing carbon sequestration. Its effect on CH_4 emissions however is variable, with both increases and decreases observed depending on soil conditions and biochar properties (Lehmann *et al.* 2011, Kulmala *et al.* 2022). Biochar was chosen as the third amendment because of the high potential for carbon sequestration by the soil when it is added to it, in addition to potential for reducing the emission rates.

In the European Union, only 3.8% of the total land area used for agriculture consists of drained peatlands, but for example in 2020, it was responsible for approximately 15.0% (41.47 Gg N_2O-N y-1) of the total annual N_2O emission (276.46 Gg N_2O-N y-1) produced by all agricultural soils (Lin *et al.* 2022, European Environment Agency 2024). For comparison, the total estimated N_2O emission in European Union in 2022, caused by nitrogen fertilizer volatilization redeposition and leaching was approximately 51,52 Gg of N_2O-N (Menegat *et al.* 2022). Due to the high GWP of N_2O, the N_2O emission from just the organic matter decomposition of drained agricultural peatlands makes up only 0.025% of the CO_2-equivalent total GHG emissions emitted by agriculture by mass (Intergovernmental Panel on Climate Change (IPCC) 2023).

Since soil amendments can modify soil properties and chemical characteristics, they also indirectly affect microbial processes and community structures which are responsible for soil GHG emissions. For example, soil pH — a major factor determining microbial activity — can be altered by amendments, impacting nutrient availability and metal solubility (Abdu *et al.* 2017). Therefore, the use of soil amendments could change the community structure or activity profile of soil microbes. The nitrogen cycle has a central role in soil nutrient and element cycling, because of providing precursors of N_2O, but also because to its importance for the broader carbon dynamics, influencing both carbon dioxide (CO_2) and methane (CH_4) production (Butterbach-Bahl *et al.* 2013). Nitrifiers, in particular, directly and indirectly produce N_2O, but can also affect heterotrophic carbon mineralization, and thus CO_2 production, by altering soil nitrogen availability and pH, which influence microbial respiration and soil organic matter decomposition processes. The activity of these same nitrifying microbes also plays a large role in determining the amount of nitrogen fertilizers lost due to fertilizer volatilization, so the processes of soil organic matter decomposition and nitrogen fertilizer volatilization are closely tied together. Organic amendments, such as biochar, on the other hand, can increase microbial activity and denitrification by providing an additional carbon source for the microbes (Ju *et al.* 2011).

In this study, we hypothesize that addition of the chosen soil amendments can significantly alter the GHG, especially N_2O, production in agricultural peat soils. We also hypothesize that the structure of soil microbial community, particularly the nitrifiers, differs between soils that react to amendments differently. The focus of our study was to study if any of the soil amendments show potential to reduce N_2O emissions in agricultural peat soils.

Material and methods

Study sites and peat properties

We chose four cultivated peat fields of which one located in Sweden (Uppsala) and the others in Finland (Kannus, Jokioinen and Ruukki) (Table 1) as the study sites for this experiment. Additionally, we chose one forested site, and one afforested peat field located near the Kannus peat field site to study if the soil microbial com-

munity structure varies within the same area, based on soil use type. These two additional sites were included, because most of the peatlands in Finland are drained for forestry, and some low productive agricultural peatlands have also been afforested. These soils are also emitting N_2O, but they can have very different microbial populations from the agricultural peat fields, and seeing if they respond differently to soil amendments used in the agricultural fields might help determine which microbial groups are responsible for possible changes in GHG emissions after amendment addition.

Topsoil (0–20 cm) was collected from each site and stored at +4°C until further processing. All of the sites were previously drained peatlands, and all of the agricultural field sites had been plowed recently before sampling. Prior to bottle incubation the soil samples were sieved through a 5.66 mm sieve and homogenized to remove roots and other alive plant material. Before the bottle incubation, soil dry water content was determined by drying soil samples at 65°C oven for 24 hours, and maximum soil water holding capacity (WHC) was determined so that the water content of each of the soils could be adjusted to 60% of their maximum water holding capacity (g H_2O / g dry soil). This was done because it is previously shown that both nitrification and denitrification are possible simultaneously in different soil microsites at 60% of maximum WHC (Bateman and Baggs 2005). Soil pH, electrical conductivity and pH were measured from soil slurry (15 g soil/50 ml milli-Q H_2O) before and after the incubation. Soil nitrate (NO_3^-), nitrite (NO_2^-) and sulfate (SO_4^{2-}) concentrations were measured from the soil H_2O extract directly after filtering (Whatman 589/3) with an ion chromatograph (Dionex ICS-2100). Soil ammonium concentration was measured with spectrophotometer using sodium-nitroprusside method (Fawcett and Scott 1960)

Table 1. Initial physical and chemical properties of the studied soils. Standard errors given as standard deviations based on three replicates that were analyzed for each soil, pH (H_2O); soil pH in H_2O slurry, EC (mS cm^{-1}); electrical conductivity in H_2O slurry, MAX WHC; maximum water holding capacity, TOC; total organic carbon content, IC; total inorganic carbon content.

	UAG (Uppsala, Sweden)	JAG (Jokioinen, Finland)	RAG (Ruukki, Finland)	KAG (Kannus, Finland)	KAF (Kannus, Finland)	KFR (Kannus, Finland)
Land use type	Agricultural peat field	Agricultural peat field	Agricultural peat field	Agricultural peat field	Afforested peat field	Peatland forest
Location	60.083°N 17.233°E	60.490°N 23.300°E	64.684°N 25.104°E	63.917°N 23.971°E	63.903°N 23.989°E	63.917°N 23.971°E
Moisture % in fresh soil	70.6±0	51±0	44.9±0.1	60.9±0	51.7±0.2	67.7±0.1
Moisture % during experiment	72.20	56.90	51.30	71.60	66.30	77.60
Organic matter %	86.3±0.1	53.1±0.2	53.1±0.2	79.7±0.8	68.9±0.7	94.7±0
Gravimetric moisture (g H_2O g^{-1})	2.4±0	1±0	0.8±0	1.6±0	1.1±0	2.1±0
MAX WHC (g H_2O g^{-1})	4.3±0	2.2±0	1.8±0.1	4.2±0.1	3.9±0.1	5.8±0
pH (H_2O slurry)	5.7±0	5.4±0	5.9±0	5.1±0	4.8±0.1	3.5±0
EC (mS cm^{-1}) (H_2O)	254.7±15.3	148.7±2.6	76.7±3.3	170±3.6	86.7±3.1	134±1.4
TOC (µg g^{-1})	444.5±17.3	143.3±4.6	98.7±13.9	186±4.8	133.6±1.7	176.8±12.8
IC (µg g^{-1})	11.5±0.7	9.2±0	1.5±0.1	4.1±0.2	2±0.4	2.7±0.4
Ammonium (mg g^{-1})	6.8±2	1.9±0.1	1.3±0.2	5.3±2.8	4±0.1	11.2±2.7
Nitrate (mg g^{-1})	248.7±7.1	95.3±2.8	2±0.4	130.9±0.3	39.6±0.2	34.8±0.2
Nitrite (mg g^{-1})	0±0	0±0	1.2±0.3	0±0	0±0	0.6±0.4
Sulfate (mg g^{-1})	85.9±3.1	26.8±1.2	83.2±0.9	54.5±1.5	21.1±0.1	36±0.6

from 1 M potassium chloride (KCl) extract that was also filtered prior to measurement. Soil slurries were placed in shaker for 1 hour at 175 rpm prior to filtration. The H_2O extracts were placed in storage at –20°C freezer and were used for determining the TOC and IC concentrations later with TOC analyzer (TOC-L Shimadzu).

Amendment treatments

We tested the effects of multiple soil amendments (Table 2, Table S2 in Supplementary Information) in the experiment. The incubation experiment was conducted using 550 ml bottles having five replicates per treatment. Approximately 30 grams of sieved soil was weighed into each bottle and adjusted to an appropriate moisture content of 60% of the maximum WHC for each soil, via the addition of milli-Q H_2O directly into the soil in the bottles. Soil amendments were added into the incubation bottles before the additional H_2O. The incubation bottles that were used had a cross-section surface area of 38.5 cm^2 and airspace volume of 516 to 544 ml depending on which soil amendment was added together with the soil. Tested amendments were calcium carbonate ($CaCO_3$), gypsum ($CaSO_4 \cdot 2\ H_2O$), biochar and foundry sand. Elemental composition of foundry sand, gypsum, and biochar was determined in commercial laboratory, but for the $CaCO_3$ treatment we used laboratory reagent $CaCO_3$ (J.T. Baker, CAS nr: [471-34-1]). Of calcium carbonate and gypsum, we added 1.54 g to each bottle and of biochar, 11.54 g, corresponding to 4000 kg ha^{-1} and 30000 kg ha^{-1}, respectively. For foundry sand treatment we added 30 grams of foundry sand into each incubation bottle. These amounts roughly correspond to the commonly used doses per hectare in agriculture (Ekholm *et al.* 2012, Berglund *et al.* 2021, Ajosenpää *et al.* 2022). The incubation bottles were loosely covered with aluminum foil and the incubation was done in dark incubator chamber at 15°C. Gas sampling was done on day 1 after adding the soils to and amendments in the incubation bottles (week 0) so the GHG production rates for the (Ajosenpää *et al.* 2022) control samples could be used in the microbial community analysis, and day 14 (week 2) to determine the effects of the soil amendment additions on the GHG production rates. Because the space in the incubation chamber was limited, we split the treatments into two incubation groups. Group 1 treatments included: control, $CaCO_3$, and gypsum, while group 2 treatments included: control, foundry sand, and biochar. The 14-day incubation was first done on group 1, and group 2 incubation was started immediately after group 1 incubation bottles were removed from the chamber after the final gas sampling. The sieved soils were stored in +4°C cold storage while waiting for the beginning of group 2 incubation. While stored in cold storage, the soils were covered with black plastic bags to protect them from the light and to minimize evaporation of soil moisture. Because the plastic covers were not gastight, and the soil was thoroughly mechanically mixed and disturbed when it was added to the incubation bottles right before the start of the incubation, we assume the soil did not have elevated levels of gasses that might have accumulated during cold storage.

On sampling day, the bottles were ventilated with fan prior to closing them with rubber stoppers and metal screw caps. After closing each bottle, 60 ml of room air was injected into the bottle to provide over pressure to enable gas sampling from the bottles. Four samples of 20 ml were taken with syringes 15, 30, 45 and 60 minutes after sealing the bottles and were injected immediately into pre-evacuated Labco® vials for analysis of gas concentrations with Agilent 7890 gas chromatograph. This method of gas sampling has been used previously in similar bottle incubation experiments (Liimatainen *et al.* 2014). After gas sampling was done, rubber septa were removed, bottles covered with aluminum foil and placed back into the incubation chamber. After the last gas sampling, soil in each bottle was divided for H_2O and KCl extractions and measurements of soil properties. For statistical analysis and interpreting the results, gas production rates from day 14 sampling were used, to allow the soil to settle and microbial community to acclimate to moisture and temperature conditions, as well as the presence of the soil amendments before measurements (Madegwa and Uchida 2021).

Table 2. Physical and chemical properties for the studied soils after the incubation. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls. Control (G1) and Control (G2) are the controls for each respective incubation group. Standard errors are given as standard deviations (n = 5). Significance levels: '**': p <= 0.01, '*':p <= 0.05. Significance levels given as difference to respective controls. pH (H_2O); soil pH in H_2O slurry, EC (mS cm^{-1}); electrical conductivity in H_2O slurry, TOC; total organic carbon content, IC; total inorganic carbon content.

	Control (G1)	Gypsum	$CaCO_3$	Control (G2)	Foundry Sand	Biochar
UAG						
pH (H_2O)	5.9±0	5.3±0**	7.6±0**	5.9±0	7.1±0.1*	6.4±0.2*
EC (mS cm^{-1})	158.4±17.5	2007.8±128.5*	250.8±17.4*	213.4±9.9	735±61.9**	175.4±44.5**
TOC (µg g^{-1})	NA	NA	NA	132.3±3.3	250.8±15**	204.6±19.8
IC (µg g-1)	NA	NA	NA	1.8±0.2	8.2±0.9**	2.1±0.5
Ammonium (mg g^{-1})	21.3±4.7	19.4±3.2	26.6±3.1	13.1±5.4	162.7±152.5	27.6±20.9
Nitrate (mg g^{-1})	145.5±1.9	154.3±1.7**	192.3±6.3**	269.5±10.8	276.1±16.1	157.4±14.2**
Nitrite (mg g^{-1})	1.8±0.6	0±0**	14.7±1**	3±0.5	7.4±1.3**	5.5±1.4**
Sulfate (mg g^{-1})	66.8±1.8	0±0**	87.5±1.2**	94±5.3	2213.3±120.7**	103.3±11.1
JAG						
pH (H_2O)	5.6±0	4.8±0.2*	7.5±0*	5.5±0	6.5±0.1*	5.9±0*
EC (mS cm^{-1})	114.6±16.1	2232±39.2**	269.5±11.6**	156.4±1.6	601.4±30*	105.6±6.5*
TOC (µg g^{-1})	NA	NA	NA	37.6±1.2	80.3±1.9**	57±5**
IC (µg g^{-1})	NA	NA	NA	0.8±0.2	1.8±0.2**	1.1±0**
Ammonium (mg g^{-1})	9.1±2.8	8.7±0.5	23.1±1.4**	3.5±0.1	3.5±0.3	3.1±0.3
Nitrate (mg g^{-1})	80.1±26.4	70.6±2	150.5±5.1*	106±2.4	108.9±13.8	47.8±10.7**
Nitrite (mg g^{-1})	2.7±0.8	0±0**	7.4±1.7*	1.2±0.6	2.2±1.2	2.2±1.1
Sulfate (mg g^{-1})	24.1±7.5	0±0**	49.8±1.3*	50.9±51.1	1382.2±170.8**	31.4±6.9
RAG						
pH (H_2O)	6.1±0	5.4±0*	7.9±0*	6±0	7.2±0.1*	6.3±0.1*
EC (mS cm^{-1})	100.2±4.8	1735.2±136.5*	207.8±8.8*	83.6±5.3	525.4±27.2*	78.2±6
TOC (µg g^{-1})	128.9±10.6	64.8±1.7**	272.4±11.3**	120.4±6.3	207.2±119.9	187.9±8.5*
IC (µg g^{-1})	1.7±0.3	1.7±0.7	36.2±1.4**	1.2±0.1	5.2±4.8	1.9±0.4*
Ammonium (mg g^{-1})	5±0.2	4.8±0.5	5.5±0.3*	9.2±2.5	5.8±0.6*	6.6±2
Nitrate (mg g^{-1})	3.2±2.9	12.6±2.2**	51.3±3.2**	8.4±1.9	17.5±1.1**	0±0**
Nitrite (mg g^{-1})	0.2±0.2	0±0	9±0.5**	0±0	1.9±0.8**	0.7±0.7
Sulfate (mg g^{-1})	145.4±19.6	37.7±75.4**	176.7±8*	128.1±15	2135±44.8**	148.2±14.7
KAG						
pH (H_2O)	5.4±0.1	4.6±0*	7.5±0.1*	5.3±0	6.7±0*	5.7±0*
EC (mS cm^{-1})	80.2±4.6	2223.4±215**	291.4±31.8**	130.6±7.1	578.8±36.6**	91.8±11.1**
TOC (µg g^{-1})	NA	NA	485±58.7	41.6±9.8	177.8±9.4**	148.4±18.7**
IC (µg g^{-1})	NA	NA	73.3±4	0.8±0.1	2.9±0.3**	1.1±0.2*
Ammonium (mg g^{-1})	20.1±0.8	25±7.2	73.4±2.3**	15.8±6.3	11.1±0.9	12.1±1.8
Nitrate (mg g^{-1})	71.7±5.2	90.5±6.2**	228.7±25.1**	161.1±7.4	152.7±6.5	23.3±11.8**
Nitrite (mg g^{-1})	3.1±0.3	0±0**	19.1±3.6**	1.1±0.6	3.3±0.2*	2.2±0.7
Sulfate (mg g^{-1})	51.4±4.7	484.8±263.1**	129.4±20.8**	39.1±2.2	181.5±13.6**	54.8±5.2
KAF						
pH (H_2O)	4.9±0.1	4.2±0*	7.5±0*	5±0.1	6.3±0*	5.5±0.1*
EC (mS cm^{-1})	50±4.1	2152±24.8**	317.6±10*	69.4±4.1	462.6±11.2*	49.4±11.6
TOC (µg g^{-1})	236.4±63.8	72.7±30.2*	372.6±108.4	51.9±3	164.7±5.2**	172.8±30.4**
IC (µg g^{-1})	3.9±1.2	1.4±0.2*	53.6±14.6**	1.2±0.1	1.6±0.2**	1±0.1
Ammonium (mg g^{-1})	15.9±1.4	25.6±6.9**	49.1±6.3**	7.6±0.6	8.4±0.4	10.1±4.4
Nitrate (mg g^{-1})	30.3±2.6	64.2±5.1**	332.9±30.7**	80.4±9	89.5±8.5	1.5±0.5**
Nitrite (mg g^{-1})	0±0	0±0	20.3±6**	0.6±0.5	2.5±0.8*	3.2±1.8
Sulfate (mg g^{-1})	17.7±3.6	2441.3±4882.5	83.7±8.9**	44.8±17.1	2773.3±214.9**	74.1±34.8
KFR						
pH (H_2O)	3.7±0	3.1±0.1*	7.5±0*	3.5±0.1	5.8±0**	4.3±0.1**
EC (mS cm^{-1})	117±2.5	2214±44.1*	230.4±5.5*	138.6±9	525.8±33.6**	66.6±5**
TOC (µg g^{-1})	147.5±6.9	105.6±7.2**	594.4±18.2**	243.7±10.3	957±59.9**	836.8±55.9**
IC (µg g^{-1})	2.3±0.2	1.5±0.3**	55.7±1.2**	2.8±0.5	4.9±0.6**	2.5±0.5
Ammonium (mg g^{-1})	20.8±4.3	128.2±14.6**	270.2±18.5**	18.6±2.2	14.9±4.3	10.5±2.3**
Nitrate (mg g^{-1})	20.8±2.2	8.6±4**	13±2.3**	34.6±5.9	35.8±3.5	0±0**
Nitrite (mg g^{-1})	0±0	0±0	16.2±0.6**	0±0	0±0	0±0
Sulfate (mg g^{-1})	9.8±1.5	554.8±1109.7	45.5±3.2**	4.7±3.6	2601±229.2**	25.1±5.7**

Microbial community analysis

To determine the structure of the soil microbial community, each soil was sampled in triplicate after homogenization and those samples were flash frozen with liquid nitrogen and stored in –80°C deep freezer until DNA extraction. The sample DNA was extracted and purified according to applied protocol based on (Yeates *et al.* 1998, Griffiths *et al.* 2000), with a minor modification shown in (Siljanen *et al.* 2019). From the purified DNA, we amplified 16S rRNA *gene* with conventional PCR (MJ Research PTC-200 PCR DNA Engine Thermal Cycler w/ Dual 48-Well Alpha Block) using 515F (GTGYCAG-CMGCCGCGGTAA, (Parada *et al.* 2016) and GACTACHVGGGTATCTAATCC, (Herlemann *et al.* 2011) primers and Maxima SYBR Green Master Mix (Bio-rad). The primers used were Illumina sequencing adapter linked. Temperature program for amplification was: initial denaturation 3 min at 95°C, followed by 25 cycles of amplification (denaturation 25 sec. at 94°C, primer annealing 30 sec at 57°C, and extension at 72°C for 1 min.). Amplified PCR products were sequenced in University of Vienna sequencing service with Illumina MiSeq equipment, paired end 250bp (PE250) method. The sequencing data was analyzed using DADA2 pipeline in R (Callahan *et al.* 2016). The output 16S rRNA *gene* sequences were aligned to Silva Project's database version 138.1. The sequencing data is available in SRA-NCBI database under the project code: PRJNA994037.

Statistical analysis

All statistical tests were made with R statistical program version 3.4.4 (R Core Team 2018). Prior to statistical analyses, data were tested for normal distribution using histograms as well as density and QQ-plots coupled with the Shapiro-Wilk normality test. To test correlation between environmental and microbial variables, and nitrification rates, we applied the Two-Way ANOVA, linear regression model and the non-parametric Spearman's correlation test. The effect of soil amendment type was determined with the student *t*-test and pairwise comparisons with Tukey HSD and for the samples which did not fill ANOVA testing predictions, the non-parametric Kruskall-Wallis test was used. The community structure of microbes in the sites were compared with the non-dimensional scaling (NMDS) ordination and environmental parameters were studied with ENVFIT function in the R package vegan based on the physical and chemical variable measurements of the control treatment replicates of each soil (Oksanen *et al.* 2018).

Results

Soil properties after incubation with amendments

All the tested soil amendments significantly impacted different physical and chemical soil properties.

Gypsum significantly reduced pH and increased EC in all soils (Table 2). It also increased NO_3^- concentration in three out of four agricultural soils (UAG, RAG, KAG) and KAF (Table 2). It reduced TOC and IC in all three of the soils that the data was available for (RAG, KAF, KFR), while having no effect on NH_4^+ or NO_3^- concentration in the agricultural soils, but reducing NH_4^+ concentration in KAF and KFR, and NO_3^- concentration in KFR. Gypsum also reduced NO_2^- concentration in three out of four agricultural soils (UAG, JAG, KAG).

Calcium carbonate significantly increased pH, EC and NO_2^- concentration in all studied soils**.** It reduced TOC in one agricultural soil (RAG) and the forested site soil (KFR). It reduced IC in one of the two agricultural soils that the data was available for (RAG), and the afforested (KAF) and forest site soil (KFR). Furthermore, it reduced NH_4^+ concentration in all soils except one of the agricultural soils (UAG) and NO_3^- concentration in all soils except the forest site soil (KFR)

Foundry sand significantly increased pH, EC, and SO_4^{2-} concentration in all soils, and TOC, and IC in all soils except one of the agricultural soils (RAG). It increased NO_3^- concentration in one of the agricultural soils (RAG) and, NO_2^- concentration in three out of four agricultural soils (UAG,

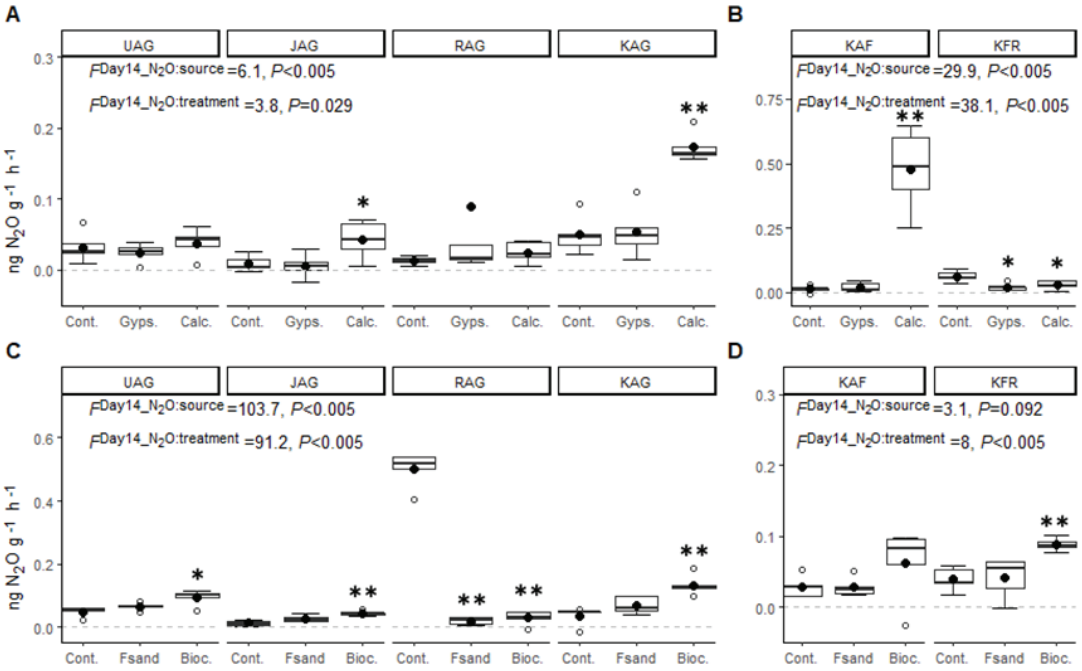

Fig. 1. N_2O production on day 14 of the experiment (ng N_2O g^{-1} h^{-1} ± standard deviation, n = 5). Significance levels: '**': p <= 0.01, '*':p <= 0.05. Significance levels given as difference to respective controls. (**a**) Incubation group 1 agricultural soils. (**b**) Incubation group 1 afforested and forest soils. (**c**) Incubation group 2 agricultural soils. (**d**) Incubation group 2 afforested and forest soils. Note the different scale in y-axis in different groups. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls.

RAG, KAG) as well as the afforested site soil (KAF). Foundry sand also significantly reduced ammonium concentration in one of the four agricultural soils (RAG).

Biochar significantly increased pH in all soils, TOC in three out of four agricultural soils (RAG, JAG, KAG) and the forest site soil (KFR). It increased IC in three out of four, (RAG, JAG, KAG), and NO_3^- concentration in one of the four agricultural soils (UAG). Biochar significantly reduced EC in three out of four agricultural soils (UAG, JAG, KAG) and the forest site soil (KFR). It also reduced NO_3^- concentration in all soils.

Greenhouse gas production results

N_2O

Calcium carbonate increased N_2O production in two of the agricultural soils (Fig. 1a) and the afforested soil (Fig. 1b), while decreasing it in forest soil (Fig. 1b). Gypsum also decreased N_2O production in the forest soil. Biochar significantly increased N_2O production in three of the four agricultural soils, as well as the forest soil (Fig. 1c, 1d). The control samples for RAG exhibited anomalously large N_2O production rate when compared to all the other agricultural control soils, so the effect of biochar and foundry sand addition in the RAG samples could not be determined with statistical certainty.

CO_2

Calcium carbonate increased the CO_2 production rate in all six soils (Fig. 2a, 2b), while foundry sand also increased it in three of the agricultural soils, as well as the forest soil (Fig. 2c, 2d). Gypsum decreased CO_2 production rate in all agricultural soils, and KAF, but increased it in KFR (Fig. 2a, 2b). Biochar decreased CO_2 production rate in all soils except RAG (Fig. 2c, 2d).

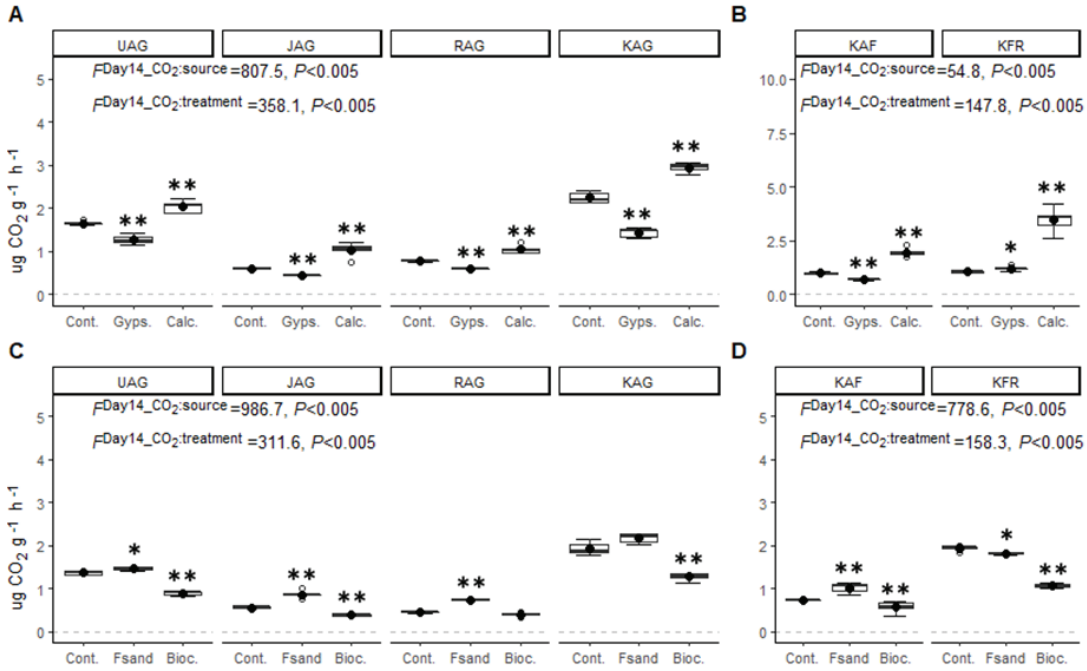

Fig. 2. CO_2 production on day 14 of the experiment (ng CO_2 g^{-1} h^{-1} ± standard deviation, n = 5). Significance levels: '**': p <= 0.01, '*':p <= 0.05. Significance levels given as difference to respective controls. (**a**) Incubation group 1 agricultural soils. (**b**) Incubation group 1 afforested and forest soils. (**c**) Incubation group 2 agricultural soils. (**d**) Incubation group 2 afforested and forest soils. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls.

CH_4

In general, all the control soils were a net sink for CH_4. Calcium carbonate reduced uptake of CH_4 in two of the agricultural soils, and both KAF and KFR (Fig. 3a, 3b), enough to turn KAG, KAF and KFR into net sources of CH_4 instead. Gypsum had similar but lesser effect, reducing uptake in UAG, KAF and KFR, but not enough to turn them into net sources of CH_4. Foundry sand increased CH_4 uptake in RAG, but the effect was not noticeable in any of the other soils (Fig. 3c). Biochar reduced uptake in UAG without turning it into net source but increased it in RAG (Fig. 3c).

Soil Nitrogen Dynamics

Net nitrification rate

Calcium carbonate significantly increased the net nitrification rate in all soils (Fig. 4a, 4b). Foundry sand increased net nitrification rate in three of the agricultural soils as well as the afforested site soil (Fig. 4c, 4d). Biochar increased net nitrification rate on one soil (Fig. 4c). Gypsum reduced net nitrification rate in three of the four agricultural soils (Fig. 4a).

Net N mineralization rate

The addition of $CaCO_3$ increased net N mineralization rate the most out of the tested amendments. It significantly increased the net N mineralization rate in all agricultural soils as well as the afforested site soil (Fig. 5a, 5b). However, it decreased the net N mineralization rate in the forest site soil (Fig. 5d). Gypsum had a similar but weaker effect, and the effect was significant in fewer soils (Fig. 5a, 5b). Biochar significantly reduced the net N mineralization rate in all soils, turning the rate negative (Fig. 5c, 5d).

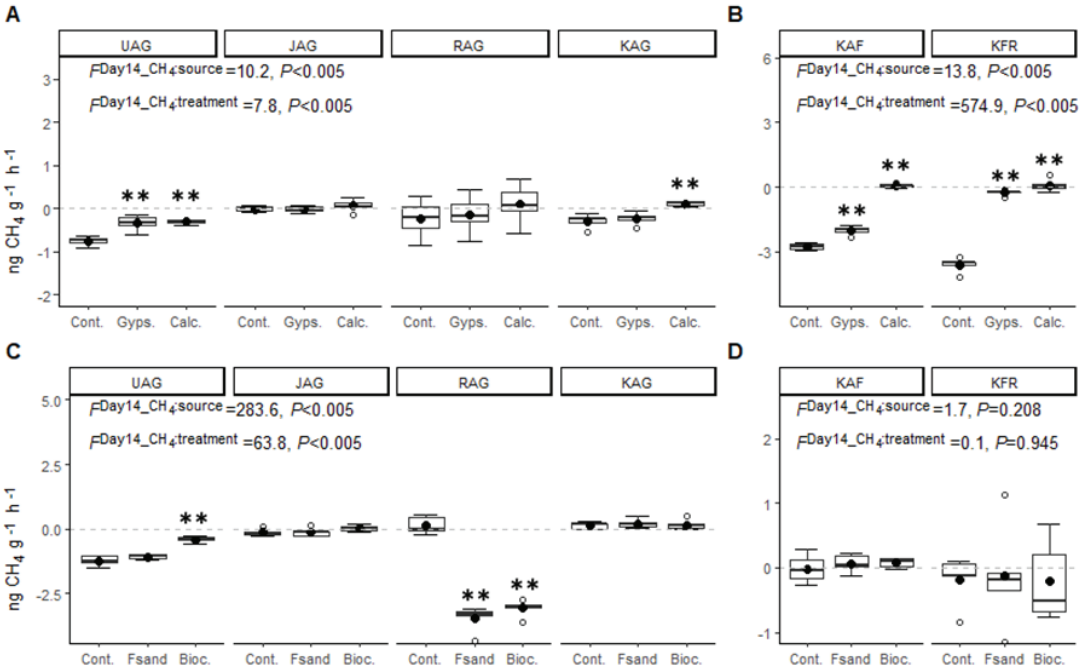

Fig. 3. CH_4 production on day 14 of the experiment (ng CH_4 g^{-1} h^{-1} ± standard deviation, n = 5). Significance levels: '**': p <= 0.01, '*':p <= 0.05. Significance levels given as difference to respective controls. (**a**) Incubation group 1 agricultural soils. (**b**) Incubation group 1 afforested and forest soils. (**c**) Incubation group 2 agricultural soils. (**d**) Incubation group 2 afforested and forest soils. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls.

Microbial community structure

Correspondence analysis

The microbial community structure was analyzed with canonical correspondence analysis; it showed that agricultural sites and non-agricultural sites are different (Fig. 6). Community structure in afforested site (KAF) is closer to most of the agricultural sites (RAG, JAG and KAG), than to the forest site (KFR). Three of the agricultural sites had structures very similar to each other, with UAG being further away from the other agricultural sites.

Environmental factor analysis, with enfit analysis, shows that day 14 N_2O production was diametrically opposed with higher pH and sulfate concentration, and most aligned with higher ammonium concentration, high soil organic matter content, high gravimetric moisture percentage, and high maximum WHC, as well as the community structure in the forest site soil (KFR). CH_4 production had the most noticeable alignment with the community structure in afforested (KAF) and forest site (KFR) soils. Production of CO_2 on day 14 was loosely aligned with high soil organic matter content and high gravimetric moisture percentage.

Community structure in the UAG site was the most aligned with higher net nitrification rate, NO_2^-, NO_3^-, as well as EC. Community structure in the other three agricultural sites (RAG, KAG, JAG) preferred higher pH and were more aligned with higher net N mineralization rate.

Relative abundance of microbial phyla

The 16S rRNA gene sequencing results show that KFR site samples had the highest relative abundance of *Archaeal* 16S sequences out of all sites (12.3%), while second highest site KAF had 1.8%. Relative abundance of

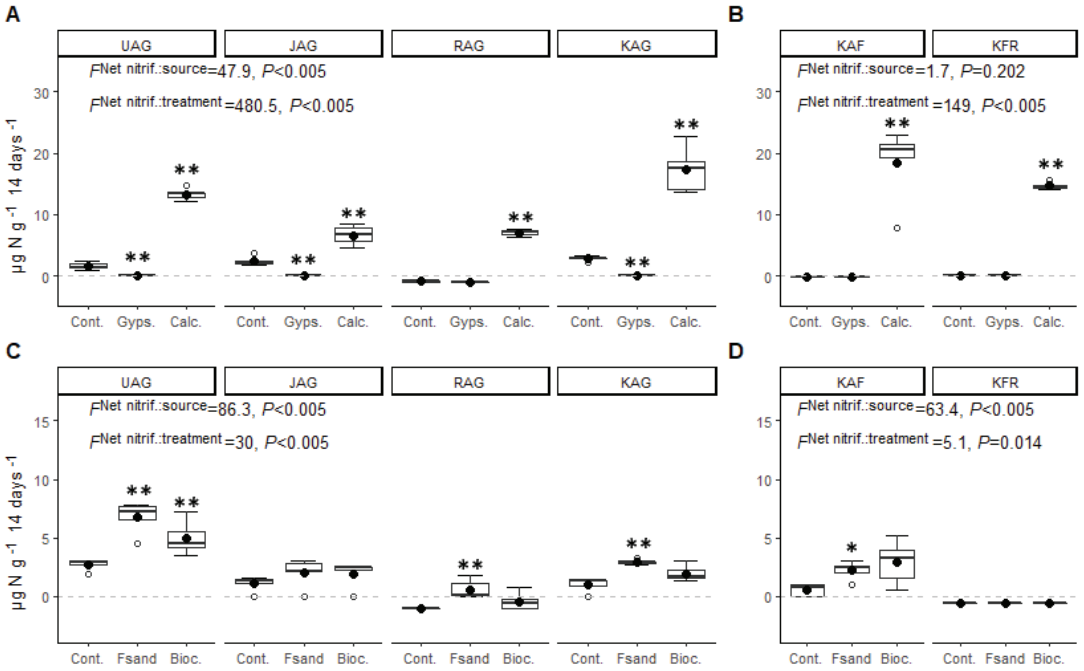

Fig. 4. Net nitrification rate during the 14-day experiment (μg N g^{-1} 14 days^{-1} ± standard deviation, n = 5). Significance levels: '**': p <= 0.01, '*':p <= 0.05. Significance levels given as difference to respective controls. (**a**) Incubation group 1 agricultural soils. (**b**) Incubation group 1 afforested and forest soils. (**c**) Incubation group 2 agricultural soils. (**d**) Incubation group 2 afforested and forest soils. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls.

sequences belonging to Archaea in agricultural sites were 0.9% (UAG), 0.9% (RAG), 1.2% (JAG), and 1.2% (KAG). 96.3% of all archaeal 16S sequences found in the samples belonged to the *Crenarchaeota* phylum, and the species level analysis showed that these microbes are mostly *Thaumarchaeota*, which are known nitrifying archaea.

Another notable difference between the sites was the larger relative abundance of the phylum *Firmicutes* in all three of the Kannus sites (2.3–4.0%) compared to rest of the agricultural sites (0.7–1.0%). UAG also had a larger relative abundance of the phylum *Nitrospirota* at 1.5%, compared to the 0.2–0.3% at the other agricultural sites. The relative abundance of *Nitrospirota* in KAF was 0.2%, and they were completely absent at 0 % in KFR.

The percentage of sequences that were filtered out for each site to preserve the readability for the graph (Fig.7) were 3.4% (UAG), 4.3% (JAG), 5.1% (RAG), 3.3% (KAG), 4.4% (KAF) and 3.3% (KFR).

Microbial population structure and GHG production rate correlation

The correlation analysis between GHG production rates and the original soil microbial community structure showed that changes caused by different soil amendments correlated with different microbial phyla. Based on these correlations, biochar and foundry sand caused similar changes in GHG production rates in different microbial communities, as did gypsum and $CaCO_3$, with the exception of N_2O production rate (Fig. S1 in Supplementary Information). Gypsum and $CaCO_3$ did not share any statistically significant correlations of N_2O with the relative abundance of different phyla (Fig. S1 in Supplementary Information).

Decreased N_2O production had strongest negative correlation with Crenarchaeota abundance. Gypsum amendment also caused the most significant reduction in N_2O production rate in the forest site soil (KFR) (Fig. 1b), in which the relative abundance of Crenarchaota was the highest (Fig. 7).

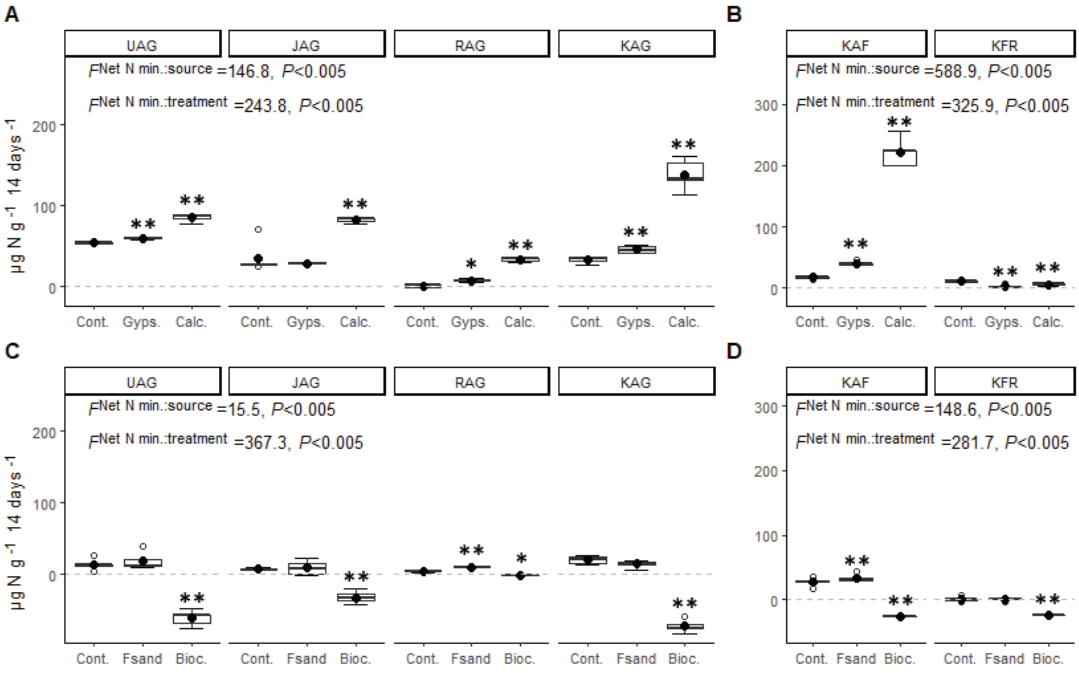

Fig. 5. Net N mineralization rate during the 14-day experiment (μg N g^{-1} 14 days^{-1} ± standard deviation, $n = 5$). Significance levels: '**': $p <= 0.01$, '*': $p <= 0.05$. Significance levels given as difference to respective controls. (**a**) Incubation group 1 agricultural soils. (**b**) Incubation group 1 afforested and forest soils. (**c**) Incubation group 2 agricultural soils. (**d**) Incubation group 2 afforested and forest soils. Significance levels shown with asterisks represent the comparison between amendment treatment means to incubation group controls.

Discussion

Soil amendments are mainly used for increasing the harvest yield, but some, like gypsum, are already being used for reducing the environmental impact of agriculture. Our results show that different amendments do indeed have different effects on the GHG production rates in the tested agricultural soils. The effect of different soil amendments on the GHG production rates should therefore be considered when multiple choices exist for any given agricultural peat soil.

Soils treated with $CaCO_3$ significantly increased N_2O production rate in two out of the four agricultural soils, and the afforested site soil, while reducing it in the forest site soil (Fig.1a, 1b). The reason for why this change can be seen only in some of the soils is not immediately clear, as the correlation between pH and N_2O production rate was not statistically significant (Table S2 in Supplementary Information). $CaCO_3$ treated soils also had the highest day 14 CO_2 production rate out of all of the soils, but we suspect that a significant portion of this $CaCO_3$ induced CO_2 production is the result of carbonate formation when the $CaCO_3$ reacts with soil water (Fig. 2a, 2b.) (Biasi *et al.* 2008). In the agricultural soils, CO_2 production had strong positive correlation with pH, TOC and IC concentrations, as well as the concentrations of all forms of nitrogen (Table S2 in Supplementary Information). Based on our findings we cannot say how large portion of the observed CO_2 production in the $CaCO_3$ treated replicates was caused by chemical reactions instead of microbial activity, as we did not use isotopes to separate the sources during the experiment, but previous literature suggests that $CaCO_3$ containing liming agents produce approximately 12% of cumulative CO_2 in the field, and up to 50% of CO2 emissions in laboratory experiments (Biasi *et al.* 2008).

Gypsum amendment significantly reduced CO_2 production in all of the agricultural soils,

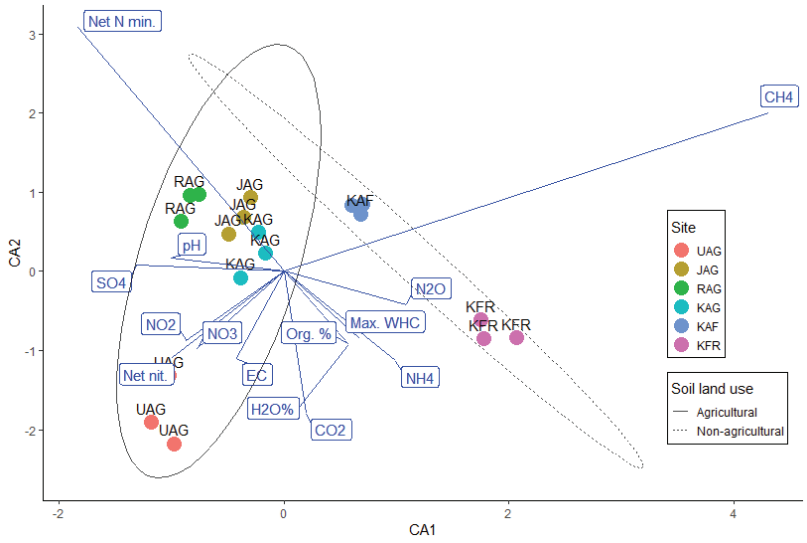

Fig. 6. Correspondence analysis (CA) of the community structure based on 16S rRNA-gene sequencing, overlayed with vectors (blue) from environmental factor analysis (Envfit, R Vegan package). Ellipses represent a 95% confidence level for multivariate *t*-distribution based on the microbial community structure CA. Only statistically significant factors ($p < 0.05$) are shown in the graph. Net N min.; Net N mineralization rate, CH_4; day 14 methane production, pH; soil pH in H_2O slurry, SO_4; sulfate concentration, NO_2; nitrite concentration, NO_3; nitrate concentration, Net nit.; net nitrification rate, EC; electrical conductivity, Org. %; soil organic matter percentage, Max. WHC; maximum water holding capacity, H_2O%; gravimetric moisture percentage, CO_2; day 14 carbon dioxide production, NH_4; ammonium concentration, N_2O; day 14 nitrous oxide production.

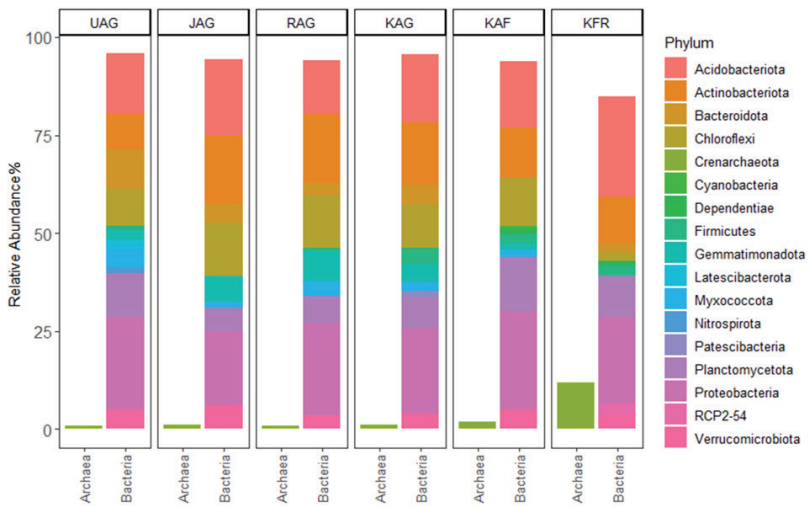

Fig. 7. Relative abundance percentage of microbial phyla in the studied soils. Site-specific relative abundances are calculated as averages of three replicates ($n = 3$). Phyla with relative abundance of less than 0.75% in all the six soils were filtered out, and archaeal and bacterial phyla were organized into separate columns, to preserve readability of the chart.

and net nitrification rate in three of the four agricultural soils (Fig. 4a). It increased net N mineralization rate in three of the four agricultural soils, but it didn't significantly increase N_2O production, which is interesting as might expect that increasing the amount of microbe-available NO_{2-} and NO_{3-} in the soil could lead to increased N_2O production if the denitrifying microbe activity is limited by nitrogen availability. Reduction in CO_2 production could be due to the fact that gypsum was the only tested amendment that decreased pH in all tested soils (Table 2). The sulphate (SO_4^{2-}) in gypsum could also be inhibiting microbial activity on its own via increased hydrogen sulphide (H_2S) production by SO_4^{2-} reducing microbes, but in our experiment, the SO_4^{2-} concentration did not have a statistically significant correlation with either N_2O production or net nitrification rate in the agricultural soils (Table S2 in Supplementary Information) (Lackner *et al.* 2020). It had a significant negative correlation with CH_4 production (Table S2 in Supplementary Information). It is to be noted, however, that the correlations were calculated from the concentrations in the soil before any amendments were added, and the excessive SO_4^{2-} from the gypsum treatment could have a drastically different effect from how the ambient SO_4^{2-} concentration in the soils correlates with GHG production rates.

Foundry sand amendment produced the strongest increase in pH after $CaCO_3$ out of all of the tested amendments (Table 2). It had similar, but not as profound, CO_2 production increasing effect as $CaCO_3$ (Figure 2c, 2d). It also increased net nitrification rate in a manner similar to $CaCO_3$. The production of CO_2 was positively correlated with higher pH, so this effect could at least partially be through increase in microbial activity (Table S2 in Supplementary Information) since foundry sand lacked the same property of forming CO_2 through purely chemical processes that $CaCO_3$ has.

Biochar amendment caused the most significant and consistent increase on N_2O production in agricultural soils out of the tested amendments (Fig. 1c, 1d). This was unexpected, as earlier studies have shown biochar amendment to decrease N_2O emissions (Kulmala *et al.* 2022). In our study, N_2O production rate did not have statistically significant correlation with any of the other variables in the day 14 soils, which makes it hard to determine the exact cause of the increased N_2O production (Table S2). Biochar addition did not increase the CO_2 production like it did for N_2O production, despite having very high organic carbon content and increasing pH in all soils. Counter to predictions it instead reduced CO_2 production (Table 2; Fig. 2c, 2d). One possible explanation could be that biochar amendment caused a shift in microbial activity towards more anaerobic processes that produce more N_2O rather than CO_2. The results of our experiment suggest that biochar has the capacity to absorb nitrogen compounds, as it reduced the net N mineralization rate to below zero in all soils, while having no significant effect on net nitrification in the majority of the soils. In contrast, both CaCO3 and foundry sand increased the net nitrification rate (Fig. 4a, 4b). Previous studies have not been conclusive on what exactly the effect of biochar addition to soil is, but because in our experiment we saw unexpected increase in N_2O production rates in the biochar treatment when compared to the control, we think that soils respond to it differently between *in vitro* and field experiments. In the field the presence of plants significantly changes the soil nitrogen dynamics when compared to a bottle incubation with only soil, possibly causing more intense competition for nitrogen compounds between plants and microbes, leading to reduced N_2O production. (Kalu *et al.* 2021, Kulmala *et al.* 2022). Previous experiments have also suggested that biochar acts as a growth surface for N_2O-reducing microbes leading to reduced N_2O release from the soil in a 56-day incubation (Liao *et al.* 2021).

Microbial community structure was significantly different between the agricultural soils, and the afforested and forest soils. Correspondence analysis (CA) showed that all agricultural soils were very similar to each other when compared to the non-agricultural soils (Fig. 6). The afforested peat field soil (KAF) that had previously been used as agricultural field had a microbial community structure that clearly fell in between the agricultural soils and the forest soil (Fig. 6). This difference was also visible in the relative abundance of different microbial

phyla in each soil. The three most acidic soils, KFR (pH 3.5), KAF (pH 4.8) and KAG (pH 5.1) had the highest relative abundance of sequences belonging to phylum *Crenarchaeota*, a phylum known to contain AOA, at 12.3%, 1.8% and 1.2%, respectively (Table 1; Fig. 7). This finding is in line with previous studies that have shown that AOA are more significant producers (direct and indirect) of N_2O than AOB in peatland soils with low pH and no inorganic nitrogen fertilization (Hink *et al.* 2017, Siljanen *et al.* 2019, Prosser *et al.* 2020). Another notable difference for nitrogen cycling microbes is the complete absence of sequences belonging to the phylum *Nitrospirota* in the forest soil (KFR), while UAG had the highest relative abundance at 1.5%. Highest relative abundance of *Nitrospirota* could explain why net nitrification rate was most aligned with RAG soil microbial community structure, and least aligned with KFR, in the CA analysis (Fig. 6). The fact that correlations between GHG production and soil chemical properties differ between the agricultural soils and the afforested and forest site soils seems to also suggest that the structure of the soil microbial community participates in determining how the GHG emissions from soil will react to different soil amendments (Table S2 in Supplementary Information).

The disparity between the correlations of different variables in the microbial community CA analysis (Fig. 6), and the supplementary correlation matrix (Table S2 in Supplementary Information), is most likely caused by the different sampling times for the physical and chemical properties of the soil. Variables for the CA analysis were measured on day 0 of the experiment from the sieved soil on the same day that the samples for 16s rRNA sequencing were collected, while the variables for the correlation matrix were measured on day 14 of the experiment from the control soils used in the incubation. When comparing the correlations between the GHG production rates from different amendment treatments and the relative abundance of different microbial phyla in the original soils, however, the amendments can be roughly divided in two groups; the gypsum and $CaCO_3$ group, and biochar and foundry sand group (Fig. S1 in Supplementary Information). We suspect this division could be explained by the fact that both biochar and foundry sand amendments alter the physical soil structure more than gypsum and $CaCO_3$ due to the volume of added amendment material, and this leads to different moisture and oxygen conditions in the soil, which in turn can affect both the CO_2 and CH_4 production rates. Nitrous oxide production in gypsum treated soils also had very strong statistically significant negative correlation with relative abundance of the *Crenarchaeota* phylum, suggesting that in soils with high relative abundance of *Crenarchaeota*, gypsum amendment could be inhibiting ammonia-oxidizing microbes, leading to lower N_2O production rates. The effect of gypsum on ammonia-oxidizing microbes has been previously studied, and it has been shown to inhibit nitrification and ammonia-oxidizing bacteria, but not archaea (Liao *et al.* 2021).

Gypsum and biochar amendments both were promising for reducing CO_2 production, but the use of gypsum as an amendment is problematic in other than coastal areas due to its high concentration of sulfate. The role of biochar in increasing N_2O production and reducing net N mineralization rate also warrant further study, as our results conflict with previous studies that have shown it to reduce N_2O production in experiments (Lehmann *et al.* 2011, Kulmala *et al.* 2022). We do recognize that studying the effect of different amendments in a bottle incubation experiment is different that studying them in the field, and that intact site typical vegetation can significantly alter the nitrogen dynamics, as plants are major users of nitrogen compounds. If the biochar is really absorbing nitrogen compounds, it could be that the nitrogen inside the biochar is more available to microbes than it is to vegetation. Soil amendments directly or indirectly change the availability of organic and inorganic carbon and affect the aeration conditions in the soil with their physical structure. In the case of biochar, they can also physically sequester nitrogen compounds and affect how much of them are available for microbial and plant activity. We do however believe that our results provide a reliable view of how the tested soil amendments interact with soil microbes alone.

Conclusively, while biochar and gypsum showed potential for reducing CO_2 production from agricultural peatland soil in this experiment, the unexpected effect of biochar on the N_2O production rates warrants further study. We also suggest that the effect of the tested soil amendments is not limited to just changing the soil pH. Based on our findings in this experiment, we plan to further study the effects of calcium carbonate, gypsum, and biochar in a follow up experiment, with longer incubation period. Further study is also needed to help us better understand how these amendments affect the structure and activity of the soil microbial community.

We also conclude that the structure of the soil microbial community has a significant effect on how the GHG emissions from soil respond to soil amendment addition, as the community structure dictates which microbial processes are present and active in the soil to take advantage of the increased availability of nitrogen compounds in the soil after amendment addition.

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Supplementary Information: The supplementary information related to this article is available online at: http://www.borenv.net/BER/archive/pdfs/ber30/ber30-021-037-supplement.pdf

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