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Factors limiting microbial N_2O and CO_2 production in a cultivated peatland overlying an acid sulphate subsoil derived from black schist

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ABSTRACT

Factors limiting the production of the greenhouse gases nitrous oxide (N_2O) and carbon dioxide (CO_2) were investigated in three incubation experiments conducted with soil from top- and subsoil horizons of a peatland which had an acid sulphate mineral subsoil derived from black schists. The effect of moisture was investigated by equilibrating undisturbed soil samples from three horizons (H2, Cg and Cr) at −10, −60 or −100 cm matric potential and measuring the gas production. In the second experiment, the effects of temperature and various substrates were studied by incubating disturbed soil samples in aerobic conditions at 5 or 20 °C, and measuring basal respiration and N_2O production before and after adding water, glucose or ammonium into the soil. In the third experiment, the effects of added glucose and/or nitrate on the denitrification in soil samples from four horizons (H1, H2, Cg and Cr were investigated by acetylene inhibition and monitoring of N_2O production during a 48-h anaerobic incubation. The production of CO_2 in the topmost peat horizon was largest at −10 cm matric potential, and it was larger than those in the mineral subsoil also at −60 and −100 cm potentials. In contrast, drainage seemed to increase N_2O production, whereas in the wettest condition the production of N_2O in the mineral subsoil was small and the peat horizon was a sink of N_2O. Lowering of temperature (from 20 °C to 5 °C) decreased CO_2 production, as expected, but it had almost no role in the production of N_2O in aerobic conditions. Glucose addition increased the aerobic production of CO_2 in peat, but it had a minor effect in the mineral horizons. Lack of C source (glucose) was limiting anaerobic N_2O production in the uppermost peat horizon, while in all other horizons, nitrate proved to be the most limiting factor. It is concluded that peatlands with black schist derived acid sulphate subsoil horizons, such as in this study, have high microbial activity in the peaty topsoil horizons but little microbial activity in the mineral subsoil. These findings are contrary to previous results obtained in sediment-derived acid sulphate soils.

1. Introduction

The main soil processes producing nitrous oxide (N_2O) are denitrification and nitrification (Davidson, 2009; Chen et al, 2015), of which denitrification is considered the dominant source (Firestone and Davidson, 1989; Skiba et al., 1993; Bremner, 1997; Saggar et al. 2013). The emission of N_2O from soil results not only in the release of a detrimental greenhouse gas (GHG) to the atmosphere, but also to losses of N from agriculture. Carbon dioxide (CO_2) production in soil is the result of aerobic and anaerobic decomposition and mineralization of organic matter by microbial activity as well as respiration by plant roots (Yiqi and Zhou, 2010). The rate of this microbial activity is regulated not only by the availability of organic matter, but also soil temperature, with increasing temperature promoting activity.

The mechanisms and controlling factors affecting N_2O production by both nitrification and denitrification are widely studied (for example Linn and Doran, 1984; Yokoyama and Ohama, 2005; Davidson, 2009; Chen, et al., 2015), but to quantify the contribution of each is complicated because the involved processes are coupled in soil. Firestone and Davidson (1989) offered a conceptual model, later dubbed the “Hole in the Pipe” or HIP, to explain factors affecting emissions on N_2O and NO. Nitrification and denitrification are symbolised by pipes through which N flows in the soil ecosystem, and the emissions of NO and N_2O are represented by leaking holes in these pipes. Magnitude of the emissions

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(size of the holes) and the N_2O:N_2 product ratio are determined largely by soil water content, but soil acidity, the activity and community composition of soil microbes, and the relative abundance of soil electron donors (decomposable organic matter and NH_4^+) and acceptors (especially O_2, NO_3^- and SO_4^{2-}) as well as diffusivity of gases in soil also play a role (Davidson et al., 2000). The importance of soil water content is based on its effect on soil aeration: the wetter the soil, the less air-filled pore space available for gas exchange between soil air and the atmosphere. Under conditions of impaired gas exchange, microbial activity in soil may deplete any available oxygen and create anoxic micro sites, where denitrification can take place. Soil acidity increases the N_2O product ratio in denitrification due to impaired N_2O reductase expression (Russenes et al., 2016).

Acid sulphate (AS) soils contain sulphidic materials in their waterlogged subsoil. When these materials come into contact with atmospheric oxygen, sulphuric acid is formed producing environmental problems, including acid and metal discharge into waterways worldwide (Dent, 1986; Michael, 2013) and particularly in Finland (Sundström et al., 2002; Virtanen, 2015). In Finland, there are at least 50,000–336,000 ha of these soils in cultivation, mainly in the coastal region of the Gulf of Bothnia, where they were formed from the sediment of the Littorina sea stage of the Baltic Sea and exposed to oxygen through land uplift due to glacial rebound, or through agricultural drainage (Yli-Halla et al., 1999). The occurrence of AS soils in Finland is the highest in Europe (Andriesse and van Mensvoort, 2006). In addition to the sediment-derived coastal AS soils, similar soils occur also in inland areas, where the bedrock contains large amounts of black schist, a sulphide containing rock not uncommon in for example in the regions of North Karelia, Kainuu and Oulu regions in Finland. Elevated metal concentrations in the soils on black schist areas were reported by Nystrand et al. (2021). In the same study, large amounts of sulphide, As, Pb and Zn in the peat topsoil overlying black schist affected soil, but not sediment derived AS soil, were also discovered, and attributed to capillary rise from the black schist affected soil materials. The peat layer overlying black schist soils also differ from that of sediment derived AS soils by being more acidic, especially if oxidized (Nystrand et al., 2021). Similarly to sediment derived AS soils, black schist derived AS soils can contain large stocks of mineral nitrogen, typically present as NH_4^+ and concentrated just below the peat layer (Yli-Halla et al., 2017).

AS soils are shown to have large GHG emissions: Denmead et al. (2010), in studying CH_4 and N_2O, detected tenfold emissions (expressed as CO_2 equivalent) compared to those from non- AS soils under sugarcane cultivation in Australia, and unusually high fluxes of N_2O, annually 2–28 kg N ha^{-1}, have been observed in a coastal mineral AS soil in Finland (Uusi-Kämppä et al., 2012; Yli-Halla et al., 2020).

Šimek et. al. (2011, 2014) investigated the chemical properties and microbial activity of a boreal clayey AS soil formed in coastal sediment and discovered abundant stocks of carbon (C) and nitrogen (N), as well as large basal and substrate induced respiration in the Cg horizon. Large N_2O emissions have also been reported (Epie et al., 2015) in the adjacent AS field. This discovery was contrary to a typical non-acid arable soil, where microbial activity was highest near the surface (Šimek et. al., 2011, 2014). Depth profiles of microbial activity in black schist derived AS soils are yet to be established as such information seems absent from current literature. In addition to microbial gas production, AS soils possess a chemical denitrification pathway, described by MacDonald et al. (2010), where the reduced iron of AS soil is oxidised by nitrate and gaseous forms of N are produced.

Peat soils have often reduced sulphidic subsoil horizons that may develop into active AS soils if the conditions turn aerobic by drainage or peat excavation. Reclamation of former peat excavation sites for agriculture may increase the harmful environmental effects by inland AS soils in future. In recent years, increasingly more research has been carried out on the harmful environmental effects of acid discharge to water systems by coastline AS soils (Joukainen and Yli-Halla, 2003; Powell and Martens, 2005), whereas the greenhouse gas emissions and properties of inland AS soils have received less attention. The removal of peat exposes the underlying black schist subsoil not only to atmospheric oxygen but also to higher temperatures and increased temperature variation, the effects of which on the microbial activity is unknown.

At the site of the current study, a former peat excavation site with a black schist derived AS subsoil, Yli-Halla et al. (2017) discovered a large NH_4^+-N stock at 50–70 cm depth, in the upper part of the AS subsoil, but only very moderate cumulative emissions of N_2O compared with typical peat and AS soils, thus identifying a need for more detailed information on the main factors governing the gas production in different soil horizons of black schist derived soils. For this reason, in the current study, the effects of soil moisture (matric potentials −10, −60 or −100 cm w. c.), temperature (5 or 20 °C) and easily available C (glucose) and N source (NH_4^+ or NO_3^-) on aerobic CO_2 and N_2O production as well as total denitrification were investigated in a series of laboratory experiments with soil samples collected from the field site investigated by Yli-Halla et al. (2017). Our aim was to determine, which mechanisms and factors mostly limit the production of CO_2 and particularly N_2O at this site, where conditions seem favourable for large emissions.

2. Materials and methods

2.1. The experimental site and soil sampling

The experimental site, Pärnänsuo field, is a former peat excavation site in Joensuu, Eastern Finland (62° 25′ N, 30° 04′ E, elevation 83 m). After the peat excavation ended in 2000, reed canary grass (*Phalaris arundinacea*) was cultivated in the field until 2010. Since 2011, the site has been in private agricultural use and drained with open ditches (Yli-Halla et al., 2017). During the current study, the field was used for seed production of timothy (*Phleum pratense*). The site has been classified as Typic Sulfosaprist (Yli-Halla et al., 2017). Beneath a varying depth of peat left over from the excavation, the Pärnänsuo subsoil contains black schist, giving it characteristics of an AS soil (Mäkelä et al., 2015; Simojoki and Kabir, 2019). The soil profile consisted of organic H1 and H2 horizons at the approximate depths of 0–10 cm and 10–45 cm, respectively, mineral subsoil Cg horizon at the depth of 45–80 cm underlain by a thick mineral Cr horizon until the depth of at least 2.5 m. Throughout the profile, soil pH is low, with 3.2 in H1, 2.9 in H2, 3.8 in Cg and 5.4 in Cr. The organic C content is 33% and 0.5% in the peat horizons and in the underlying mineral subsoil, respectively.

Soil sampling took place on two separate occasions: the disturbed soil samples were sampled in May 2015, and undisturbed soil cores in September 2015. Within the Pärnänsuo field, both undisturbed and disturbed samples were taken from the GHG emission measurement site 3 (see Yli-Halla et al., 2017 for more details about the site). Four replicate undisturbed soil cores were taken near the gas emission chambers at the site, within the distance of 10 m from each other, into 200-cm^3 steel cylinders. The samples were taken from three soil horizons: the depths of 20–25 cm (with the top of the steel cylinder at 20 cm depth) representing the peat horizon (H2), 60–65 cm representing the upper mineral subsoil (Cg), and 80–85 cm representing the lower mineral subsoil (Cr).

In addition, approximately one litre of structurally disturbed soil (where natural structure of the soil was not preserved) was collected with a large Eijkelkamp Edelman auger (diameter 10 cm) in triplicate from the depths of 0–10 cm, representing upper peat (H1), 15–30 cm representing lower peat (H2), 45–60 cm (Cg) and in duplicate from 90 to 110 cm (Cr). The samples were packed into double plastic bags, and the bags containing lower subsoil samples (Cr) were submerged to prevent oxidation. The samples were stored at 5 °C until the experiments were conducted.

2.2. Experiments

In this study, three different experiments were carried out to

investigate the effects of soil moisture, temperature as well as C and N substrates on the CO_2 and N_2O production of different horizons of the soil profile in oxic and anoxic conditions. Experiment 1 was carried out in oxic conditions using undisturbed soil cores equilibrated at three different matric suctions. Experiment 2 was carried out in oxic conditions using disturbed soil samples incubated at two different temperatures and with different added substrates. Experiment 3 was carried out using disturbed soil samples with different added substrates to study total denitrification by acetylene inhibition method in anoxic conditions. The experiments are described in detail below.

2.2.1. Experiment 1

In Experiment 1, in order to study the effects of moisture status on the oxic CO_2 and N_2O production in soil, 200–cm^3 undisturbed soil cores from three horizons were saturated, and then equilibrated at −10, −60 or −100 cm matric potentials (10, 60 and 100 hPa at 20 °C) on sand beds in triplicate (Fig. 1). These matric potentials corresponded to 97, 79 and 76 % water filled pore space (WFPS) in peat, 87, 85 and 84 % in upper mineral soil and 85, 84 and 82% in deep mineral subsoil. Separate soil samples were used for each matric potential. The gas production rates were determined after approximately 2 weeks, when the soil samples had stabilized at their targeted matric potentials.

2.2.2. Experiment 2

In Experiment 2, temperature sensitivity of the greenhouse gas (GHG) production of soil was studied by incubating disturbed soil samples from four different horizons at 5 and 20 °C temperatures and 60% water filled pore space for the determination of basal and substrate-induced gas production for CO_2 and N_2O (Fig. 2).

Before starting the Experiment 2, dry matter content of soil in each horizon was determined in triplicate. Based on this, fresh soil equivalent to 2.5 g of dry matter of peat and 10 g of dry matter of mineral soil material, respectively, were weighed into 120-ml incubation bottles for each treatment in triplicate. Soil moisture content was adjusted to correspond to 60% of water-filled pore space in order to support maximum aerobic microbial activity (Linn and Doran, 1984). To maintain equal headspace in the incubation bottles, the soil volume was adjusted to 10 ml by pressing it with a clean test tube. The bottles were transferred to their respective incubation temperatures (controlled temperature rooms at +5 °C and +20 °C, in dark) without caps.

Basal oxic rates of CO_2 and N_2O production were determined by measuring the amount of CO_2 and N_2O after a 24 h sampling cycle in closed bottles at 5 or 20 °C three times during the approximately 1-week period after the start of incubation, in order to monitor the stabilization of soil microbial activity before adding the different substrate solutions (Fig. 2). During the first week, however, no temporal trend in the CO_2 production rates (BR, basal respiration) was observed in any horizon and the soil microbial activity was considered stable. Then, solutions containing water (control), glucose or ammonium were added to the respective bottles to study their effects on gas production. Freshly prepared glucose was added as 4 mg C g^{-1} dry soil (Šimek et al., 2011) and ammonium sulphate was added as 0.2 mg N g^{-1} dry soil (corresponding roughly to a N fertilization rate of 100 kg ha^{-1}) in a 1-ml solution with a Finnpipette (Labsystems) into the incubation bottles. Then, moisture was adjusted again by adding required amount of milli-Q water into the incubation bottles. The bottles were flushed with air, sealed with butyl rubber stoppers, and incubated at their respective incubation temperatures for 24 h. Gas samples were collected and analysed by GC in the same way as described for the basal gas production above.

2.2.3. Experiment 3

In Experiment 3, four different experimental solutions were added to disturbed soil samples from different horizons in order to study the effects of C and N substrates on the total denitrification in anoxic conditions at room temperature (Fig. 3), using the acetylene inhibition method commonly used to investigate the microbial community present in the soil at a given moment (Tiedje, 1979; Tiedje 1994, Šimek et al. 2011; Sanchez-Garcia et al. 2016, Malique et al. 2019) and considered a valid method particularly for assessing controlling factors of denitrification in terrestrial ecosystems (Groffman et al., 2006). These experiments typically last only one to two hours (Šimek 2011; Malique et al. 2019), but in the current study, a slighty longer approach of 48 h was selected to better observe the gas production, similarly to Senbayram et al. (2019) and Xu et al. (2019), who continued the incubation even longer, but discovered the N_2O production reaching a peak near 48 h. The treatment solutions included control (milli-Q water), 1 cM glucose, 1 cM KNO_3, and a combination of 1 cM glucose and 1 cM KNO_3. 2.5 g DM for peat soils and 10 g DM of mineral soils were weighed into 120-ml inbucation bottles. Butyl rubber septum caps were used to seal the bottles.

Anoxic conditions in the bottles were created by triple evacuation and flushing of bottles with helium (He) with a customized evacuation apparatus which consisted of one compressor, one pressure meter, He (grade 4.6) gas line with the pressure set to 1.6 bar. After the evacuation, approximately 95 ml of N_2 gas (grade: 5.0a) and 9.5 ml (10% of the headspace volume) of C_2H_2 (grade 2.6) gas was passed to the bottles through the connecting syringe. Both N_2 and C_2H_2 gas cylinder was equipped with stainless steel tubing for connection with incubation bottles to prevent oxygen contamination.

A volume of 25 ml of each given treatment solution (degassed by grade 5.0 N_2 gas) was injected into the respective incubation bottles. Then the bottles were shaken at 150 rpm in the shaker (IKA LABORTECHNIK KS250 basic) during the 48-h determination of total denitrification by acetylene inhibition method, except for the gas samplings at

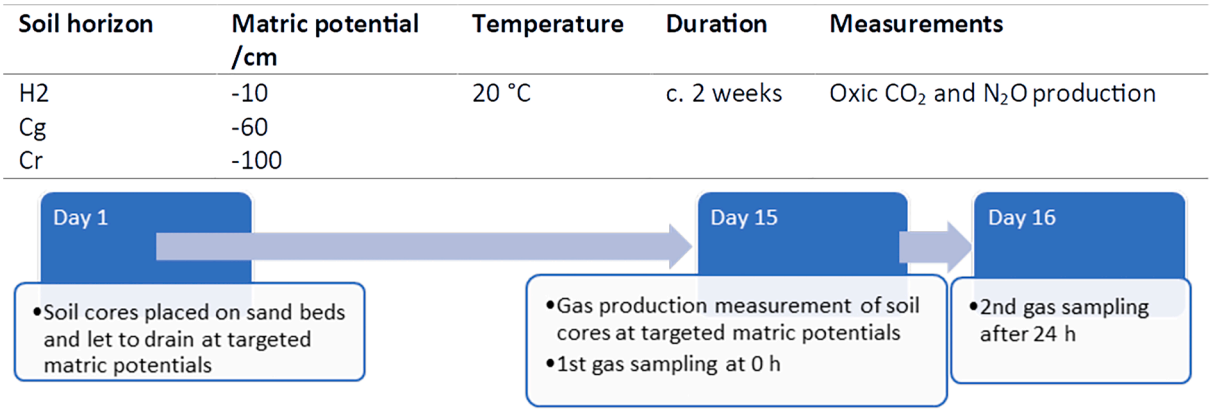

Fig. 1. The experimental setup and timeline of Experiment 1 to study the effects of soil moisture on the oxic production of CO_2 and N_2O in different soil horizons (H2 is Lower Peat, Cg Upper mineral subsoil and Cr Deep mineral subsoil.)

Soil horizon	Temperature °C	Substrate added	Duration	Measurements
H1 H2 Cg Cr	5 20 °C	water glucose ammonium	10 days	Oxic CO_2 and N_2O production (basal) Oxic CO_2 and N_2O production (substrate-induced) Soil mineral nitrogen content

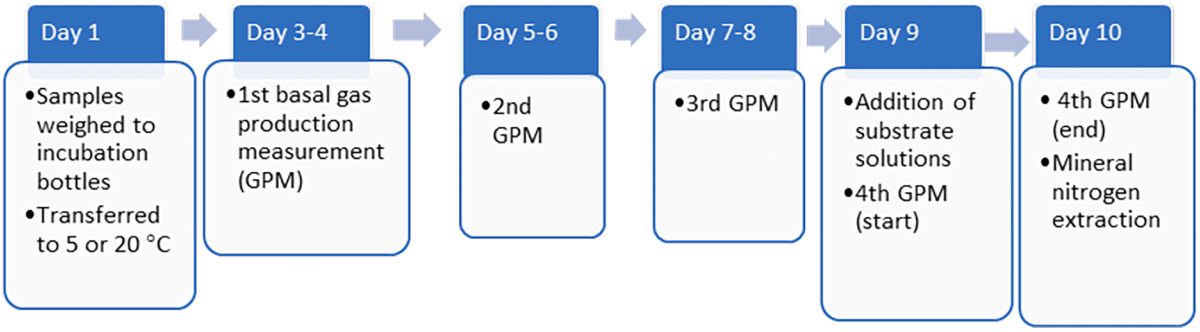

Fig. 2. Experimental setup and timeline of Experiment 2 to study the effects of temperature and added C and N substrates on the oxic production of CO_2 and N_2O in different soil horizons (H1 is Upper peat, H2 Lower Peat, Cg Upper mineral subsoil and Cr Deep mineral subsoil.).

Soil horizon	Substrate	Temperature	Duration	Measurements
H1 H2 Cg Cr	water glucose nitrate nitrate + glucose	room temperature (20 °C)	3 days	Anoxic denitrification rate by AIM Anoxic CO_2 production during AIM (not shown) Soil mineral nitrogen content

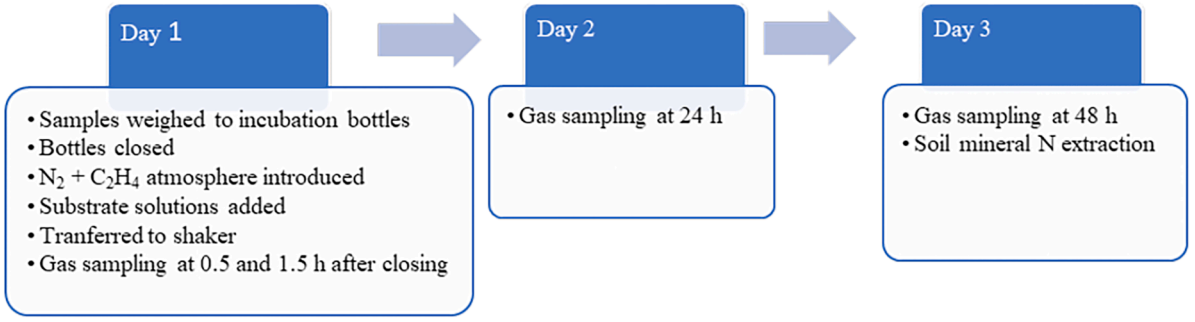

Fig. 3. Experimental setup and timeline of Experiment 3 to study the effects of added C and N substrates on the total denitrification rate in different soil horizons by acetylene-inhibition method (AIM) in anoxic conditions (H1 is Upper peat, H2 Lower Peat, Cg Upper mineral subsoil and Cr Deep mineral subsoil.)

0.5, 1.5, 24 and 48 h.

2.3. Measurements and analyses

2.3.1. Gas production rates

In all experiments, the gas production rates of soil were determined by sealing soil samples into close containers and taking gas samples from the headspace to monitor the gas concentrations with time. In experiments 1 and 2, the gas production rate was determined with two time points (0 and 24 h), and in Experiment 3, there were four time points (0.5, 1.5, 24 and 48 h) The composition of gas samples was analysed with a gas chromatograph (Agilent Technologies 7890B GC custom, Santa Clara, CA, United States), equipped with a thermal conductivity detector (TCD) for CO_2, and an electron capture detector (ECD) for N_2O in the same way as described by Penttilä et al. (2013). Nonlinearity of ECD was corrected with an empirical correction function determined separately using known concentrations of N_2O (data not shown). The gas production rate was calculated as the product of headspace volume and the linear temporal slope of gas concentration during the measurement divided by the dry mass of soil. For technical reasons, however, there were some differences in the containers used and gas samples taken in different experiments as detailed below.

In Experiment 1, after stabilization to their targeted matric potentials (Fig. 1), the soil samples were transferred into 350 ml glass containers that were closed gas tight with metal lids equipped with three-way valves for gas sampling. Headspace was flushed with compressed air before closing the containers to ensure a standardised atmosphere in each container at the beginning of measurement. The gas concentrations of 8-ml samples of headspace air taken immediately after closing and after 24 h incubation into He-flushed and evacuated glass vials (3-ml

Labco Exetainers® with double septa, Labco Ltd., Buckinghamshire, UK) were analysed by gas chromatography and used for calculating the temporal slope of gas concentration change in the headspace.

In Experiment 2, before closing the bottles for measurement, they were flushed with compressed air with an ambient CO_2 concentration, in order to standardize the headspace air composition at the beginning of each measurement, and then sealed. An 8-ml gas sample from the headspace was taken after 24 h, as also described by Šimek et. al. (2011, 2014), and analysed by GC as described above for Experiment 1. The rate of CO_2 and N_2O production during the incubation period was calculated as the difference between the concentration of CO_2 and N_2O in bottles containing soil samples and those without soil. Cases, where the calculated CO_2 production became negative (seven samples out of 72), were treated as unreliable and disregarded from the calculations.

The total headspace volume of bottle was calculated by subtracting the volume of soil material from the total volume of the bottle (as estimated by measuring the volume of water required to fill the bottle). Bunsen's solubility coefficients at respective temperatures (1.45 and 0.942 for CO_2 and 1.14 and 0.629 for N_2O at 5 °C and 20 °C, respectively, according to Gerrard (1980) and Gliński and Stępniewski (1985)) were used for estimating the dissolved amounts of CO_2 and N_2O in the liquid phase in equilibrium with the measured gas-phase concentrations. The results were expressed as μg of CO_2-C or N_2O-N per kg of dry soil per hour. Results expressed as μg of CO_2-C or N_2O-N per kg of soil C content per hour are presented as supplementary data.

The temperature coefficients (Q10 values, indicating the rate of change in a process as a result of increased temperature by 10 °C) were calculated from the average production rates of a given treatment at 5 °C and 20 °C during the 1-wk period before adding the different treatment solutions, thus providing triplicate Q10 values for each horizon. The Q10 value was calculated for the temperatures of 5 and 20 °C according to Eq. (1):

$$Q10 = \left(\frac{R2}{R1}\right)^{10°C/(T2-T1)} \quad (1)$$

with T1 = 5 °C, T2 = 20 °C, and R1 and R2 the rate of gas production rates at 5 and 20 °C, respectively.

In Experiment 3, the rates of total denitrification in soil were determined in anoxic conditions by the acetylene inhibition method (Smith et al., 1978; Smith and Tiedje, 1979; Yoshinari and Knowles, 1976; Yoshinari et al., 1977). The gas concentrations were determined for the samples taken from the headspace at 0.5 h, 1.5 h, 24 h and 48 h after adding the treatment solutions. In addition, the pressure in the headspace was determined before and after of each gas sampling by a portable battery-operated pressure meter fitted with a needle probe (Tensimeter, Soil Measurement Systems, Arizona, USA). The total headspace volume of N_2O was corrected by multiplying with pressure (atm) measured before and after each respective sampling (0.5 h, 1.5 h, etc.). The measured gas-phase amounts of N_2O were corrected for the gas dissolved in the liquid phase of treatment solutions and soil pore water according to Bunsen's solubility co-efficient as described previously. The emissions of N_2O at time intervals of 0–0.5 h, 0.5–1.5 h, 1.5–24 h and 24–48 h were measured and the cumulative emission with time was calculated by summing the emissions of individual time intervals. The average rate of emission was calculated from a linear slope of cumulative emission with time by using the slope function of MS Excel. The results were expressed as μg of N_2O per kg dry soil per hour. Results expressed as μg of N_2O-N per kg of soil C content per hour are presented as supplementary data.

2.3.2. Soil mineral nitrogen content

Mineral N (NH_4^+-N and NO_3^--N) was determined after both oxic Experiment 2 and anoxic Experiment 3 for each incubation bottle. Mineral N was extracted with 2 M and 1 M KCl solutions in oxic and anoxic experiments, respectively. In the anoxic potential denitrification experiment, the use of 2 M KCl as an extracting solution was not possible as it would have been diluted too much by the water suspensions of added substrates solutions, and the solubility of KCl did not allow adding of more concentrated KCl solution to achieve 2 M KCl concentration in the suspension. Instead, 2.8 M KCl solution was added into the incubation bottle so as to approach a final concentration of 1 M KCl in the suspension (soil slurry). For the samples of the peat layer, 1:10 (2.5 g dry soil: 25 ml KCl) and for the samples from mineral soil material 1:2.5 (10 g dry soil: 25 ml KCl) soil-to-solution extraction ratios were used in both experiments. Mineral N (NO_3^- and NH_4^+) was determined by colorimetry with a flow-injection auto-analyser (Lachat QuickChem® 8000, USA and Lachat QuickChem methods 12-107-06-2-A and 12-107-04-1-E). Known standard samples were analysed at regular intervals between the samples and used to correct for any temporal drifts in the sensitivity of methods during long sequences. The soil moisture content was taken into account for the calculation of extractant-to-soil ratio and the contents of NO_3^- and NH_4^+ in soil were given as mg N per kg dry soil.

2.4. Statistical analyses

A logarithmic transformation was performed for the N_2O data in basal gas production, substrate-induced gas production and potential denitrification experiment, as well as CO_2 data in the substrate-induced gas production to ensure homoscedasticity and normality. The transformation was omitted in the moisture experiment (Experiment 1) due to numerous negative emissions. Results were analysed with IBM SPS Statistics programme version 24, using the linear mixed model analysis to assess the effect of the various treatments on the observed gas production. The experimental treatments were assigned to the model as fixed factors. In addition, the treatment means were compared within each soil horizon and, when relevant, horizon-temperature-combinations with the least significant difference test. Residual plots were assessed visually to check for any violation of the linearity, homoscedasticity or normality assumptions, discovering none.

3. Results

3.1. Gas production of undisturbed core soil samples at different matric water potentials

The production of N_2O and CO_2 had different patterns with soil moisture and horizons. The production of CO_2 was most abundant in the peat horizon (H2), especially when wet, and decreased with depth (Fig. 4). In contrast, the production of N_2O had the maximum in the upper mineral subsoil horizon (Cg) near field capacity (matric potential −100 cm water column), whereas the production of N_2O in the lower mineral subsoil horizon (Cr) was small at all studied potentials (Fig. 5).

The production of CO_2 in peat horizon (H2) was at least one or two orders of magnitude higher than that in the mineral subsoil horizons (Fig. 4). Soil moisture changes also had a varied effect on CO_2 production of different horizons ($F_{6,\ 26} = 10.3$, $p < 0.001$). The moisture changes affected the production of CO_2 only in the peat layer. By far the greatest production was observed in the peat horizon, when the soil was wet (matric potential −10 cm water column), but as the matric potential increased, CO_2 production in peat decreased, but the 60 and 100 cm potentials were indistinguishable from one another (Fig. 4). In the mineral horizons, CO_2 production remained close to zero under all moisture conditions, and no differences could be observed between the horizons or any matric potential. When examining soil CO2 production relative to soil C content in Experiment 1, it was discovered that the Cg horizon had the greatest production, while the other two examined horizons maintained a similar production level (Fig. 4). The difference in production at −10 cm w.c. matric potential in peat did not reach significance although the trend was still visible (Fig. 4).

N_2O production of different soil horizons reacted differently to the moisture changes, as shown by the statistically significant ($F_{6,26} = 6.62$,

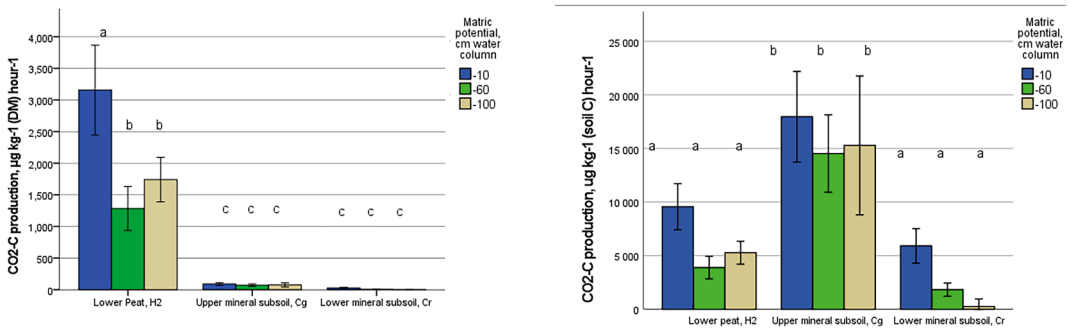

Fig. 4. CO_2 production in undisturbed core soil samples from different soil horizons at different matric potentials (cm) relative to soil dry matter (left) and soil C content (right) (Mean ± SE, N = 4). Means denoted with the same letter do not differ (LSD $p < 0.05$).

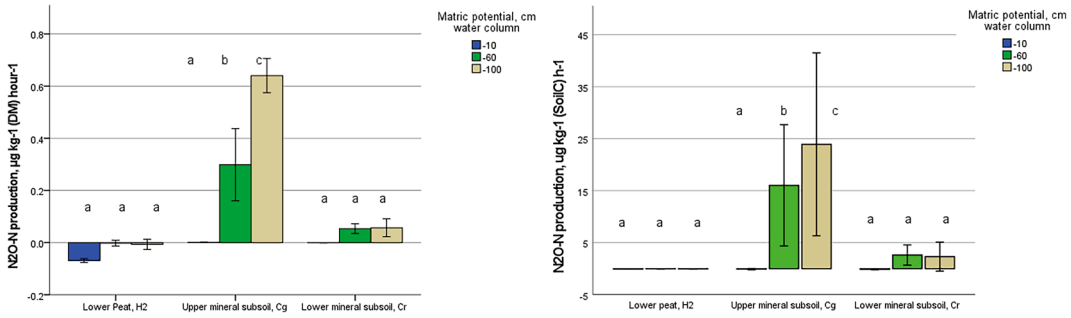

Fig. 5. N_2O production in undisturbed core soil samples from different soil horizons potentials (cm) relative to soil dry matter (left) and soil C content (right) (Mean ± SE, N = 4). Means denoted with the same letter do not differ (LSD $p < 0.05$).

$p < 0.001$) interaction between horizon and matric potential. The clearest response to increased matric potential by drainage of water (10 to 100 cm water column) was observed for the top mineral soil (Cg), where the production of N_2O increased with increasing potential (Fig. 5). Peat acted as a sink for N_2O, when the soil was wet. At higher matric potentials, the net production was close to zero. The deep mineral subsoil (Cr) was a small source of N_2O, but it was not significantly affected by the changes in soil moisture. N2O production in Experiment 1, expressed relative to soil C content, was greatest in Cg while the other two examined horizons were indistinguishable from one another

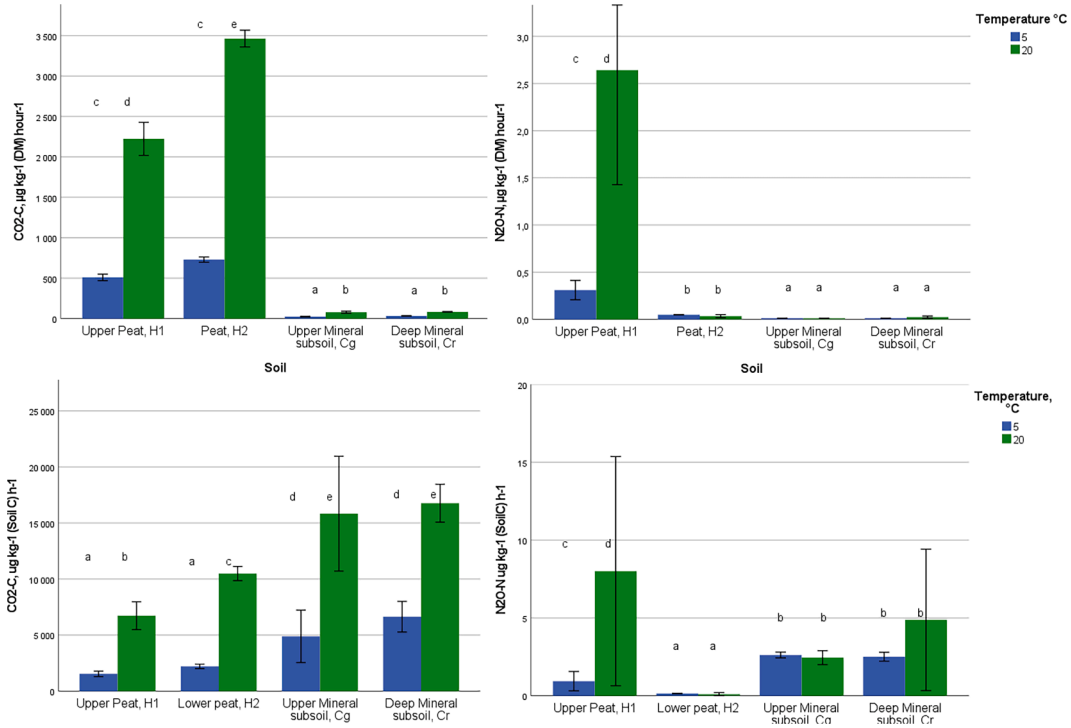

Fig. 6. Basal respiration and N_2O production in the different soil horizons at 5 and 20 °C relative to soil dry matter (above) and soil C content (below). (Mean ± SE, N = 3). Means denoted with the same letter do not differ (LSD $p < 0.05$).

(Fig. 5). No changes in statistical significance were detected in N2O production when examined relative to soil C compared to the respective examination relative to soil dry matter content.

3.2. Effect of temperature on basal respiration and N_2O production

Under aerobic conditions of constant moisture at 60% WFPS, CO_2 production in the four soil horizons had a different response to temperature ($F_{3,59} = 104.8p < 0.001$ for the interaction between soil and temperature). Since the interaction between soil horizon and temperature was significant, the effect of temperature was examined within the peat horizons and mineral horizons separately. The highest CO_2 production was observed in the lower peat horizon (H2) under both temperatures (Fig. 6). The CO_2 production was greater at 20 °C compared to 5 °C in all horizons, ($F_{1,32} = 106.1$ and 270.1 for the topmost and lower peat, respectively, $p < 0.001$ and $F_{1,27} = 15.6$ and 21.7, $p = 0.001$ and < 0.001 for upper and deep mineral subsoil, respectively), although the magnitude of the response was smaller in the mineral soil. The same was reflected in the Q10 values, with both peat horizons approaching a Q10 value of three, while in both mineral horizons remained slightly below a Q10 value of two (Table 1). CO_2 production in the mineral horizons was small, as also found with undisturbed core soil samples at different matric water potentials (−1 to −100 cm w.c.) representing considerably wetter soil conditions. For the peat horizons, CO_2 production rate at 20 °C was similar to the production in Experiment 1 at 10 hPa. While examining relative to soil C content, both mineral horizons (Cg and Cr) exhibited larger CO_2 production than either peat horizon (H1 and H2), but the two mineral horizons were indistinguishable from one another, as were the CO_2 productions of the peat horizons at 5 °C. In 20 °C, H2 exhibited larger CO_2 production, as when examined relative to soil dry matter content. The effect of increased temperature was clearly visible within all examined horizons as greater CO_2 production (Fig. 6).

N_2O production of different soil horizons under constant moisture conditions responded differently to a change in temperature ($F_{3,63} = 2.75$, $p < 0.05$ for the interaction between soil and temperature). Only the topmost peat horizon (H1) showed increased N_2O production with increased temperature, while no such effect could be observed for the other horizons. This was supported by the fact that the Q10 values were near one in all horizons apart from the topmost peat exhibiting a value over four (Table 1). Interestingly, unlike with undisturbed soil cores at a wetter range of different matric water potentials (−1 to −100 cm w.c.), both peat horizons now produced clearly more N_2O than either mineral horizon, where the production was negligible (Fig. 6). When examined relative to soil C content, the only observable temperature effect on N_2O production occurred in upper peat (H1) (Fig. 6).

3.3. Substrate induced aerobic respiration and N_2O production

The response of N_2O and CO_2 production to added glucose or ammonium depended both on soil horizon and temperature. Glucose induced large CO_2 production in peat horizons (Fig. 7), whereas confirming any significant effects on N_2O production were complicated by large variation in the data.

When glucose or ammonium was added into the soil samples as substrates, a significant response in N_2O production was observed only in the topmost horizon (H1) for added glucose over added ammonium, and only at 20 °C (Fig. 8). In all other horizons, production remained at the same level as it was before the addition of substrate solutions. The only significant substrate induced effect on N_2O production was observed for glucose at 20 °C, when examined relative to soil C content (Fig. 8). In relation to soil C content, the level of N_2O production was similar in all other horizons apart from H2, which showed less production than the other horizons (Fig. 8).

The addition of glucose increased CO_2production in both peat horizons, at 20 °C as well as 5 °C ($F_{2,24} = 14.3p < 0.001$ for the effect of substrate in lower peat (H2) at 20 °C and $F_{2,24} = 39.8$, $p < 0.001$ for the upper peat (H1)), the respiration reaching the mean of 13000 ± 3400 and 9700 ± 800 μg CO_2-C $kg^{-1}h^{-1}$ in the glucose treated upper and lower peat, respectively. The corresponding results at 5 °C were $F_{2,24} = 17.5$, $p < 0.001$ for lower and $F_{2,24} = 16.5$, $p < 0.001$ for upper peat. A significant glucose response was also observed in the upper mineral soil ($F_{2,22} = 19.8$, $p < 0.001$ and $F_{2,22} = 10.2$, $p < 0.001$ at 20 and 5 °C, respectively). Substrate induced respiration in the upper mineral soil at 20 °C was 1600 ± 500 μg CO_2-C $kg^{-1}h^{-1}$, corresponding to roughly a tenfold increase compared to the control. The only response to added ammonium was observed in the upper mineral soil at 5 °C.

Substrate induced CO_2 production was greatest in Cg at 20 °C, when examined relative to soil C content. All significant substrate induced effects observed relative to soil dry matter content were also significant when examined relative to soil C content (Fig. 7).

Mineral N content varied in different soil horizons (Table 2). Ammonium was most abundant in the lower peat horizon (H2), but it was also present in the mineral soil layers (Cg, Cr). In the upper peat (H1), only a low amount of ammonium was present, unless added during the experiment. Nitrate, however, was present in the upper peat (H1), while in the other horizons it was negligible.

3.4. Potential denitrification

The potential for denitrification of each soil horizon was investigated by adding either nitrate, glucose or their combination to the soil under anaerobic conditions, and monitoring the production rate of N_2O. Upper peat (H1) had by far the largest N_2O production that decreased with depth. The mineral subsoil layers (Cg, Cr) did not differ significantly (Fig. 9 and Table 3). Rate of production of N_2O in the upper peat horizon (H1) is not limited by either nitrate or glucose. However, in the lower peat (H2), upper mineral (Cg) and lower mineral (Cr) horizons added nitrate increased N_2O production rate significantly ($P < 0.05$). Moreover, N_2O production rate in the lower mineral subsoil horizon (Cr) increased significantly ($P < 0.05$) by added glucose. Interactions of added glucose and nitrate were not significant in any of the horizons (Table 3). When examined relative to soil C content, anaerobic N_2O production was at a similar level in all horizons apart from H1, where the production was larger (Fig. S6 and Table S1). Nitrate remained the limiting factor in all but the topmost horizon (H1), and the effect of glucose was only significant in Cr when examined relative to soil C content, in the same manner as when examined relative to soil dry matter content.

In the same manner as in the substrate induced respiration experiment, nitrate content in all other horizons except the top peat horizon (H1) was negligible unless nitrate was added to soil in an experimental solution (Table 4). When glucose was added to the upper peat horizon (H1), the amount of nitrate decreased clearly, and that of ammonium more slightly. Ammonium was the dominant form of mineral nitrogen in all horizons beneath top peat. Nitrate addition caused the amount of ammonium to fall by more than half in the lower mineral subsoil horizon (Cr).

Table 1
Q10 values of CO_2 and N_2O production in the different soil horizons. Means denoted with the same letter do not differ (LSD $p < 0.05$).

	Q10 (CO_2)		Q10 (N_2O)	
Soil	Mean[A]	SEM	Mean[A]	SEM
Upper Peat (H1)	2.67 [a]	0.03	4.23 [a]	0.77
Peat (H2)	2.82 [a]	0.05	0.99[b]	0.02
Upper Mineral subsoil (Cg)	1.91[b]	–	0.96[b]	0.06
Deep Mineral subsoil (Cr)	1.86[b]	0.07	1.49[b]	0.44

[A] N = 3 except for N = 1 in the horizon Cg

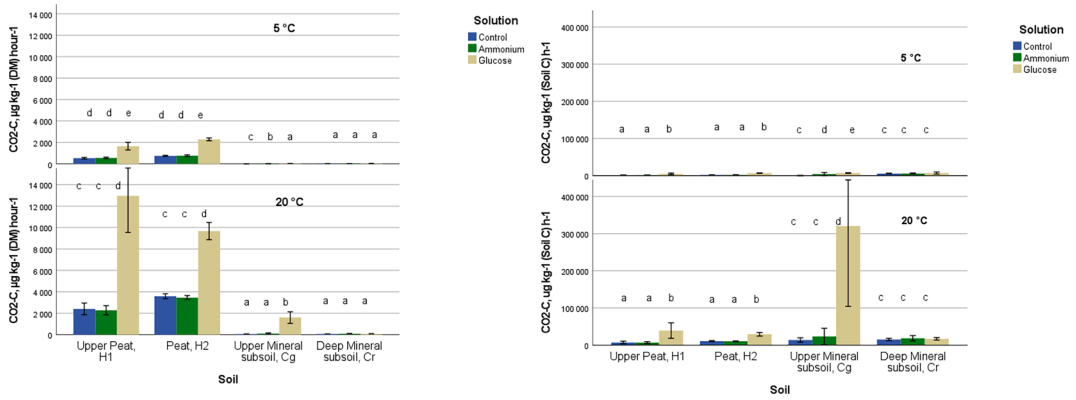

Fig. 7. Aerobic CO_2 production rate of soil horizons by different added substrate solutions at 5 (above) and 20 °C (below)) (Mean ± SE, N = 3) relative to soil dry matter (left) and soil C content (right). Means denoted by the same letter within a given temperature do not differ at $P < 0.05$.

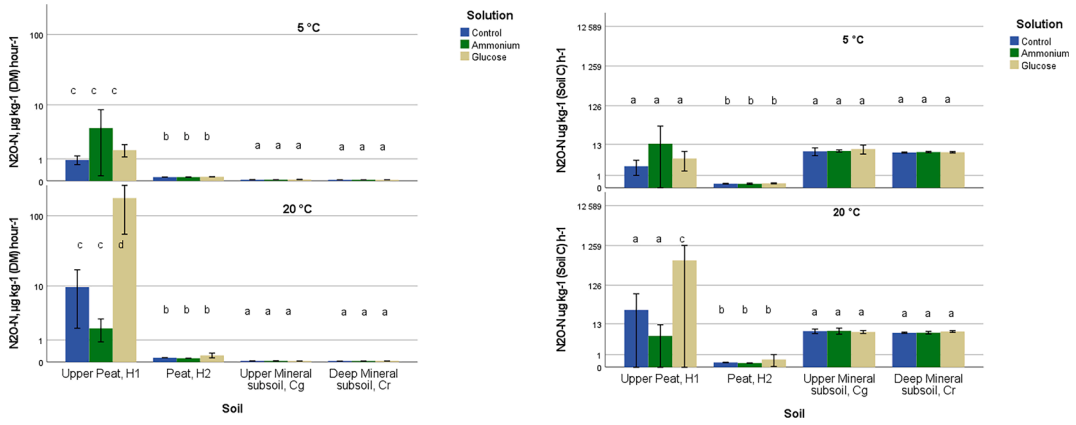

Fig. 8. Aerobic production rate of N_2O by different substrate solutions added to different soil horizons at 5 (above) and 20 °C(below) (Mean ± SE, N = 3) relative to soil dry matter (left) and soil C content (right). Means denoted by the same letter within a given temperature do not differ at $P < 0.05$.

Table 2
Mineral N contents[a] of soil horizons in different experimental treatments after Experiment 2 (the aerobic incubation experiment).

		NH_4^+ (N mg kg^{-1} soil)			NO_3^- (N mg kg^{-1} soil)		
Soil horizon	Temp	Control	Glucose	$NH4^+$	Control	Glucose	$NH4^+$
Upper Peat (H1)	5	10 ± 2	6.2 ± 0.5	277 ± 16	92 ± 0.4	54.7 ± 1.1	118 ± 26
	20	9 ± 4	3.0 ± 0.6	246 ± 10	89 ± 2	36 ± 3	93.2 ± 0.5
Lower Peat (H2)	5	101 ± 5	94 ± 11	284 ± 10	4 ± 2	2.7 ± 1.4	3 ± 3
	20	111 ± 13	37 ± 15	254 ± 22	3 ± 2	2.9 ± 1.3	3 ± 2
Upper Mineral (Cg)	5	37 ± 18	35 ± 11	291 ± 34	0.6 ± 0.3	0.16 ± 0.02	0.9 ± 0.3
	20	29 ± 13	18 ± 12	260 ± 20	0.55 ± 0.14	0.14 ± 0.05	0.68 ± 0.11
Deep Mineral (Cr)	5	63 ± 2	68 ± 4	261 ± 53	0.40 ± 0.04	0.23 ± 0.01	0.9 ± 0.3
	20	81 ± 4	78 ± 11	279 ± 22	0.7 ± 0.2	0.7 ± 0.5	2.4 ± 1.7

[a] Mean ± SEM, N = 3. Temp, incubation temperature (°C)

4. Discussion

4.1. Factors limiting CO_2 and N_2O production in different soil horizons under aerobic conditions

The largest microbial activity, measured as CO_2 and N_2O production rates, was observed in the peat layers. This was expected, as peat horizons contained over 60-fold more organic C than the mineral subsoil horizons. However, when the current results were examined relative to soil C content, the production rate of CO_2 in Experiments 1 and 2 became similar, or even larger in the mineral horizons (Cg and Cr) compared to peat (Figs. S1 and S3), than when calculated relative to soil dry matter content which may indicate different quality and availability of carbon compounds in different layers. The high microbial activity and decomposition of large organic C pools makes cultivated peat soils often large sources of greenhouse gases (see e.g. Maljanen et al., 2007; Couwenberg, 2011; Lind et al., 2019). Moreover, due to greater amounts of plant roots, organic and inorganic nutrients, the activity of microbes in the surface soil horizons is generally higher compared with deeper soil layers (Speir et al., 1984).

The CO_2 production rates were well within the range of values measured in a microcosm study for a set of lowland peatland horizons covering a wide range of humification (Kechavarzi et al., 2010). The fact that the respiration rates in the lower peat horizon (H2) were higher than those in the upper peat horizon (H1) is opposite to the findings of Scanlon and Moore (2000), who found that the rate of respiration decreased with increasing depth in a peat soil profile. High ammonium contents in the lower peat horizon are probably caused by a high

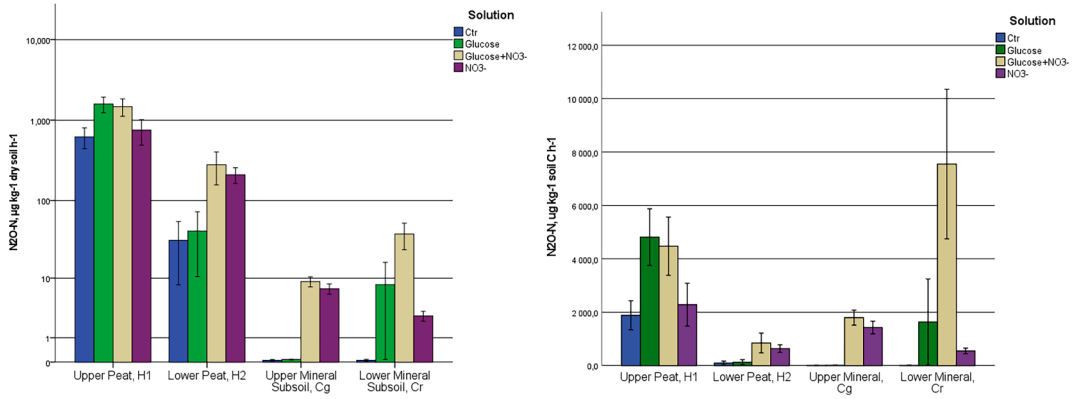

Fig. 9. Anaerobic production of N_2O in the soil horizons with different substrate additions relative to soil dry matter (left) and soil C content (right) (Mean ± SE, N = 4).

Table 3
The geometric means of anaerobic N_2O production[A], and the relative effects and statistical significances of glucose and nitrate additions on it, in different soil horizons (μg N_2O-N $kg^{-1}h^{-1}$).

Soil horizon	Mean	Nitrate	$F_{1,48}$	p	Glucose	$F_{1,48}$	P
Upper Peat (H1)	889.20[c]	0.97	0.002	0.965	2.50	1.526	0.223
Lower Peat (H2)	34.51[b]	**33.81**	22.510	<0.001	1.88	0.723	0.399
Upper Mineral (Cg)	0.67[a]	**131.22**	43.181	<0.001	1.44	0.238	0.628
Lower Mineral (Cr)	0.81[a]	**93.11**	37.335	<0.001	**7.48**	7.351	0.009

[A] Statistical tests were carried out by a MIXED procedure with log-transformed data; geometric means and relative effects were obtained by back-transformation (10^x). Means denoted by a different lowercase letter differ significantly ($P < 0.05$, *t*-test). Each F value tests the simple effects of nitrate and glucose, respectively, within each soil horizon. The interactions nitrate × glucose at given horizons were not significant. Significant effects are bolded.

Table 4
Mineral N contents in each soil horizon and substrate solution treatment after Experiment 3 (the potential denitrification experiment) (mean ± SE, N = 4).

	NH_4^+ (N mg kg^{-1} soil)				NO_3^- (N mg kg^{-1} soil)			
Soil	Control	Glucose	Glucose + NO_3^-	NO_3^-	Control	Glucose	Glucose +NO_3^-	NO_3^-
Upper Peat (H1)	19.4 ± 0.6	6.6 ± 0.4	31.4 ± 1.3	41 ± 2	106 ± 9	10 ± 2	1068 ± 173	1504 ± 68
Lower Peat (H2)	177 ± 15	120 ± 20	188 ± 26	181 ± 21	6 ± 0.5	8.8 ± 1.5	1761 ± 172	1668 ± 232
Upper Mineral (Cg)	46 ± 8	44 ± 9	45 ± 10	41 ± 11	0.07 ± 0.12	−0.33 ± 0.01	329 ± 9	304 ± 11
Lower Mineral (Cr)	171 ± 16	209 ± 18	81 ± 6	80 ± 9	1.35 ± 0.15	6 ± 5	436 ± 41	487 ± 84

respiration-coupled ammonification rate. This is tentatively attributed to more favourable conditions for microbial activity in the lower peat, as uppermost soil horizons are generally more prone to water stress and other disturbances, such as temperature fluctuations, that may change the microbial community and reduce microbial respiration.

Another possible explanation may be that the higher input of slowly decomposable litter (such as hay straw from current agricultural activity) cause substrate limitation for microbial respiration in the upper peat horizon compared with the relatively more decomposed and more easily decomposable organic matter in the lower peat horizon. This assertion is supported by Kan et al. (2020), who discovered lower basal C mineralization rate under residue retention and no-till treatments. The notion of substrate limitation was supported by high glucose-induced respiration observed in both peat horizons, especially in the upper one. The respiration of the lower peat horizon (H2) was increased by approximately three-fold with the addition of glucose, and the response was even greater in the upper peat (H1). It is also possible that soluble organic compounds have leached from the upper peat horizon to the lower one, resulting in higher basal respiration in the lower peat, and a stronger response to added available C in the upper.

Leaching of easily decomposable carbon compounds in the soil profile was also supported by the high glucose induced respiration expressed relative to soil C observed in Cg at 20 °C, exceeding that of either peat layer by almost an order of magnitude (Fig. 6). No such effect was present in Cr. This suggests that the microbial community present in Cg, just beneath the peat layer, is well adapted to utilising easily decomposable carbon compounds, while the deepest horizon, Cr, lacks this property, or that there is another limiting factor at work in Cr besides C. Besides deposition of these compounds by leaching from the overlying layers, easily decomposable carbon compounds may also have been introduced through rhizodeposition (both root exudates and dead root biomass) to the upper mineral subsoil horizon (Cg). In the lower mineral subsoil, below to the mean ground water level during the growing seasons (82 cm in 2014 and 73 cm in 2015 after Yli-Halla et al., 2017), rhizodeposition from the roots of current agricultural crops likely had a minimal role.

For CO_2 production, the results from different experiments tended to be well in line with one another: production under similar conditions produced similar results. Most abundant CO_2 production was found when the soil was wet at the temperature of 20 °C. Under these conditions, without any amendments, the lower peat horizon produced approximately 3500 μg of CO_2-C kg^{-1} DM h^{-1}, with a similar result in the upper peat horizon. With an average dry bulk density of 0.13 kg dm^{-3}, this is equivalent to 0.46 mg CO_2-C $dm^{-3}h^{-1}$. Assuming a peat layer of 45–60 cm, this would correspond to about 50–67 kg ha^{-1} d^{-1} or 18–24 Mg ha^{-1} in 12 months. Assuming a Q10 value of 2.7 in the peat layer,

and a mean annual temperature of 2.6 °C (Maljanen et al. 2003), this emission corresponds to 3.2–4.2 Mg ha^{-1} annually. These values are close to annual field emissions of CO_2-C compiled by Maljanen et al. (2007) for various cultivated peat soils under grass (4.1 ± 2.8 Mg ha^{-1}), even if the comparison to field measurements is a gross simplification. However, the temperature range in this experiment does mirror the range of a boreal organic soil, as reported by Maljanen et al. (2003), who observed temperatures between approximately 3 and 20 °C during the growing season at 5 and 20 cm depth.

Both mineral subsoil horizons (Cg, Cr) exhibited only very modest microbial activity as expected based on their low pH and organic C contents. Basal respiration of the mineral layers (Cg, Cr) in this experiment was clearly higher at 20 °C compared to 5 °C. With a mean Q10 value of 1.9, as discovered in this study for the mineral subsoil horizons, the basal respiration rates of 30–80 μg $kg^{-1}h^{-1}$ at 5–20 °C were broadly similar to those in the most acidic subsoil Bg horizons of a coastal AS soil and a control soil (approximately 100–200 μg $kg^{-1}h^{-1}$ at 25 °C) reported by Šimek et al. (2011). On the other hand, the basal respiration rates of Cg and Cr horizons were smaller by an order of magnitude than those in organic rich and circumneutral C horizons of coastal AS soils (Šimek et al., 2011, Šimek et al., 2014). Such a large difference in microbial activity reflecting the pH and organic C contents of C horizons can be viewed as new information on the differences between AS soils of contrasting formation histories.

Both black schists and sulfidic compounds of coastal AS soils produce acidity upon oxidation and in both soils jarosite $KFe_3(SO_4)_3(OH)_6$ has been observed in the most acidic horizons (Šimek et al., 2011; Virtanen, 2015; Yli-Halla et al., 2017). Many black schist sites in Finland, including the site of the current study, have been under industrial peat excavation. As a result, oxidation may proceed more rapidly compared to coastal AS sites as the sulfidic material is close to the new soil surface and is quite suddenly exposed to oxidation upon removal of the peat layer. Therefore, the production of acidity is abundant, and owing to lower content of SOM and coarser soil texture in the mineral soil horizons of the black schist site, the pH may decrease more rapidly and reach more extreme values than in coastal AS soils, resulting in unfavorable conditions for microbial activity.

CO_2 production in the acidic and organic-poor mineral subsoil horizons (Cg, Cr) remained in the range of approximately 0–100 μg CO_2-C $kg^{-1}h^{-1}$ despite any treatments they were subjected to, apart from the moderate increase (1600 μg CO_2-C $kg^{-1}h^{-1}$) in production in the Cg horizon when glucose was added. This result is in contrast to those by Šimek et al. (2011), who found large substrate-induced respiration in the reduced organic-rich subsoil of a coastal, sediment-derived AS soil with the rates exceeding those in this study by more than two orders of magnitude. To sum up, the results suggest that the microbial community present in the subsoil of a black schist-derived AS soil is less abundant and less responsive to substrates than that found in a sediment-derived AS soil, such as in the study of Šimek et al. (2011). This is due to lack of C, resulting in a lower diversity in the microbial community as well as unfavourable conditions due to extremely low and rapidly changing pH values.

When expressed relative to soil C content, CO_2 production in Experiment 1 was larger in Cg than in Cr (Fig S1), although this difference was not apparent when examined relative to soil dry weight. In experiment 2, both mineral horizons (Cg and Cr) exhibited similar CO_2 production rates on both approaches. This suggests that when the natural soil structure was disturbed, conditions for microbial activity were improved in Cr, while no such effect was observed in Cg. This could be due to release of protected soil carbon to easily decomposable forms in Cr when soil structure was disturbed. A similar response was discovered by Salomé et al. (2010), who concluded that access to substrate was the main regulatory mechanism in C mineralization in subsoil. Schnecker et al. (2015) came to a similar conclusion when they discovered a lack of correlation between subsoil enzyme activity and SOM content, but deduced that spatial separation or physical stability of SOM regulate substrate availability.

For aerobic N_2O production, different experiments produced somewhat conflicting results. With all the experiments conducted with disturbed soil samples, both peat horizons were sources of N_2O, with the topmost horizon (H1) with the highest nitrate content he largest source. However, the gas production measurements with 200-cm^3 undisturbed soil cores at different moisture contents showed that in wet conditions, peat acted as a sink for N_2O, and in drier conditions the net N_2O production was close to zero. The mineral subsoil horizons with small initial nitrate contents showed close to zero N_2O production in most experiments, except in Experiment 1 with undisturbed core samples, where the largest N_2O production was observed in the upper mineral subsoil (Cg), and decreasing soil moisture increased N_2O production significantly with more N_2O released when the soil was allowed to drain from saturation to field capacity (−100 cm matric potential). The various impacts of soil moisture on the N_2O production can be explained by the combined effect of the slow diffusion of N_2O and the high degree of reduction of N_2O to N_2 in nearly saturated wet soils. It is generally accepted that the product ratio N_2O/N_2 of denitrification approaches zero at water-filled pore space exceeding 90% (Davidson et al., 2000), even if the relationship between the N_2O production and soil moisture content is in reality a function of soil structure that ultimately determines the effective gas diffusion coefficient and especially the amount and shape of large air-filled pores at a given moisture content. The relationship between soil moisture and gas production was probably not similar in our undisturbed and disturbed samples as the soil structure and size of samples differed, causing changes in the rate of N_2O diffusion and consequently the time available for the reduction of N_2O during diffusion from soil into the headspace. As a consequence of large sample size and high WFPS in undisturbed core samples, the reduction of N_2O seems more probable in the undisturbed core samples than in the disturbed samples. In addition, as the observed N_2O production in the H2 horizon was not very large according to Experiment 2, any N_2O produced in undisturbed H2 samples has probably been reduced to N_2 in Experiment 1 at all investigated matric potentials (−10, −60 and −100 cm, corresponding to %WFPS of 97, 79 and 76 respectively), due to the small air space and a long distance for N_2O to diffuse from the soil to headspace. This did not occur in the disturbed samples used in Experiment 2 due to smaller sample size and lower WFPS (60%).

The same mechanism may provide an explanation for the observation in the Cg horizon in Experiment 1, where a decrease in N_2O production occurred as a consequence of increased moisture. In addition, the low microbial activity in the acidic and organic-poor mineral subsoil horizons (Cg, Cr) no doubt contributed to the low levels of N_2O production. The discovery of low microbial activity in wet subsoil horizons provides an explanation to the surprisingly modest cumulative emissions of N_2O discovered by Yli-Halla et al. (2017) at this site. The groundwater level reported by Yli-Halla et al. (2017) was approximately 80 cm below soil surface, which would give the Cg horizon a matric potential range from 0 to −35 cm in the field. Out of the moisture levels studied here, this corresponds best with −10 cm matric potential, where N_2O production was negligible. A similar discussion gives a matric potential range of −35 to −80 cm for H2, corresponding to −60 cm matric potential in Experiment 1, where no N_2O production was observed in the H2 horizon. The non-existent correlation between N_2O emission and soil temperature observed in their study is also in line with the current findings.

The addition of glucose resulted in consumption of mineral N in H1 horizon in 5 and 20 °C, and in H2 at 20 °C, but had no such effect in the subsoil horizons. The decrease of both ammonium and nitrate could be due to N immobilization as a result of glucose-induced microbial growth. On the other hand, the decrease in nitrate could be also partly due to denitrification, as suggested by the observed increase in N_2O production in H1 at 20 °C after glucose addition. The same pattern of glucose response to denitrification in H1 was observed in Experiment 3, although statistical significance was not quite achieved. Since the

addition of ammonium caused no significant changes in either nitrate content or N_2O production in any horizon, it seems that ammonium does not limit nitrification or N_2O produced through nitrification in the soil profile.

4.2. Factors limiting denitrification in anoxic conditions

Experiment 3 (the potential denitrification experiment) was carried out to determine if the Pärnänsuo soil profile had the capacity for denitrification and if the capacity varied among different horizons. The upper peat horizon (H1) emitted much more N_2O compared to other soil horizons, and the emission from this horizon was not limited by either nitrate or glucose. This indicates that the microorganisms producing N_2O have abundant supply of N and C in the upper peat horizon. Even though the lower peat (H2) horizon emitted much less N_2O than the upper peat (H1) horizon (about 25-fold of that in the lower peat), the production of N_2O was much higher in both peat horizons than in the mineral horizons. However, this difference was greatly reduced when the results were examined relative to soil C content, when there was no observable difference between H2, Cg and Cr in mean N_2O production, highlighting the significance of soil C, although H1 remained the largest N_2O source (Fig. 9).

The results clearly showed, like in the aerobic experiments, that anoxic denitrifying microbial activity was far more abundant in the peat horizons (H1, H2) than in either mineral horizon (Cg, Cr). This is due to the much higher organic matter content in peat than in mineral soil, and it is in line with numerous studies (Duxbury et al., 1982; Simojoki and Jaakkola, 2000; Maljanen et al., 2003; Pihlatie et al., 2004; Maljanen et al., 2007) concluding that organic soils emits N_2O with a very high rate compared to mineral soils. Carbon of organic soils acts as an electron donor and exerts indirect influence on the production of N_2O in soils (Firestone et al., 1980; Firestone, 1982). The very low rate of production of N_2O in our acidic and organic-poor mineral subsoil horizons can be attributed to the lower rate of denitrification and possibly lower number of denitrifying microorganisms in the subsoil compared with the peaty topsoil horizons (Parkin and Meisinger, 1989; Luo et al., 1998). A difference could also be seen between the two observed peat layers: there was more activity in the upper peat horizon (H1) that also exhibited a different response pattern to the added substrates compared with other horizons, either peat (H2) or mineral soil horizons (Cg, Cr). This response pattern remained even if observed relative to soil C content (Fig S6).

The substrate-induced production of N_2O in the upper peat horizon (H1) was statistically insignificant after glucose or nitrate additions in anoxic conditions which indicates that denitrification in the upper peat of our soil is not limited by either available organic C or nitrate. This is opposite to the results of many other studies (Burford and Bremner, 1975; Myrold and Tiedje, 1985; Weier et al., 1993; Senbayram et al., 2012). The lack of response to nitrate suggests that the given horizons already contain nitrate at sufficiently high concentrations not to be limiting denitrification. The relatively high mineral N contents of upper peat horizon (H1) supports this, as the upper peat was the only horizon, where nitrate was present even if it was not added to soil as an experimental solution. The lack of response to nitrate in the H1 horizon and the very high response to nitrate in the underlying three horizons agrees with the conclusion of Ryden (1983) that denitrification rate is not dependent on nitrate when the NO_3^- concentrations exceeded 5–10 mg (N) kg^{-1} soil.

In the lower peat (H2), upper mineral (Cg) and lower mineral (Cr) horizons with initially low nitrate contents, added nitrate increased N_2O production, confirming that nitrate is the limiting factor for denitrification in these horizons. Ammonium contents in all of these horizons are high, and nitrate contents are low. The low nitrate contents can be broadly attributed to limited nitrification because of high water table in the field and impaired aeration with increasing soil depth, as nitrification is an aerobic process. Under field conditions, H2 and Cg generally have high matric potentials in the range between 0 and −70 cm w.c., and Cr is submerged for most of the year.

The fact that denitrification in the upper mineral horizon (Cg) exhibited a clear and significant response to added nitrate, rather than to glucose, indicates that the denitrification of upper mineral subsoil horizon is limited by nitrate, not by C availability. Moreover, the lack of response to added glucose under anoxic conditions together with a very low basal N_2O production indicates very low or no denitrifying microbial activity in the upper mineral subsoil horizon (Cg). The very high response of denitrification to added nitrate in both mineral subsoil horizons (Cg, Cr) suggests the possibility of N_2O production by the reaction of nitrate with sulphides in the same way as has been suggested (Macdonald et al., 2010) for sediment-derived AS soils. In boreal AS sediments, there is an abundance of metastable sulphides (Boman et al., 2008). Macdonald et al. (2010) reported that, in AS soils, nitrate instead of O_2 can oxidize the sulphides of underlying soil horizons, producing sulphate and N_2O.

In contrast to the upper mineral subsoil (Cg) horizon, the lower mineral subsoil (Cr) horizon exhibited statistically significant response not only to nitrate but also to glucose, indicating that, besides nitrate, denitrification in the Cr horizon is limited by the lack of easily available C. This is in line with the studies of McCarty and Bremner (1992), Murray et al. (2004), Peterson et al. (2013) who found that potential denitrification in the subsurface soils is highly responsive to added C and reported that subsoil denitrification is limited by the lack of easily available C. Such a different response to glucose in Cg and Cr subsoil horizons is tentatively attributed to the higher pH in Cr (5.4, compared with 3.8 in Cg), making it a more favourable habitat for denitrifying soil microbes.

5. Conclusions

According to this study, soil moisture variation and additions of C and N have contrasting effects on the aerobic and anoxic production of CO_2 and N_2O in different soil horizons of a black schist-derived organic AS soil. The production of CO_2 in the topmost peat horizon is largest near saturation (at −10 cm matric potential) and larger compared with the mineral subsoil horizons throughout the matric potential range −10 to −100 cm. Near saturation, however, the production of N_2O in the mineral subsoil horizons is small and the peat horizon can become a sink of N_2O, whereas the N_2O production in the upper mineral subsoil horizon increases with decreasing soil moisture content. Low temperature can limit CO_2 production, but it has almost no role in the production of N_2O in aerobic conditions. Glucose addition can increase the aerobic production of CO_2 in peat, but it has only minor effects at a low temperature and in mineral subsoil horizons. Anaerobic N_2O production is not limited either by available C or nitrate in the upper peat horizon but is limited by nitrate in other soil horizons, and available C is the limiting factor in the lower mineral subsoil horizon (Cr) only. While the N_2O production rates of mineral soil in this study were small, a clear risk for greenhouse gas production through denitrification was however discovered if the soil was exposed to excess nitrate. To prevent this, any fertilizer application should be planned to avoid leaching of nitrate to the subsoil layers. It is concluded that peatlands with an AS subsoil derived from black schist, such as in this study, have high microbial activity in the organic topsoil horizons but little microbial activity in the mineral subsoil horizons. These findings are contrary to those from previous research conducted on sediment-derived coastal AS soils which are richer in available C in the subsoil horizons. The findings presented in this paper give a solid base for further studies on the gas exchange of an organic black schist-derived acid sulphate soil, and the mechanisms governing its gas production. To deepen our understanding of these processes, a detailed examination of the temporal variation of emissions and the variation of soil air composition within the soil profile *in situ* has been carried out in the same field and will be presented in a follow-up paper.

Declaration of Competing Interest

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

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