Community structure, ecophysiology, and regulation of new, acid-tolerant denitrifiers as cause of high N_2O emissions from cryoturbated peat circles of acidic tundra soils

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Summary

Denitrification is the main source of N_2O emissions from cryoturbated peat circles in the Eastern European Arctic tundra. These permafrost-affected bare peat circles lack vegetation, have a high amount of available nitrate, and a low carbon to nitrogen ratio. Together with a soil moisture of ~ 70%, these are optimum conditions for denitrification and associated microorganisms. An acidic *in situ* pH of these peat circles is hypothesized to be a major controlling factor influencing denitrification and triggering the release of N_2O as emitted end product of denitrification, as the final enzymatic step of denitrification is impaired by a low pH. This thesis aimed to test if the ecophysiology of new and acid-tolerant denitrifiers affects the emissions of N_2O from cryoturbated peat circles.

Microcosm experiments with soil from bare peat circles and surrounding vegetated peat plateau were conducted to test denitrification potentials of both soils. Additionally, the influence of pH on denitrification was tested. Nitrate reduction and associated production of gaseous N-products by denitrification were detected in both soils. In peat circle soil microcosms, independent of pH, the released end product was N_2. Though nitrate reduction was slower at pH 4. In peat plateau microcosms N_2 was as well the emitted main end product at pH 6, whereas at pH 4 N_2O was emitted. Therefore, data indicate the potential for denitrification in both soil types, as well as complete denitrification potential at acidic pH in peat circles.

Microcosms experiments supplemented with labeled acetate were conducted to reveal the active, organic acid utilizing archaeal and bacterial community of denitrifiers based on 16S rRNA stable isotope probing coupled to Illumina MiSeq amplicon sequencing. Results suggest *Burkholderiaceae* as key nitrate reducers and acetate assimilators. This is supported by a new species within the *Burkholderiaceae* (*Caballeronia*) isolated from peat circle soil that encodes diverse nitrate and nitrite reductases. Potential key denitrifiers responsible for the reduction of N_2O to N_2, the detected end product and product of complete denitrification, are *Sphingobacteriacea*. Altogether, collected data suggest an electron donor limitation in peat circle soil leading to high *in situ* emission of N_2O, since peat circle soil demonstrated the potential for complete denitrification despite an acidic pH.

Keywords: denitrification, N_2O, permafrost, peat soil, *Burkholderiaceae*

Zusammenfassung

Denitrifikation ist die Hauptquelle von N_2O-Emissionen aus kryoturbierten Torfkreisen der osteuropäischen arktischen Tundra. Diesen vom Permafrost betroffenen kahlen Torfkreisen mangelt es an Vegetation, sie haben eine hohe Menge an verfügbarem Nitrat und ein niedriges Kohlenstoff-zu-Stickstoff-Verhältnis. In Kombination mit einer Bodenfeuchtigkeit von ~ 70% ergeben sich so optimale Bedingungen für die Denitrifikation und die damit assoziierten Mikroorganismen. Es wird angenommen, dass ein saurer *in situ* pH-Wert dieser Torfkreise ein wichtiger Kontrollfaktor ist, der die Denitrifikation beeinflusst und der Auslöser für die Freisetzung von N_2O als emittiertes Endprodukt der Denitrifikation ist, da der letzte enzymatische Schritt der Denitrifikation durch einen niedrigen pH-Wert beeinträchtigt wird. Ziel dieser Arbeit war es zu testen, ob die Ökophysiologie neuer und säuretoleranter Denitrifizierer die N_2O-Emissionen aus kryoturbierten Torfkreisen beeinflusst.

Mikrokosmos-Experimente mit Boden aus kahlen Torfkreisen und dem umgebenden bewachsenen Torfplateau wurden durchgeführt, um die Denitrifikationspotentiale beider Böden zu testen. Zusätzlich wurde der Einfluss des pH-Wertes auf die Denitrifikation getestet. Die Nitratreduktion und die damit verbundene Produktion von gasförmigen N-Produkten durch Denitrifikation wurde in beiden Böden nachgewiesen. In Torfkreis-Bodenmikrokosmen war das freigesetzte Endprodukt, unabhängig vom pH-Wert, N_2. Wobei die Nitratreduktion bei pH 4 langsamer verlief. In Torfplateau-Bodenmikrokosmen bei pH 6 war ebenfalls N_2 das freigesetzte Hauptendprodukt, während bei pH 4 N_2O emittiert wurde. Die Daten weisen daher auf das Potential zur Denitrifikation in beiden Bodentypen hin, sowie auf das Potential zur vollständigen Denitrifikation bei saurem pH-Wert in Torfkreisen.

Mikrokosmos-Experimente, supplementiert mit markiertem Acetat, wurden durchgeführt, um die aktive organische Säuren nutzende archaeelle und bakterielle Gemeinschaft von Denitrifizierern auf der Grundlage eines 16S rRNA basierenden Stabilen-Isotopen-Beprobungs-verfahren gekoppelt an eine Illumina MiSeq Amplikon-Sequenzierung, zu enthüllen. Die Ergebnisse deuten auf *Burkholderiaceae* als wichtige Nitratreduzierer und Acetatassimilierer hin. Dies wird durch eine neue Art innerhalb der *Burkholderiaceae* (*Caballeronia*) unterstützt, die aus Torfkreisboden isoliert wurde und diverse Nitrat- und Nitritreduktasen kodiert. Potenzielle Hauptdenitrifizierer, die für die Reduktion von N_2O zu N_2, dem nachgewiesenen Endprodukt und Produkt der vollständigen Denitrifikation, verantwortlich sind, sind *Sphingobacteriacea*. Die Datenlage in ihrer Gesamtheit deutet auf eine Limitierung von Elektronendonoren im Torfkreisboden, die zu einer hohen *in-situ*-Emission von N_2O führt, da der

Torfkreisboden trotz eines sauren pH-Wertes das Potenzial für eine vollständige Denitrifikation aufweist.

Schlagwörter: Denitrifikation, N_2O, Permafrost, Torfboden, *Burkholderiaceae*

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List of Abbreviations

C	carbon
CH_4	methane
CO_2	carbon dioxide
DNRA	dissimilatory nitrate reduction to ammonium
GHG	greenhouse gas
N	nitrogen
N_2	molecular nitrogen
N_2O	nitrous oxide
NH_4^+	ammonium
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
OTU	operational taxonomic unit
PC	cryoturbated peat circle soil
PP	peat plateau soil

1 General introduction

1.1 Permafrost regions

Subsurface that stays below 0 °C for at least two consecutive years is defined as permafrost (Everett, 1989) and occurs primarily in regions with cold winter temperatures and shallow snow cover, which results in a long-term negative annual heat energy balance of land surface. Permafrost is a characteristic feature of the ice and glacier free circum-Arctic region (Brown et al., 1997).

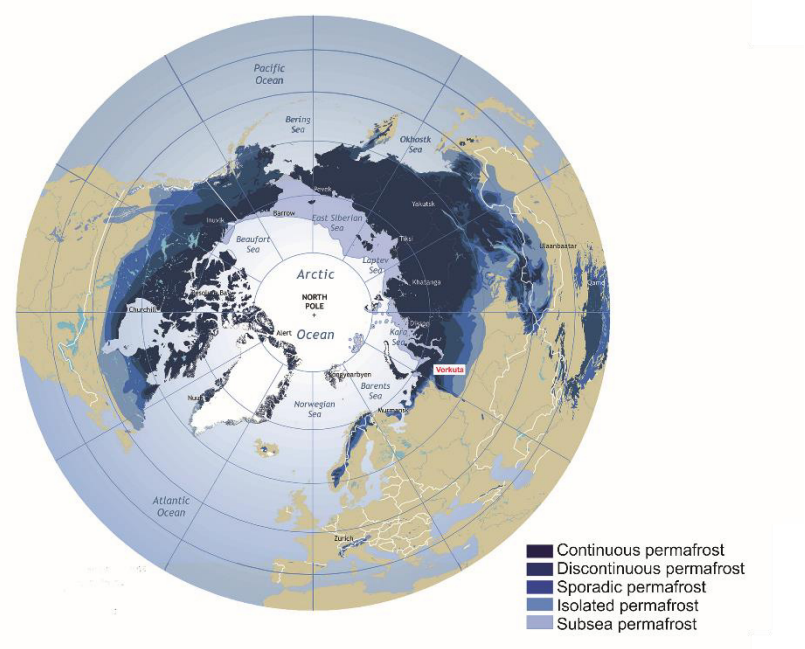

Figure 1: Circum-Arctic permafrost and ground ice map. Modified after (Brown et al., 1997); International Permafrost Association.

Approximately 16-25% of the global soil surface area are covered by permafrost (Figure *1*), including large peatland areas, e.g. up to 80% of the West Siberian surface area, and are estimated to store 50% of the global below ground organic carbon, a potential donor for the generation of reactive N- from less reactive N-species (Anisimov, 2007; Tarnocai et al., 2009). High carbon content is corelated with high organic N content, therefore northern peatlands are assumed to be large N reservoirs (Post et al., 1985), storing an estimated 67 Pg N in the upper 3 m of soil (Harden et al., 2012). This is 500 times more than the annually globally loaded N to soils as fertilizer (Bouwman et al., 2013; Stocker et al., 2018), with northern peatland soils alone storing approximately 10% of the global soil organic matter N (Limpens et al., 2008; Loisel et al.,

2014). All these low-temperature environments are colonized by cold-adapted microorganisms represented by all three domains of life: Archaea, Bacteria, and Eukarya (Casillo et al., 2019).

1.2 Greenhouse gases

Greenhouse gases (GHGs) re-emit energy they absorb from the lower atmosphere and thereby alter Earth's climate. Indeed, GHGs are the main drivers of climate change (Stocker et al., 2018). Warming induced by GHGs is mainly due to anthropogenic CO_2 emissions, though not alone. Further gases affecting climate are CH_4, N_2O, hydrofluorocarbons (HFCs), sulphur hexafluoride (SF_6) and perfluorocarbons (PFCs) (Montzka et al., 2011). They can have an effect on climate decades, even up to millennia, after their emission, depending on their persistence in the atmosphere. Non-CO_2 GHG emissions associated with anthropogenic sources will continue to rise and further increase global warming, since they are often linked to food and energy production (Montzka et al., 2011). The global warming potential of GHGs is based on their influence on climate, which is defined through their ability to absorb infrared radiation and their atmospheric lifetime, integrated over time (Montzka et al., 2011). This climate influence is then expressed in relation to an equivalent mass of CO_2 (Montzka et al., 2011). N_2O is the third most important greenhouse gas after CO_2 and CH_4. Its atmospheric concentration increased from 270 to 319 ppm between 1750 and 2005, which is critical not only due to its long atmospheric lifetime and its role as ozone depleting substance (Forster et al., 2007; Ravishankara et al., 2009), but also because of its high global warming potential, that is 300 times higher than that of CO_2 on a 100 year basis (Forster et al., 2007; Spahni et al., 2005).

1.2.1 Greenhouse gases and climate change

In the European north of Russia significant permafrost degradation is occurring. Moreover, through the end of the century, the projected regional climate warming is almost twice that of the global average (Anisimov et al., 2013; Romanovsky et al., 2017). Between 1975 and 2005 permafrost with a thickness of 10 to 15 m thawed completely in the Vorkuta area (Oberman, 2008) (Figure *1*). Hence, the southern permafrost boarder retreated north by approximately 80 km and the continuous permafrost boarder has moved north by 15 to 50 km (Oberman, 2008).

Moreover, in the past several decades taliks, unfrozen soil layers within permafrost, have developed, and the thickness of existing closed taliks increased in the Vorkuta region from 6.1 to 6.7 m (Romanovsky et al., 2010). Major factors, despite climatic factors, that explain permafrost and active layer trends at larger scales are local vegetation and soil variability (Streletskiy et al., 2015). For instance, well-drained landscapes like sandy tundra and blow outs, with little organic material, show more pronounced permafrost temperature responses to atmospheric variability compared to peatlands, mires, and bogs (Melnikov et al., 2004; Streletskiy et al., 2012; Vasiliev et al., 2008). Recent studies reported significant warming of active layer soil temperatures in the tundra landscape of the Vorkuta area from 1997 to 2018, that changed from -3.8°C to -1.9°C, accompanied by a near-surface permafrost thaw (Vasiliev et al., 2020). In addition, the permafrost table was lowered to 1.2 m below the ground surface in 2015 from 0.6 m in 1999 (Vasiliev et al., 2020). Though, permafrost warming was rather moderate and ranged from 0.04 to 0.05°C yr^{-1} (Vasiliev et al., 2020). The region is situated in a metastable stage of permafrost degradation, after ground ice in the transient layer is completely thawed and permafrost degradation is enhanced (Vasiliev et al., 2020). Warming air temperature and added winter insulation, together with active layer thickening, permafrost table lowering, and an increasing ground temperatures, drive the observed permafrost degradation (Vasiliev et al., 2020).

As a result of climate change and subsequent permafrost thaw, processes of the N-cycle are assumed to be fueled by the increase of available N (Anisimov, 2007; Marushchak et al., 2011). This is supported by studies investigating the effect of warming and permafrost thaw in *in situ* experiments along with higher temperatures experiments that result in increased N_2O emissions (Voigt et al., 2017b, 2017a; Yang et al., 2018).

1.2.2 Nitrous oxide and its formation

Soils, especially agricultural and tropical rainforest soils, are the main source of N_2O emissions and for 60-70% of the annually emitted N_2O (Behrendt et al., 2019; Christensen, 2009; Conrad, 1996; Denman et al., 2007; Marushchak et al., 2011; Mosier et al., 1998; Repo et al., 2009). Both abiotic and biotic processes can lead to the formation of N_2O. Chemodenitrification is an abiotic process that can contribute to the emission of N_2O under low pH and oxygen limited conditions, though the main products of the process are NO and NO_2 (Kresovic et al., 2009; van Cleemput,

1998). Furthermore, the process will most likely be outcompeted by the faster microbial denitrification (Kresovic et al., 2009; van Cleemput, 1998). Biotic processes leading to the emission of N_2O include nitrification, dissimilatory nitrate reduction to ammonium (DNRA) and denitrification. Ammonia oxidizing archaea, performing the first step of nitrification, are well known for their contribution to N_2O emissions from aerated soil and sediment (Siljanen et al., 2019). Although N_2O is not formed enzymatically and originates form the non-enzymatic conversion of the released intermediates NO and hydroxylamine (NH_2OH) (Zhu-Barker et al., 2015). In anaerobic soils and sediments, the emissions of N_2O can be attributed to DNRA or denitrification, depending on the C/N ratio and the pH (Rütting et al., 2011; Tiedje et al., 1983; Yoon et al., 2015). However, in terms of DNRA N_2O is only a byproduct, produced via the non-specific interaction of nitrate reductase with NO_2^- (Philippot and Hojberg, 1999; Smith and Zimmermann, 1981). For further details see section 4.2.3 Dissimilatory nitrate reduction and associated organisms in Horn and Hetz, 2021.

1.3 Denitrification

Denitrification is defined as the sequential reduction of NO_3^- or NO_2^- to molecular dinitrogen gas via the gaseous intermediates NO and N_2O:

$$\mathrm{NO_3^{-\,(+5)} \rightarrow\ NO_2^{-\,(+3)} \rightarrow\ NO^{(+2)}\ \rightarrow\ N_2O^{(+1)} \rightarrow\ N_2^{(0)}}$$

Microorganisms can possess all or only particulate enzymes associated with denitrification, hence, many truncated forms exist and N_2O is not only an obligate intermediate of this process, but also a possible end product (Cofman Anderson and Levine, 1986; Stein and Klotz, 2016). For further details see section 4.2.3 Dissimilatory nitrate reduction and associated organisms in Horn and Hetz, 2021.

1.3.1 Denitrifying microorganisms

Denitrifiers can be found within all three domains of life (Archaea, Bacteria, and Eukarya), showing a high phylogenetic and functional variability. More than 60 genera are known to harbor denitrifiers (Philippot et al., 2007; Zumft, 1997), the major genera being the bacterial Alpha-, Beta-, Gamma-, and Epsilonproteobacteria, and the Firmicutes. Within the Eukarya, denitrification is mainly limited to fungi, even though it has also been reported to occur in some foraminifer species (Kraft et al., 2011). However, fungi generally lack the nitrous oxide reductase and the released end product is N_2O (Chen et al., 2014; Lavrent'ev et al., 2008; Morozkina and Kurakov, 2007; Mothapo et al., 2015; Takaya, 2009).

The majority of denitrifiers are facultative, heterotrophic anaerobes that use sugars and/or organic acids as electron donors but are generally not capable of growing by fermentation. However, reduced S-compounds, H_2, NH_4^+, NO_2^-, or Fe^{2+} can be used as alternative electron donors by autotrophic denitrifiers (Shapleigh, 2006; Tiedje, 1988; Zumft, 1992). Another possible form of denitrification, though biochemically challenging, is the methane dependent denitrification, that oxidizes CH_4 with NO_3^-/NO_2^- via denitrification (Thauer and Shima, 2008), e.g. by the bacterium "*Candidatus* Methylomirabilis oxyfera" of the candidate phylum NC10 (Ettwig et al., 2010). Under oxygen limiting conditions, many of the classical "autotrophic" ammonia oxidizers are capable of denitrification as well by converting NH_4^+ to NO_2^-, which is then sequentially reduced to NO and further to N_2O (Colliver and Stephenson, 2000). In addition, many heterotrophic nitrifiers are capable of aerobic denitrification, using the periplasmic nitrate reductase *napA* and either one of the nitrite reductases *nirS* or *nirK*. The end product of aerobic denitrification is primarily N_2O (Ji et al., 2015; Stein, 2011). For further details see section 4.2.3 Dissimilatory nitrate reduction and associated organisms in Horn and Hetz, 2021.

1.3.2 Enzymes involved in denitrification

Complete denitrification from NO_3^- to N_2 involves four reductions catalyzed by seven enzymes (Zumft, 1997). The reduction of NO_3^-, common to denitrification and nitrate ammonification, is catalyzed by the nitrate reductase Nar or Nap (Zumft, 1997). The reduction of NO_2^- is performed by the nitrite reductase NirK or NirS, the key enzyme of denitrification, and results in the first gaseous product of the process, NO (Zumft, 1997). In the next step, NO is reduced to N_2O by the

NO reductase cNor or qNOr, with qNor also being used by non-denitrifying prokaryotes for NO detoxification (Zumft, 2005). The last step of denitrification, the reduction of N_2O to N_2, is catalyzed by the only known enzyme capable of this reaction, the nitrous oxide reductase Nos (Jones et al., 2008; Zumft, 1997). For further details see section 4.2.3 Dissimilatory nitrate reduction and associated organisms in Horn and Hetz, 2021.

1.3.3 Factors influencing denitrification in soils

Denitrification can act as source or sink of N_2O, depending on several factors, including pH, the C/N ratio, as well as oxygen and substrate availability, and the microbial community (Bergaust et al., 2010; Bru et al., 2011; Dorsch et al., 2002; Enwall et al., 2005; Holtan-Hartwig et al., 2000; van Cleemput, 1998). Acidity, early growth phase, and high NO_3^-/organic carbon ratios stimulate release of N_2O during denitrification (Baggs et al., 2010; Van Breemen and Feijtel, 1990). Indeed, in certain systems denitrification is impaired by a pH below 5 and associated with an increased product ratio of N_2O to N_2 (Cuhel et al., 2010; Simek et al., 2002). This product ratio can also reflect the relative abundance of the bacterial community capable of N_2O reduction in the environment (Graf et al., 2014; Hallin et al., 2012; Philippot et al., 2009). Highest known N_2O emissions from natural terrestrial ecosystems originate in the tropics, where a high supply of mineral nitrogen and favorable soil moisture occur, both supporting conditions for N_2O production (Potter et al., 1996; Repo et al., 2009; Werner et al., 2007). In contrast, low N_2O emissions have been reported from pristine terrestrial ecosystems at northern latitudes (Martikainen et al., 1993; Potter et al., 1996), where cold humid conditions (Shaver et al., 1992) and low atmospheric deposition of N (Dentener et al., 2006) slow the mineralization of organic matter. Therefore, plants and microorganisms compete for available nitrogen, which is scarce. Biological processes are generally N limited in northern latitudes (Jonasson et al., 1999), leading to low N_2O emissions from these systems. However, such a view is now changing for certain permafrost-affected systems as outlined below. For further details see section 4.2.3 Dissimilatory nitrate reduction and associated organisms in Horn and Hetz, 2021.

1.4 Major goals

Cryoturbated peat circles of the Arctic tundra are “hot spots” of N_2O emissions, contrary to the surrounding unturbated peat plateau soil (Marushchak et al., 2011; Repo et al., 2009). N_2O, also known as laughing gas, is a potent greenhouse gas with an atmospheric lifetime of approximately 114 years and a global warming potential about 300-fold higher than CO_2 (Forster et al., 2007; Spahni et al., 2005). Permafrost affected systems are considered as reactive concerning global warming (Stocker et al., 2018). New, acid-tolerant nitrate reducers and denitrifiers inhabit these systems and are potential sources of N_2O emissions. The reduction of NO_3^- to NO_2^- is part of the denitrification process. Denitrification is the sequential reduction of N-oxides like NO_3^- and NO_2^- to N_2O and molecular N_2 via NO. N_2O is formed as an intermediate during denitrification, but, depending on denitrification regulation, can also be consumed and further reduced to N_2 (Zumft, 1997). Major factors influencing N_2O production and consumption are the availability of nitrate and oxygen, as well as pH. It is assumed that the ratio of N_2O to N_2 rises as the pH drops below 6.5, therefore leading to an increase in N_2O emissions at low pH (Cuhel et al., 2010; Simek and Cooper, 2002). It is furthermore assumed that the N_2O-reductase is inhibited at low pH and therefore N_2O is the emitted end product of denitrification (Liu et al., 2014). Even though denitrification is a major N_2O-forming process and denitrification is an important process controlling N_2O-emissions in peat soils, the ecophysiology of active denitrifiers is mostly unknown. Therefore, denitrifiers and nitrate reducers associated with N_2O-emissions, as well as diverse environmental factors, were investigated.

The main hypothesis of this work was that the ecophysiology of new and acid-tolerant denitrifier communities affects the emission of N_2O from cryoturbated peat circles. For this purpose, (i) the phylogeny of active, organic acid utilizing denitrifiers based on 16S rRNA, (ii) the relative effect of bacteria and archaea, and (iii) the effect of environmental factors on new, acid-tolerant key-denitrifiers in cryoturbated peat circles was investigated and identified. Furthermore (iv) key-denitrifiers were isolated and characterized.

1.5 Structural note

The research objectives were addressed in four different experiments, represented as manuscripts and discussed therein. Original ideas for the experiments and incubations set ups

were conceived together with the co-authors of these manuscripts. Experiments were conducted by myself, as well as analysis, if not stated otherwise. I contributed significantly to the discussion and interpretation of results, first drafts of manuscripts were written by me and refined with the co-authors.

2 List of Publications and manuscripts

2.1 Published articles in peer-reviewed journals

Hetz SA, Poehlein A, Horn MA. Whole-Genome Sequences of Two New *Caballeronia* Strains Isolated from Cryoturbated Peat Circles of the Permafrost-Affected Eastern European Tundra. Cameron Thrash J (ed.). *Microbiol Resour Announc* 2020;**9**:30–2.

2.2 Published book chapters

Horn MA, Hetz SA. Microbial nitrogen cycling in permafrost soils: implications for atmospheric chemistry. In: Liebner S, Ganzert L (eds.). *Microbial Life in the Cryosphere and Its Feedback on Global Change*. Berlin, Boston: De Gruyter, 2021.

2.3 Manuscripts in preparation

Hetz SA, Horn MA (2020). *Burkholderiaceae* are key ^{13}C-acetate assimilators during complete denitrification in acidic cryoturbated peat circles of the arctic tundra. *Front Microbiol*

Hetz SA, Horn MA (2021). Key denitrifiers and acetate assimilators in permafrost affected acidic peatlands under nitrate reducing conditions.

Hetz SA, Horn MA (2021). Nitrate fuels pH dependent complete denitrification in acidic permafrost affected peatland.

2.4 Published abstracts at national and international conferences

1. Hetz SA, Horn MA (2016). Nitrous oxide production in peat circles of the arctic tundra is driven by available organic carbon limited denitrifiers. **16th International Symposium on Microbial Ecology** (ISME-16).

Pristine permafrost-affected acidic peat circles of the Eastern European Tundra contain up to 2 mM nitrate and emit nitrous oxide (N_2O) in the range of heavily fertilized agricultural soils. N_2O is a greenhouse gas and ozone depleting substance. Thus, its origin and fate are of major concern. During anoxia, denitrification, which is the sequential reduction of nitrate via nitrite, nitric oxide, and N_2O to dinitrogen gas (N_2), is the main process yielding N_2O. Denitrification is regulated by organic carbon to nitrate ratios, pH, and the N_2O/N_2 ratio. The assembly of a functional N_2O-reductase of neutrophilic model denitrifiers is blocked at acidic pH. Diverse novel denitrifiers are associated with the N_2O production of peat circles and it is hypothesized that peat circle denitrifiers are (i) adapted to low pH and capable of complete denitrification, and (ii) operating under substrate limitation. Anoxic slurry incubations were conducted at *in situ* near pH 4. The effect of [^{13}C]- and [^{12}C]-acetate on denitrification was tested with and without nitrate and with and without acetylene. Acetate with nitrate stimulated denitrification by 150% and nitrate stimulated acetate consumption concomitant to CO_2 production rates by 200% relative to control treatments. In the absence of acetylene, nitrate was consumed but N_2O was not detectable, suggesting complete denitrification. Differential RNA stable isotope probing is ongoing to identify the denitrifying key players in peat circles. The data indicate that peat circle denitrifiers produce large amounts of nitrate derived N_2 via complete denitrification at pH 4 under substrate limited conditions.

2. Hetz SA, Horn MA (2016). Denitrifiers limited by available organic carbon drive nitrous oxide production in peat circles of the arctic Tundra despite their capability for nitrous oxide consumption. **XI. International Conference on Permafrost** (ICOP 2016).

Pristine permafrost-affected acidic peat circles (pH approximates 4) in the Eastern European Tundra have recently been discovered to harbor up to 2 mM of pore water nitrate (NO_3) and emit significant amounts of nitrous oxide (N_2O) in the range of heavily fertilized agricultural fields. N_2O is a greenhouse gas and ozone depleting substance. Thus, processes releasing and consuming N_2O are of major concern. Under anoxic conditions, N_2O is primarily produced by denitrification, which is the sequential reduction of nitrate via nitrite, nitric oxide, and N_2O to dinitrogen gas (N_2) in the absence of oxygen. Denitrification can act as both source and sink of N_2O. Denitrifiers are facultative aerobes that respire N-oxides rather than oxygen when oxygen becomes limited. Diverse new denitrifiers are associated with the N_2O production of peat circles.

Microbially available organic carbon to nitrate ratios and pH regulate denitrification and the N_2O/N_2 ratio. High nitrate and pH-values lower than 6.5 increase the N_2O/N_2 ratio. Indeed, the assembly of a functional N_2O-reductase of neutrophilic model denitrifiers is blocked at acidic pH.

It is hypothesized that (i) peat circle denitrifiers are adapted to low pH and capable of complete denitrification to N_2, and (ii) high nitrate pore water concentrations in and N_2O fluxes of peat circles are due to denitrifiers operating under substrate (i.e., microbially easily degradable organic carbon) limited conditions. Anoxic slurry incubations with peat circle soil were conducted at *in situ* near pH 4 and under more neutral conditions at pH 6. Soil slurries were supplemented with and without nitrate in the presence or absence of acetylene (inhibitor of the N_2O-reduction to N_2). Supplemental nitrate was quickly consumed and N_2O produced in the absence of oxygen at both pH-values. In treatments with acetylene, almost 100 % of supplemented nitrate-N was recovered in N_2O-N. N_2O was essentially not detected in the absence of acetylene. Ammonium, ferrous iron, sulfate and methane remained stable throughout the incubation or were below the detection limit, indicating that dissimilatory nitrate reduction to ammonium, iron and sulfate reduction as well as methanogenesis were marginal. Thus, the denitrifier communities present in peat circles are capable of complete denitrification at low pH.

In a second set of incubations, the effect of [^{13}C]- and [^{12}C]- acetate on denitrification was tested in anoxic soil slurries at pH 4 with and without nitrate and in the presence of acetylene. Substrates were supplemented in pulses. Acetate was consumed without appreciable delay with nitrate and stimulated denitrification by 150% relative to nitrate only treatments. Nitrate stimulated acetate consumption and CO_2 production rates by 200% relative to acetate only treatments. Recovery of [^{13}C]-acetate carbon in [^{13}C]O_2 in nitrate and acetate supplemented slurries approximated 30-40%. [^{13}C]-labeled organic acids were insignificant, suggesting a substantial assimilation of acetate carbon by peat circle microbes. Recovery of nitrate-N in N_2O-N approximated 50%, likewise indicating assimilation. 65% of the total CO_2 was [^{13}C]O_2, suggesting that acetate carbon was preferentially dissimilated relative to peat derived organic carbon. In the absence of acetylene, results were similar to incubations in the presence of acetylene with the exception that N_2O was not detectable, suggesting complete denitrification. Experiments are ongoing to identify the denitrifying key players in peat circles by stable isotope probing.

The combined data indicate that peat circle denitrifiers are substrate limited, thus producing large amounts of nitrate derived N_2O despite their remarkable capability to reduce N_2O and complete denitrification at pH 4.

3. Hetz SA, Horn MA (2018). Complete denitrification at pH 4 in peat circles of the arctic Tundra is primarily driven by acetate assimilating *Burkholderiaceae*. **17th International Symposium on Microbial Ecology** (ISME-17).

The assembly of a functional nitrous oxide (N_2O) reductase of classical neutrophilic model denitrifiers is impaired at pH < 6 resulting in increased N_2O/N_2 (dinitrogen gas) ratios. Accordingly, acidic peat circles (pH 4) in the Eastern European Tundra, with up to 2 mM pore water nitrate, emit the greenhouse gas N_2O like heavily fertilized agricultural soils in temperate regions. The main process yielding N_2O under anoxic conditions is denitrification, i.e. the sequential reduction of nitrate to N_2O and N_2. Organic carbon to nitrate ratios and pH are crucial factors impacting denitrification and N_2O/N ratios. Active key denitrifiers of peat circles are important but hitherto unknown. Thus, it is hypothesized that acid tolerant peat circle denitrifiers are new, impaired by pH and unable to reduce N_2O. Anoxic microcosms +/- supplemental nitrate and +/- acetylene (N_2O reductase inhibitor) at *in situ* pH 4 were used to test the effect of [^{13}C]- and [^{12}C]-acetate on denitrification and N_2O production. Relative to unsupplemented controls, nitrate alone stimulated N_2O production by 1000 % and supplemental acetate with nitrate stimulated N_2O production by 330 %, with rather than without acetylene, suggesting complete denitrification at pH 4. *Burkholderiaceae*, other Proteo-, and Actinobacteria as well as Verrucomicrobia, were identified as key acetate assimilating denitrifiers in peat circles via 16S rRNA SIP. Collective data indicate that peat circles host new complete denitrifiers capable of N_2O reduction at pH 4 that operate under substrate limitation in peat circles and thus produce large amounts of nitrate derived N_2O.

4. Hetz SA, Horn MA (2018). *Burkholderiaceae* are primary acetate assimilating denitrifiers in peat circles of the arctic tundra capable of complete denitrification at pH 4. **70th Annual Conference of the Association for General and Applied Microbiology** (VAAM 2018).

Acidic peat circles (pH 4) in the Eastern European Tundra harbor up to 2 mM nitrate and emit the greenhouse gas nitrous oxide (N_2O) like heavily fertilized agricultural soils in temperate regions. The sequential reduction of nitrate via nitrite, nitric oxide, and N_2O to dinitrogen gas (N_2), called denitrification, is the main process yielding N_2O under anoxic conditions. Crucial factors altering denitrification and impacting the N_2O/N_2 ratio are organic carbon to nitrate ratios (OC/N) and pH. Low pH (< 6) blocks the assembly of a functional N_2O-reductase of classical neutrophilic model denitrifiers and like a low OC/N results in increased N_2O/N_2 ratios. Active key denitrifiers of peat circles are important but unknown to date. Thus, it is hypothesized that peat circle denitrifiers are (i) new, (ii) adapted to low pH and capable of complete denitrification and (iii) operate under substrate limitation. The effect of [^{13}C]- and [^{12}C]-acetate on denitrification was tested in anoxic microcosms +/- supplemental nitrate and +/- acetylene at *in situ* near pH 4. Acetate with nitrate stimulated denitrification by 150% and nitrate stimulated acetate consumption and CO_2 production rates by 200% relative to unsupplemented controls. In the absence of acetylene, N_2O was not detectable, suggesting complete denitrification at pH 4. 16S rRNA SIP coupled to Illumina MiSeq v3 amplicon sequencing suggested *Burkholderiaceae*, and other Proteo-, Actinobacteria as well as Verrucomicrobia as key acetate assimilating denitrifiers in peat circles. The combined data indicate that peat circle denitrifiers are operating under substrate limiting conditions due to recalcitrant old peat material and produce large amounts of nitrate derived N_2O despite their capacity for complete denitrification at pH 4.

5. Hetz SA, Horn MA (2018). Acetate assimilating *Burkholderiaceae* from acidic peat circles of the arctic Tundra drive N_2O consumption. **23rd European Nitrogen Cycle Meeting** (ENC 2018).

Acidic peat circles (pH 4) in the Eastern European Tundra harbor up to 2 mM pore water nitrate and emit the greenhouse gas N_2O like heavily fertilized agricultural soils in temperate regions. Denitrification, i.e. the sequential reduction of nitrate to N_2O and N_2, is the main process yielding N_2O under anoxic conditions. Crucial factors impacting denitrification and N_2O/N_2 ratios are organic carbon to nitrate (OC/N) ratios and pH. The assembly of a functional N_2O reductase of classical neutrophilic model denitrifiers is blocked at pH < 6 resulting in increased N_2O/N_2 ratios. Active key denitrifiers of peat circles are important but unknown to date. Thus, it is hypothesized that acid tolerant peat circle denitrifiers are new, impaired by pH and unable to reduce N_2O. Anoxic microcosms ± supplemental nitrate and ± acetylene at *in situ* near pH 4 were used to test

the effect of [^{12}C]- and [^{13}C]-acetate on denitrification and N_2O production. Relative to unsupplemented controls with endogenous nitrate, acetate with nitrate stimulated denitrification by 30%. In the absence of acetylene, N_2O was not detectable, suggesting complete denitrification at pH 4. Comparative 16S rRNA SIP coupled to Illumina MiSeq amplicon sequencing suggested *Burkholderiaceae*, other Proteo-, and Actinobacteria as well as Verrucomicrobia as key acetate assimilating denitrifiers in peat circles. Furthermore, data indicate that peat circle denitrifiers operate under substrate limiting conditions due to old peat material, thus producing large amounts of nitrate derived N_2O, yet being capable of N_2O reduction at pH 4.

3 Main results and general discussion

3.1 Recapitulation of findings

In order to test the hypothesis that the ecophysiology of new and acid-tolerant denitrifier communities affects the emission of N_2O from cryoturbated peat circles, different research objectives were deployed. Reduction potentials for NO_3^- and N_2O of acidic permafrost affected peatland soil of the Eastern European Arctic tundra were investigated. Therefore, two soil types, peat circle (PC) and surrounding peat plateau (PP), were sampled and soil slurries were incubated under nitrate reducing conditions at *in situ* near pH 4 and more neutral pH 6. For an easy differentiation between complete denitrification to N_2 and incomplete denitrification to N_2O, the acetylene-inhibition technique was used. Acetylene inhibits the N_2O reductase, leading to N_2O as the released end product of denitrification during incubations (Yoshinari et al., 1977). Furthermore, to assess the phylogeny of active, organic acid utilizing denitrifiers the stable isotope probing (SIP) technique coupled to 16S rRNA analysis via Illumina MiSeq amplicon sequencing was applied. Therefore, PC soil slurry microcosms were either directly supplemented with (labeled) ^{13}C-acetate and incubated, leaving indigenous NO_3^- to be used by microorganisms, or soil slurries were preincubated and then supplemented with both NO_3^- and ^{13}C-acetate at the start of incubation. In an additional approach, key denitrifiers of PC and PP soil were isolated.

Depletion of supplemented NO_3^- to PC soil slurry microcosms occurred faster at pH 6 than at pH 4, though at both pH values the end product of denitrification was N_2. In addition, detected NH_4^+ at the end of incubation was near detection limit, recovery of supplemented NO_3^- as $NH_4^+ < 1\%$. The recovery as N_2-N from supplemented NO_3^- was 15% higher at pH 4 than at pH6, reaching approximately 64% and 49%, respectively. Fermentation potentials, as indicated by negligible amounts of organic acids detected, were low for PC microcosms. In PP soil slurry microcosms depletion of supplemented NO_3^- occurred faster at pH 6 as well, but still ~ 5 times slower than in PC microcosms (2.5 vs 13 days) and with delay. Again, the main end product of denitrification was N_2, with a recovery of 70% from supplemented NO_3^-, though N_2O accounted for an additional 12%. PP microcosms incubated at *in situ* near pH 4 emitted N_2O independent of acetylene, suggesting N_2O as the sole end product of denitrification that accounted for approximately 70% of supplemented NO_3^-. The fermentation potential of PP soil was higher, since organic acids were detected in higher, though not significant, amounts compared to PC

incubations. PP microcosms from pH 6 showed the highest fermentation potential with the detection of produced formate and acetate after the incubation period.

PC microcosms with *in situ* near pH 4 showed depletion of both NO_3^- and acetate without delay. Based on the acetylene-inhibition technique, the sole end product of denitrification was N_2, approximating 40% of supplemented NO_3^-, and suggesting complete denitrification at acidic pH. N recovery as NH_4^+ was < 0.1%. Supplemented labeled ^{13}C-acetate could be recovered in the form of ^{13}C-CO_2 reaching ~30%, leaving a substantial amount of labeled ^{13}C for potential incorporation into nucleic acids for the SIP analyses. After incubations were completed and terminated, phylogenetic analysis, i.e. Illumina MiSeq amplicon sequencing and bioinformatic processing, was performed. Sequences retrieved by DNA-SIP using a universal primer pair for sequence analysis, revealed a relative abundance of Archaea within samples of up to 7%, though Bacteria dominated throughout all samples with mean relative abundances of 94%. Archaeal operational taxonomic units (OTUs), as revealed by RNA-SIP, all affiliated with the phylum Crenarchaeota and were dominated by the order Nitrososphaerales, independent of incubation conditions. The bacterial community was more diverse than the archaeal one, with the pre-incubation community being dominated by the classes Alpha- and Gammaproteobacteria, Verrrucomicrobiae, and Bacteroidia. After the incubation members of the family *Burkholderiaceaea* (Gammaproteobacteria) had the highest relative abundances, reaching up to 50% in heavy fractions of ^{13}C-acetate supplemented microcosms, independent of NO_3^- supplementation. Key denitrifiers, as indicated by differential analysis of count data, were OTUs affiliated with the genus *Mucilaginibacter* within the family *Sphingobacteriaceae.*

The isolation approach resulted in the finding of two isolates belonging to a new *Caballeronia* species, belonging to the *Burkholderiaceaea*, the highly abundant family at the end of SIP incubations.

3.2 General discussion

Slow mineralization of organic matter (Shaver et al., 1992), combined with a general limitation of bioavailable N (Jonasson et al., 1999), lead to the assumption that permafrost regions of the Arctic are insignificant in terms of N_2O emissions. When, a decade ago, bare cryoturbated peat circles in the subarctic Eastern European Russian tundra were reported to emit N_2O in the range

of heavily fertilized and tropical rainforest soils (Denman et al., 2007; Marushchak et al., 2011; Potter et al., 1996; Repo et al., 2009; Siljanen et al., 2019; Werner et al., 2007), this dogma needed to be reconsidered.

Identified key reasons for the large N_2O emissions from PC are the lack of vegetation, a low C/N ratio of the peat and a favorable moisture content (Repo et al., 2009). Microbial processes in PC depend on N from the mineralization of organic matter, since there is no considerable external N input from such as fertilization or atmospheric deposition (Marushchak et al., 2011). Gross N mineralization from bare PC soil is up to 6-fold higher than from vegetated PP soil. Due to the absence of plants, the mineralized N is readily available for microorganisms without competition (Marushchak et al., 2011). Nitrifiers are directly profiting from the high mineralization rates and the low NH_4^+/NO_3^- ratio in PC soil is indicative for nitrification derived NO_3^- that is further available for denitrification (Marushchak et al., 2011). Previous studies showed that the lack or even the removal of plants can results in an increased mineral N content, right up to increased N_2O emissions from soils (Maljanen et al., 2004; Marushchak et al., 2011). This phenomenon can also be reversed, as a restored peatland showed a decrease in N_2O emissions after plant coverage was increased (Silvan et al., 2005). The low C/N ratio in PC soil can likely be attributed to the minerotrophic origin of the peat, an advanced stage of decomposition, where N is enriched relative to C when peat is decomposed (Kuhry and Vitt, 1996). Another factor controlling N_2O emissions is soil moisture. It regulates the redox conditions, hence nitrification and denitrification (Klemedtsson et al., 1988). The optimum soil moisture for denitrification is between 60-70%, whereas nitrification occurs as main source of N_2O emissions from drier soils (Abbasi and Adams, 2000; Bateman and Baggs, 2005; Dobbie et al., 1999). Therefore, PC soil with a soil moisture of approximately 70% as used for the experiments, provides ideal conditions for denitrifiers. Altogether, the lack of vegetation, relatively high N mineralization rates, high NO_3^- concentrations, low C/N ratio and soil moisture content of ~ 70%, likely result in high *in situ* N_2O emission from cryoturbated PC soil (Diáková et al., 2016; Marushchak et al., 2011; Palmer et al., 2010; Repo et al., 2009). But why is N_2O and not N_2 the emitted end product from PC soil? It is hypothesized that internal N-cycling fuels the large N_2O emission, since a large proportion of excess N is available for denitrifiers (Diáková et al., 2016), hence, NO_3^- will probably not be limited for PC denitrifiers and it is not mandatory for energy yields to utilize N_2O as terminal electron acceptor (Zumft, 1997; Zumft and Kroneck, 2007).

Compared to PC soil, NO_3^- concentration of vegetated PP soil is generally low and the NH_4^+/NO_3^- ratio is high, hence the NO_3^- availability for denitrifiers is restricted (Marushchak et al., 2011; Repo et al., 2009). Denitrifiers not only compete for NO_3^- with plants, but also with microorganisms capable of dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje et al., 1983). Factors regulating the differential electron flow towards denitrification and DNRA are NO_3^- concentration, pH, and the C/N ratio. DRNA being favored under NO_3^- limited conditions (Bleakley and Tiedje, 1982; Fazzolari et al., 1998). When comparing energy yields from DNRA and denitrification, the potential energy per electron donor (e.g. acetate) is higher for denitrification, but energy yield per mole NO_3^- is slightly higher for DNA (Tiedje et al., 1983). Under *in situ* conditions, DNRA might therefore outcompete denitrification in PP soil. Nevertheless, results from microcosm experiments suggest supplemented NO_3^- stimulated the indigenous denitrifier community of PC soil and lead to the release of N_2O or N_2, dependent on the pH, as end products of denitrification. This hypothesis is supported by the findings of a structural gene marker analysis in PP soil that revealed the genetic potential of the PP microbial community for (complete) denitrification (Palmer et al., 2012). Though, the abundances of detected marker genes for denitrification as well as the denitrifier communities differ phylogenetically from PC and PP soil (Palmer et al., 2012). In conclusion, contrasting N_2O emissions from cryoturbated bare PC and unturbated vegetated PP soil (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009; Siljanen et al., 2019) are the result of low NO_3^- concentrations of vegetated PP soil and the dissimilar denitrifiers communities, rather than pH (Bru et al., 2011).

Nevertheless, pH is a well-known factor regulating denitrification and its emitted products. Neutrophilic model organisms like *Paracoccus denitrificans* accumulate and release N_2O at pH < 7 (Bergaust et al., 2010). Results from recent studies lead to the hypotheses that the main cause preventing N_2O reduction in soils with acidic pH is the preclusion of a successful assembly of a functional N_2O reductase (Liu et al., 2014). Contrasting to field measurements and *in situ* incubation experiments (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009; Siljanen et al., 2019), the end product of denitrification in all PC microcosm experiments was N_2, as indicated by the acetylene-inhibition technique, and not N_2O. There are reports of acidic peatlands acting as temporary sinks for atmospheric N_2O from both *in situ* measurements and microcosm experiments (Kolb and Horn, 2012; Marushchak et al., 2011; Palmer et al., 2010; Palmer and Horn, 2012). How is this possible, if the assembly of a N_2O reductase is impaired by low pH? The explanation for this phenomenon might be microsites. Active denitrifiers, when

present in high density, might cluster together, similar to biofilm formation, resulting in a higher pH within these microsites which enables the assembly of a functional N_2O reductase (Liu et al., 2014). Though, in general, the ratio of N_2O to total N gases emitted is higher in acidic soils compared to more pH-neutral soils (Simek and Cooper, 2002). Furthermore, the phylogenetic diversity, reflected in the soil microbial community structure, is affected by pH (Fierer and Jackson, 2006; Lauber et al., 2009). *Rhodanobacter* sp., e.g. *Rhodanobacter denitrificans*, are known acid tolerant bacteria capable of complete denitrification and have been reported from acidic subsurface environments associated with denitrification (Green et al., 2010; Van Den Heuvel et al., 2010). Approximately two-thirds of cultured denitrifiers harbor the gene for nitrous oxide reductase (*nosZ*) and are capable of complete denitrification, i.e. the utilization of N_2O as terminal electron acceptor when NO_3^- is limited (Zumft, 1997; Zumft and Kroneck, 2007). Collected data from the current studies, as well as from previous studies (Palmer et al., 2012), suggest that N_2O will be used as terminal electron acceptor by acid-tolerant denitrifiers in PC soil, hence PC soil can act as temporary sink for N_2O emissions, though this might not be relevant under *in situ* conditions due to the high supply of NO_3^-. Well known for their direct contribution to N_2O emissions during nitrification under (micro)oxic conditions in Arctic soils are ammonia oxidizing Archaea (Siljanen et al., 2019). Members of the Thaumarchaeota, recently re-integrated into the Crenarchaeota, include ammonia oxidizers preferring acidic pH < 5.5 and low NH_4^+ concentrations (De La Torre et al., 2008; Gubry-Rangin et al., 2010; Lehtovirta-Morley et al., 2011; Prosser and Nicol, 2008), *in* situ conditions that can be found in PC soil (Marushchak et al., 2011; Repo et al., 2009). Niche differentiation of ammonia oxidizing Archaea, as reported from Arctic soils, is shaped by soil moisture and N content, comprising a high β-diversity of Thaumarchaeota/Crenarchaeota (Alves et al., 2013). Positive correlations between NO_3^- concentrations and *amoA* (ammonia monooxygenase subunit A) gene abundance were reported from permafrost affected peat soil surfaces from Finland and Siberia that emit N_2O in the range of or even higher than managed peatland soils from northern countries (Siljanen et al., 2019). In the conducted SIP experiments, the archaeal community structure was not affected by any of the applied incubation conditions, though the importance of ammonia oxidizing Archaea as *in situ* source of N_2O emissions from northern peatlands, including cryoturbated PC soil, has to be considered and needs to be investigated further.

The microbial community from PC soil as reported from SIP experiments before supplementation is consistent with those from previous reports (Palmer et al., 2012). After incubation, the OTU with the highest relative abundance affiliated with *Burkholderiaceae*.

Screening of bacterial genomes, including several genomes from *Burkholderiaceae* representatives, revealed the presence of *nosZ* as well as *nirK* genes in diverse *Burkholderiaceae* (Sanford et al., 2012), thus the genetic potential of these organisms for denitrification. *Burkholderia* sp. isolated from *Sphagnum* tissue covering a Finnish acid mire, were reported to have their pH optimum at ~5 and produce N_2O after NO_3^- supplementation during incubation, independent of the presence or absence of acetylene. Every isolate harbored the gene for the nitrate reductase NarG, while neither typical nor atypical N_2O reductase genes could be amplified and detected. Suggesting these *Burkholderia* sp. as incomplete denitrifiers (Nie et al., 2015). Isolated new *Caballeronia* strains within the *Burkholderiaceae* from PC soil encode diverse nitrate and nitrite reductases, as well, but no genes encoding for a N_2O reductase could be detected (Hetz et al., 2020). Data emphasizes on *Burkholderiaceae* being important nitrate reducers and potential key players for high *in situ* N_2O emissions from PC soil. Potential N_2O reducers in microcosm experiments with PC soil are *Mucilaginibacter*. These bacteria have been found and isolated in acidic and or permafrost-affected soils, including an acidic *Sphagnum* peat bog in Siberia (Pankratov et al., 2007), the Arctic tundra of Finnish Lapland (Männistö et al., 2010), and the High Arctic tundra of Norway (Jiang et al., 2012). Though, the role of *Mucilaginibacter* in nitrate reduction and denitrification in these soils has yet to be determined, current results suggest participation in nitrate reduction and denitrification of this genus in investigated PC soil under incubation conditions. Known for their ability to reduce N_2O to N_2 under acidic conditions are members of the genus *Rhodanobacter* (Van Den Heuvel et al., 2010) and nitrite reductase sequences closely related to *Rhodanobacter* sp. were previously retrieved from an acidic (pH ~4) Finnish palsa peat, with relative abundances of OTUs in amplicon libraries of 5% in the upper 20 cm of soil (Palmer and Horn, 2012). Relative abundances of *Rhodanobacter* sp. were lower in the current studies, reaching up to 2% in total as revealed by DNA SIP. Again, the role of these organisms as N_2O-reducers in PC soil has yet to be determined, though under applied incubation conditions *Rhodanobacter* sp. might have contributed to the reduction of N_2O to N_2.

In conducted microcosm incubations for SIP analysis, N_2O could only be detected in the presence of acetylene, when the final step of denitrification, the reduction of N_2O to N_2, was inhibited. Investigated apparent Michaelis-Menten kinetics of nitrate-dependent denitrification in anoxic microcosms revealed that PC denitrifiers were saturated with less than half of the NO_3^- concentrations occurring *in situ*, therefore suggesting a limitation of electron donor availability that restricts denitrification in cryoturbated PC soil (Palmer et al., 2012). Hence, when acetate

was supplemented for SIP analysis, a possible electron donor limitation was avoided and denitrification not restricted, leading to complete denitrification under these incubation conditions (Figure 2). This is supported by a study conducted with the bacterial denitrifier *Alcaligenes faecalis* in a steady state culture. By the addition of the electron donor acetate to a starved culture, the *A. faecalis* culture immediately reduced accumulated NO_2^-, and N_2 production was increased (Schalk-Otte et al., 2000). Therefore, *in situ* electron donor limitation in cryoturbated PC soil might favor the emission of N_2O, despite the molecular potential of the microbial community for the further reduction of N_2O to N_2.

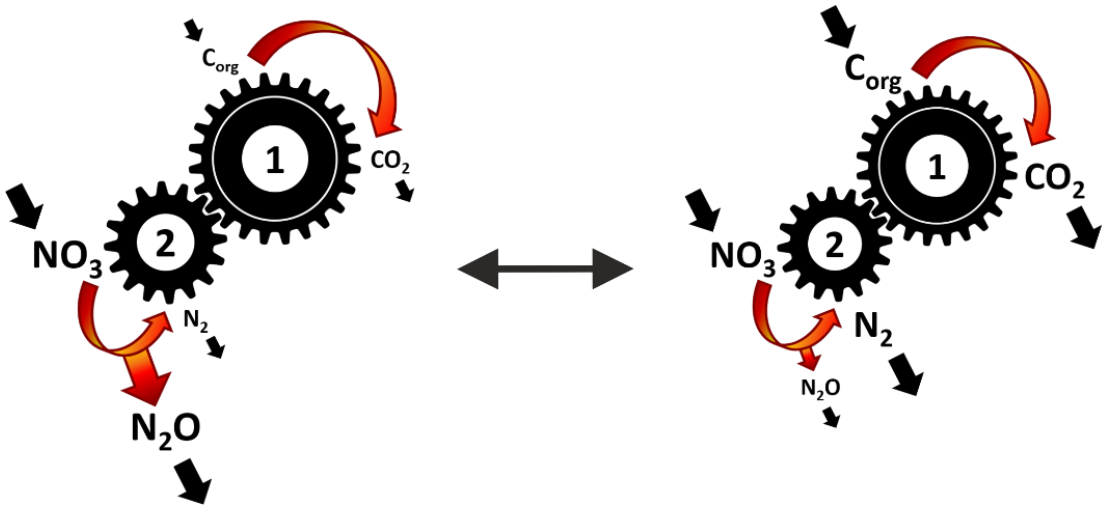

Figure 2: Proposed model of environmental factors determining N_2O emissions from cryoturbated peat circles. Width and length of arrows and font size indicative for substrate input and product output. C_{org} – organic carbon.

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5 Manuscripts

5.1 Published articles in peer-reviewed journals

5.1.1 Whole-Genome sequences of Two New *Caballeronia* Strains Isolated from Cryoturbated Peat Circles of the Permafrost-Affected European Tundra

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GENOME SEQUENCES

Whole-Genome Sequences of Two New *Caballeronia* Strains Isolated from Cryoturbated Peat Circles of the Permafrost-Affected Eastern European Tundra

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ABSTRACT Annotated genomes of *Caballeronia* strains SBC1 and SBC2 from acidic permafrost suggest a new species with a facultative lifestyle via oxygen and nitrate respiration. Thus, a contribution to nitrogen cycling in cold and low-pH environments is anticipated.

Cryoturbated peat circles (PCs) (62°57′E, 67°03′N) contain up to 2 mM pore water nitrate, emit large amounts of nitrous oxide (1, 2), and host new nitrate reducers (3). SBC1 and SBC2 were isolated from serial PC sediment (pH 4.2) dilutions by plating on semisolid modified R2A medium (1:10 diluted DSMZ 830 medium, 0.5% [wt/vol] K_2HPO_4, 7 g liter^{-1} Gelrite [pH 6]) and incubating for 7 days at 15°C. Single colonies were picked and purified by restreaking four times onto the same medium.

High-molecular-weight DNA (HWD) for Nanopore sequencing was isolated with the MasterPure complete DNA and RNA purification kit (Biozym, Hessisch Oldendorf, Germany) from cells grown in liquid modified R2A medium (pH 5). HWD quality was checked on a Bioanalyzer 2100 using the DNA 12000 kit (Agilent Technologies, Waldbronn, Germany), and HWD was quantified with the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies GmbH, Darmstadt, Germany); 1.5 μg HWD was used for library preparation employing the ligation sequencing kit 1D (SQK-LSK109) and the native barcode expansion kit (EXP-NBD114, barcode 15; Oxford Nanopore Technologies, Oxford, UK). Sequencing was performed for 72 h on the MinION Mk1B system with a SpotON flow cell R9.4.1 using MinKNOW v19.10.1, with Guppy v3.3.3 for base calling and demultiplexing. Totals of 67,997 reads with an average length of 4,873 bp (N_{50}, 27,443 bp) for SBC1 and 330,181 reads with an average length of 9,102 bp (N_{50}, 15,651 bp) for SBC2 were obtained.

Genomic DNA for Illumina shotgun sequencing was isolated via PCI extraction (4) and checked via spectrophotometry (DS-11; DeNovix, Inc., Wilmington, DE, USA). Illumina shotgun libraries were prepared using the Nextera XT DNA sample preparation kit, sequenced on a MiSeq system using reagent kit v3 with 600 cycles (2 × 300 bases; Illumina, San Diego, CA, USA), and resulted in totals of 3,249,515 (SBC1) and 2,281,633 (SBC2) paired-end reads per strain. Illumina reads were quality filtered using Trimmomatic v0.39 (5). Unicycler v0.4.6 (6) was used to perform a hybrid assembly, resulting in a closed circular chromosome (4,010,354 bp) and 4 closed plasmids (280,710 to 1,996,666 bp) for SBC1 and a closed circular chromosome (3,989,243 bp) and 7 closed plasmids (120,676 to 1,990,521 bp) for SBC2, as validated using Bandage v2.1 (7). Coverage was determined using Qualimap v2.2.1 (8) by mapping Illumina and Nanopore reads on the closed genomes using Bowtie 2 v2.3.5.1 (9) and minimap2 (10), respectively. Coverages for SBC1 and SBC2 were 98.4× and 62.4× (Illumina) and 62.8×

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This is contribution 1 from the Institute of Microbiology, Leibniz University Hannover, to gain insights into hitherto unknown nitrate reducers from permafrost sediments.

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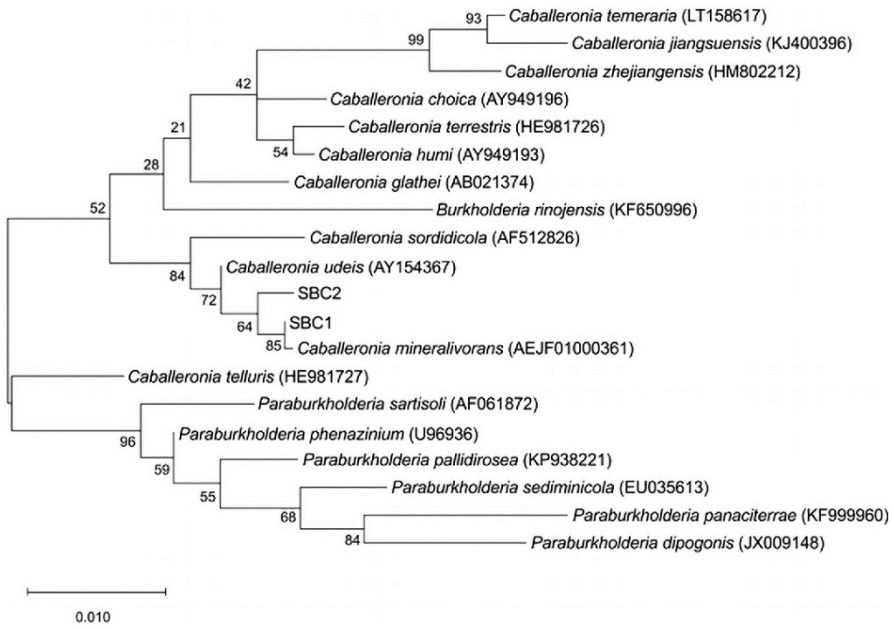

FIG 1 Maximum composite likelihood tree of 16S rRNA genes aligned with MUSCLE (17) and rooted by midpoint rooting. Branches are scaled in terms of the expected number of substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying the neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood and the Tamura-Nei model (18) and then selecting the topology with a superior log likelihood value. The closest relative of SBC1 and SBC2 was *C. mineralivorans* from a fungal ectomycorrhizosphere in acidic and nutrient-poor forest soil (19). Tree construction was conducted with MEGA X, and GenBank accession numbers of 16S rRNA gene sequences are provided in parentheses next to species names (20).

and 162.8× (Nanopore), respectively. The overall GC contents (BioEdit v7.0.5.3 [11]) of SBC1 and SBC2 were 59.69% and 59.52%, respectively. Annotation with Prokka v1.14.0 (12) revealed the presence of 5 rRNA operons for both genomes, with 8,050 and 8,520 predicted protein-encoding genes and 59 and 60 tRNA genes for SBC1 and SBC2, respectively. Default parameters were used for all software.

SBC1 and SBC2 were affiliated with *Caballeronia mineralivorans* (Fig. 1); 53 to 57% of SBC1 and SBC2 genomes aligned with the *C. mineralivorans* genome, and the average nucleotide identity using the MUMmer algorithm (ANIm) (JSpeciesWS [13]) was 88.3%. The ANIm of SBC1 compared to SBC2 was 99.7%, suggesting that SBC1 and SBC2 represent a new species of the genus *Caballeronia* (14, 15). SBC1 and SBC2 encode multiple nitrate reductases of the *narG*, *napA*, and *nasA* types, as well as nitrite (*nirBD*) and nitric oxide (*norV*) reductases (Pathway Tools v23.0 [16]).

Data availability. These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession numbers CP049156.1 (chromosome) and CP049157.1 to CP049160.1 (plasmids) for SBC1 and under the accession numbers CP049316.1 (chromosome) and CP049317.1 to CP049323.1 (plasmids) for SBC2. BioProject accession numbers for SBC1 and SBC2 are PRJNA604524 and PRJNA604525, and SRA accession numbers are SRP250914 and SRP250916, respectively.

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5.2 Published book chapters

5.2.1 Microbial nitrogen cycling in permafrost soils: Implications for atmospheric chemistry

Horn MA, Hetz SA. Microbial nitrogen cycling in permafrost soils: implications for atmospheric chemistry. In: Liebner S, Ganzert L (eds.). Microbial Life in the Cryosphere and Its Feedback on Global Change. Berlin, Boston: De Gruyter, 2021. p.53-112.

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5.3 Manuscripts in preparation

5.3.1 *Burkholderiaceae* are key ^{13}C-acetate assimilators during complete denitrification in acidic cryoturbated peat circles of the arctic tundra

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Burkholderiaceae are key ^{13}C-acetate assimilators during complete denitrification in acidic cryoturbated peat circles of the arctic tundra

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Key words: 16S rRNA stable isotope probing, nitrous oxide, climate change, permafrost affected soils

Running title: Acetate assimilators in cryoturbated peat

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Manuscript max 12 000 words

Abstract

Cryoturbated peat circles (pH 4) in the Eastern European Tundra harbor up to 2 mM pore water nitrate and emit the greenhouse gas N_2O like heavily fertilized agricultural soils in temperate regions. The main process yielding N_2O under oxygen limited conditions is denitrification, that is the sequential reduction of nitrate to N_2O and N_2. N_2O reduction to N_2 is impaired by pH <6 in most model denitrifiers and environments. Key denitrifiers of peat circles are important but their identity on 16S rRNA level is hitherto unknown. Thus, we hypothesized that the peat circle community is new and essentially unable to reduce N_2O due to a low *in situ* pH. 16S rRNA analysis indicated a diverse active community primarily composed of the bacterial classes Alphaproteobacteria, Acidimicrobiia, Acidobacteria, Verrucomicrobiae and Bacteroidia, as well as the archaeal class Nitrososphaeria. Euryarchaeota were not detected. $^{13}C_2$- and $^{12}C_2$-acetate supplemented anoxic microcosms with endogenous nitrate and ± acetylene at an *in situ* near pH of 4 were used to assess acetate dependent carbon flow, denitrification and N_2O production. Initial nitrate and acetate were consumed within 6 and 11 days, respectively, and primarily converted to CO_2 and N_2, suggesting complete acetate fueled denitrification at acidic pH. Stable isotope probing coupled to 16S rRNA analysis via Illumina MiSeq amplicon sequencing identified acetate consuming key players of the family *Burkholderiaceae* during complete denitrification. The archaeal community was stable during the incubation. The collective data indicate that peat circles (i) host acid-tolerant denitrifiers capable of complete denitrification at pH 4, (ii) hitherto unidentified parameters rather than pH are responsible for high N_2O emissions *in situ*, (iii) *Burkholderiaceae* are key acetate assimilators during denitrification thus being prominent candidates for acid-tolerant denitrifiers in peat circles.

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential about 300 times higher than CO_2 and a long atmospheric half-life of estimated 120 years (Prather et al., 2015; Stocker et al., 2018). The main source of N_2O is microbial denitrification, i.e. the sequential reduction of nitrate (NO_3^-) or nitrite (NO_2^-) via the intermediates nitric oxide (NO) and N_2O to dinitrogen gas (N_2) under the exclusion of oxygen (Zumft, 1997). Different reductases are involved in this process. The first step in the process is facilitated via the dissimilatory nitrate reductase *narG*, a membrane bound enzyme (Zumft, 1997). The reduction of NO_2^- to NO can be executed by either the cytochrome cd_1 dependent nitrite reductase *nirS* or one of three known types of copper-dependent nitrite reductases *nirK* (Helen et al., 2016; Zumft, 1997). The cytotoxic gas NO can then be further reduced to N_2O via the nitric oxide reductases *cNor*, associated with cytochrome *c*, the copperdependent CuqNOR or the quinol dependent qNOR (Zumft, 2005). The last step of denitrification is catalyzed by the copper-containing N_2O-reductases NosZ, the only known enzyme capable of this reaction (Jones et

al., 2008; Zumft, 1997). Since many organisms only possess the genetic potential to perform parts of the whole denitrification process, truncated forms lacking N_2O reductases exist, which can lead to the release of N_2O not only as intermediate but as end product, contributing to N_2O emissions from soils (Cofman Anderson and Levine, 1986; Stein and Klotz, 2016).

Tropical rainforest soils have the highest known N_2O emission potentials (Potter et al., 1996; Werner et al., 2007). These soils offer ideal conditions for denitrification, with a high supply of mineral nitrogen and an optimum soil moisture (Breuer et al., 2000) . In contrast, permafrost affected soils are traditionally viewed as sources of the greenhouse gas methane rather than N_2O, nitrogen limited and not contributing significantly to the global N_2O budget (Nadelhoffer et al., 1991).Permafrost soils cover approximately 17 % of Earth's surface (Gruber, 2012), and only a decade ago cryoturbated peat circles were found to emit N_2O in the range of temperate agricultural and (sub)tropical rainforest soils during growing season (1.9-31 mg N_2O m^{-2} d^{-1}) (Marushchak et al., 2011; Repo et al., 2009). Such peat circles are thus significant sources of N_2O accounting for up to 0.6% of annual global N_2O emissions (Christensen, 1993; Denman et al., 2007; Repo et al., 2009) . A low C/N ratio of old peat material, an oxic/anoxic interface, the lack of vegetation as competitor for nitrogen, high nitrification activities, and intermediate water content, account for high NO_3^- concentrations of up to 2 mM in the pore water of peat circles, which is one of the main sources of N_2O in soils and readily available for denitrifiers (Repo et al., 2009; Siljanen et al., 2019). A major parameter determining the emission ratio of N_2O/N_2 from soils is pH, leading to an increased release of N_2O relative to N_2 at low pH due to an inhibition of N_2O reduction (Bergaust et al., 2012; Cuhel et al., 2010; Simek and Cooper, 2002) (Cuhel et al., 2010; Simek and Cooper, 2002), suggesting that a low pH is a major reason for high N_2O emissions of peat circles.

Bacterial community analysis revealed that peat circle denitrifiers are only distantly related to known denitrifiers (Palmer et al., 2012). Functional gene analysis identified the genetic potentials for complete denitrification to N_2. Phylogenetic affiliations of *nosZ* genes showed a high relative abundance of Alphaproteobacterial *nosZ* (*Mesorhizobium* sp.), of which 60 % were only distantly related to *nosZ* of cultured microorganisms, indicating a new, specific, and acid-tolerant denitrifier community with little N_2O reduction capacity in these soils (Palmer et al., 2012). In contrast, unturbated vegetated peat soils from the same study site with the same acidic pH, do not emit N_2O *in situ* (Marushchak et al., 2011; Repo et al., 2009). Phylogenetic functional gene data show that denitrifier communities differ between bare cryoturbated and vegetated unturbated peat soils, and are likely accountable for contrasting N_2O emissions between soils rather than soil pH alone (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009). However, functional gene based phylogeny might be biased due to horizontal gene transfer and gene duplication events. 16S rRNA genes as phylogenetic markers are thus preferable for the analysis of community structure and to verify phylogenetic novelty. Interactions of microbes impacting nitrous oxide fluxes, e.g. via competition for electron donors

and carbon sources demand the analysis of the whole microbial community rather than denitrifiers alone.

Denitrifiers use low molecular weight organic carbon (LMWOC) as carbon source and electron donors in many peatland systems and sediments (Boylan et al., 2020; Castaldelli et al., 2013). LMWOC represents common intermediates in the anerobic feed chain (Beulig et al., 2018). The anaerobic feed chain is also referred to as intermediary ecosystem metabolism to highlight the complex network of trophically interacting physiological groups of microorganisms finally leading to methane in the absence of alternative electron acceptors other than CO_2 (Wüst et al., 2009). The intermediary ecosystem metabolism includes hydrolysis of biopolymers to monomers, primary and secondary fermentations, acetogenesis, and finally methanogenesis. Acetate is one of the most often detected intermediates in peatlands and a prominent methane precursor (Zeikus et al., 1975). Methanogenesis and most of the other reactions are catalyzed by methanogenic Euryarchaeota and Bacteria, respectively. When alternative electron acceptors like nitrate are present, intermediary electron and carbon flow is diverged from methanogenesis to nitrate reduction and/or denitrification as terminal electron accepting processes (Tiedje, 1988). Indeed, acetate was shown to stimulate denitrification in peat circles (Palmer et al., 2012). Although acetate is undoubtedly an important intermediate in intermediary ecosystem metabolism, acetate derived carbon and electron flow and divergence to CO_2 and/or nitrate in cryoturbated peat circles is unclear to date. Key players catalyzing such reactions in peat circle sediments are likewise unknown. Thus, we hypothesize that the peat circle community couples acetate consumption to denitrification and is unable to reduce N_2O due to a low *in situ* pH. Therefore, the main objectives of this study were 1) to determine the diversity of peat circle Bacteria and Archaea by 16S rRNA analysis, 2) analyze acetate derived carbon and nitrate derived nitrogen flow in anoxic microcosms, and 3) determine key acetate assimilators during active denitrification by stable isotope probing.

Materials and Methods

Sampling site

The study site is located in the Northeastern European Tundra in Russia within the discontinuous permafrost zone (67°03'N, 62°57'E, 100 m a.s.l.) with a mean annual air temperature of -5.6 °C (Marushchak et al., 2011). Samples were taken from cryoturbated peat circles, which were previously described (Biasi et al., 2014; Hugelius et al., 2011; Repo et al., 2009). Generally, the carbon to nitrogen (C/N) ratio is low (23 ± 2) in cryoturbated peat circles (Repo et al., 2009; Supplementary Methods) and during growing season high amounts of N_2O are emitted (1.9-31 mg N_2O m^{-2} d^{-1}) (Repo et al., 2009). Soil from the upper 10 cm of three different peat circles was sampled in summer 2014, placed in gas-tight ZipLoc bags, and stored at 4 °C until further processing. Experiments were conducted within three months after

sampling. Soil moisture content was determined via weighing soil samples before and after drying at 60 °C for one week, and was 74 %.

Preparation of microcosms, incubation and sampling

Sediment of three sampled peat circles was pooled and homogenized, and larger debris was removed prior to incubation. Soil slurries with an *in situ* near pH of 4.4 were prepared by mixing soil with deionized water (1:12) to a final volume of 300 ml in 500 ml veral bottles. The bottles were sealed with an airtight rubber stopper. Microcosms were prepared in triplicates for each treatment and incubated in the dark at 15 °C. The gas phase consisted of 100 % nitrogen. In order to differentiate between complete and incomplete denitrification to N_2O, microcosms with and without acetylene (10 % vol/vol headspace) were prepared. Acetylene blocks the N_2O reductase, hence N_2O cannot be further reduced to N_2 (Yoshinari et al., 1977). Microcosms were supplemented with 600 µM nitrate as soon as endogenous nitrate was gone. Isotope labeling of microorganisms was initiated by supplementing ^{13}C-labelled [$^{13}C_2$]-acetate (99 atom-%, Sigma-Aldrich, MO, USA) to a final concentration of 400 µM, which was refed three times to maximize ^{13}C-labelling of acetate assimilators. Control microcosms received unlabeled acetate. N_2O, nitrate, and acetate were determined at regular intervals (Palmer et al., 2010). For the microbial community analyses, microcosms were sampled at the start and the end of incubation (16 day time span). 20 ml slurry sample were immediately suspended in 2.5 ml RNA stabilization buffer (100 mM sodium acetate, 100 mM EDTA, pH 5.2) together with 1 ml 20% SDS, 64 µl mercaptoethanol, and 2 ml equilibrated phenol. In order to avoid decomposition of nucleic acids, samples were flash-frozen in liquid nitrogen and stored at -80 °C until further processing.

Analytical methods and statistics

Gases (N_2O, CH_4, CO_2) were measured via gas chromatography coupled to electron capture, flame ionization, and thermal conductivity detection, respectively (Horn et al., 2003a; Hunger et al., 2011; Palmer et al., 2010) [$^{13}C/^{12}C$]-isotope ratios of CO_2 were determined by GC combustion-isotope ratio mass spectrometry (GC-C-IRMS; BayCEER – Laboratories for Isotopic-Biogeochemistry, University of Bayreuth, GER). Liquid samples were analyzed for soluble organic compounds via high performance liquid chromatography (Palmer et al., 2010; Supplementary Materials and Method). Determination of ^{13}C labeled soluble compounds was done via HPLC-ESI-MS (BayCEER – Atmospheric Chemistry, University of Bayreuth, GER). Sulphate, nitrate, nitrite, ammonium, and iron(II) were measured by colorimetric assays (Cataldo et al., 1975; Gadkari, 1984; Harrigan and McCance, 1966). Statistical analyses were performed in OriginPro 2020 version (OriginalLab Corporation, Northhampton, MA, USA). Prior to statistical tests basic data analyses were performed, including visual inspection of all measured variables coupled with the Shapiro–Wilk normality test. To test for the treatment

effect, i.e. differences between controls and supplemented microcosms, analysis of variance (ANOVA) was used.

RNA extraction and density gradient fractionation

Prior to nucleic acid extraction, it was assured that all solutions and glassware were RNAse free by either treatment with DEPC or heat sterilization (180 °C, 8h), respectively. Utilized plasticware was certified DNase- and RNase-free. Nucleic acids were extracted in triplicates, samples analyzed included t_0 (before incubation) and t_{end} (after incubation) of treatments incubated without acetylene. The coextraction of DNA and RNA followed a modified protocol of Griffiths (Griffiths et al., 2000). Prior to extraction, a wash step modified after Placella (Placella et al., 2012) was implemented to remove humic substances that were highly present in the samples. Pure RNA was retrieved by treating pooled nucleic acid extracts with DNAse I (RNase free, New England Biolabs, MA, USA). Digestion success was verified via 16S rRNA gene amplification and visualization on agarose gel. RNA was quantified with RiboGreen (Thermo Fisher Scientific, MA, USA), and 500 ng of RNA per sample were loaded onto a CsTFA gradient buffer (Whiteley et al., 2007). After isopycnic density gradient centrifugation for 67 h at 20 °C at 130,000 g_{av}, 10 fractions of each sample were collected, and RNA precipitated for subsequent community analyses (Lueders et al., 2003). Buoyant density (BD) of each fraction was determined by weighing of fractions obtained from a blank gradient. Heavy and light fractions were defined according to values from literature, with heavy fractions ranging between 1.818 and 1.824 g ml^{-1} and light fractions ranging between 1.770 and 1.784 g ml^{-1} (Lueders et al., 2003).

Denaturing Gradient Gel Electrophoresis (DGGE) analysis of density-resolved rRNA

RNA fractions were pairwise pooled resulting in 5 pooled fractions (1-2, 3-4, 5-6, 7-8, and 8-10) per gradient and subjected to reverse transcription (SuperScript IV, Thermo Fisher Scientific, MA, USA) according to the manufacturer's protocol. Pooled fractions recovered from CsTFA gradients were compared by Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting to determine the success of labelling prior to amplicon sequencing. Primers Bact340F (TAC GGG AGG CAG CAG; Li et al., 2010) and 907R (CCG TCA ATT CMT TTG AGT TT; Muyzer et al., 1995s) were used to amplify the 16S rRNA gene for DGGE, with the forward primer containing a G+C rich sequence at the 5' end (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC; clamp; Muyzer et al., 1993). PCR reactions were carried out as 40 µl reactions, containing 1x SensiMix™ SYBR® & Fluorescein (Bioline, London, UK), 500 nM of each primer and 4 µl of template cDNA. Initial denaturation was performed at 94 °C for 8 min. Denaturation, annealing and elongation were at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, respectively, with a total of 35 cycle, followed by a final elongation at 72 °C for 5 min. Amplification was checked on a 1 % agarose gel. Amplicons

were then resolved on a 35 – 65 % DGGE gradient gel (63 V, 60 °C, 16.5 h) and imaged after the run was complete (Horn et al., 2003b).

16S rRNA gene amplification and amplicon sequencing

Paired end Illumina MiSeq amplicon sequencing of the archaeal and bacterial 16S rRNA gene of representative pooled fractions was performed. Amplicon libraries for Archaea and Bacteria were generated with primer pairs A519F (CAG CMG CCG CGG TAA; Wang and Qian, 2009)/Arch1017R (GGC CAT GCA CCW CCT CTC; Yoshida et al., 2005) and 341F (CCT ACG GGN GGC WGC AG; Herlemann et al., 2011)/805R (GAC TAC HVG GGT ATC TAA TCC; Herlemann et al., 2011), respectively. Both forward and reverse primers were fused to adapter sequences at their 5' end (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G, respectively). For each PCR, 40 µl reactions were set up, containing 1x SensiMix™ SYBR® & Fluorescein (Bioline, London, UK), 500 nM of each primer and 4 µl of template cDNA. Initial denaturation was performed at 94 °C for 8 min. Denaturation, annealing and elongation were at 94 °C for 40 s, 53 °C for 40 s and 72 °C for 50 s for amplification of bacterial 16S rRNA derived cDNA, with a total of 30 cycles. For amplification of archaeal 16S rRNA derived cDNA, denaturation, annealing and elongation were at 94 °C for 30 s, 55.5 °C for 30 s and 72 °C for 30 s, with a total of 30 cycles. Terminal elongation was at 72 °C for 5 min, for both protocols. PCR products were checked for right amplicon size on a 1 % agarose gel and then purified with the GeneRead Size Selection Kit (Qiagen, Hilden, GER), before sequencing on the Illumina MiSeq platform (v3 chemistry) at the University of Göttingen (Genomic and Applied Microbiology, University of Göttingen, Germany). Sequence data was retrieved as .fastq files.

Sequence processing

Sequence analysis was performed using mothur v.1.39.5 (Schloss et al., 2009) and a modified standard operational protocol for MiSeq data (Kozich et al., 2013). After paired-end joining of sequence reads, datasets were randomly subsampled to 20,000 sequences per sample. Then sequences were filtered by amplicon length, and sequences with ambiguous bases as well as duplicate sequences were removed. Next, sequences were aligned according to a reference database (Silva database v138; https://www.arb-silva.de/download/arb-files/; Quast et al., 2012; Yilmaz et al., 2014), formatted to be compatible with mothur (© 2019 Patrick D. Schloss, PhD, https://mothur.org/blog/2020/SILVA-v138-reference-files/#legalese), with maximum homopolymer length ≤ 8. Sequences were pre-clustered, allowing one difference for every 100 bp of sequence, and chimeras were identified and removed using VSEARCH (v2.6.0; Rognes et al., 2016). After classification against the Silva database (Silva database v138; Quast et al., 2012; Yilmaz et al., 2014) non-target sequences (e.g., fragments of mitochondria, eukaryota) were removed. Operational taxonomic units (OTUs) were assigned at 97 % similarity level

using the OptiClust algorithm (OTU assembly using metrics to determine the quality of clustering). Coverage and α-diversity indices were calculated using the Inverse Simpson diversity index (Simpson, 1949), and Bray-Curtis dissimilarity matrices were used for comparison of β-diversity (Bray and Curtis, 1957). Quantification and statistical inference of systematic changes between conditions were tested with the Differential analysis of count data (DESeq2 package; Love et al., 2014). All other analyses were performed with the Microbiome Analyst pipeline (Dhariwal et al., 2017) or Galaxy server (https://usegalaxy.eu/). Sequence data was deposited in the NCBI Sequence Read Archive under the bioproject accession number PRJNA608855.

Results

Acetate-driven carbon flow

Acetate was below the detection limit (i.e., < 20 µM) in anoxic cryoturbated peat circle sediment incubations prior to supplementation. Initially supplemented acetate was consumed without apparent delay and primarily converted to CO_2 in all treatments (Figure 1 A-D; Table 1). Traces of CH_4 were detected, suggesting a marginal role of methanogenesis for carbon and electron flow (Figure 5). Acetate was refed after day 11, when initial acetate was depleted in treatments without acetylene, and on days 14 as well as 15 (Figure 1 A, C). Acetate consumption accelerated with incubation time. Acetate and CO_2 concentrations were similar in all treatments at most of the timepoints (p = 0.05, ANOVA). CO_2 concentrations differed significantly between treatments with and without acetylene at the end of the incubations only. 0.72 to 1.01 mM acetate were supplemented in total by the end of the incubation (). Carbon recoveries based on total C-flow suggest that 56 and 83% of acetate carbon was oxidized to CO_2 during phase I and II, respectively (Table 2).

Mean proportions relative to total acetate of $^{13}C_2$- (m/z=61; fully labeled) and $^{13}C_1$-acetate (m/z=60) in ^{13}C-acetate treatments at day 1 were 82.6 ± 1.2 % and 12.9 ± 0.5 %, respectively, suggesting a moderate portion of ^{12}C in treatments with $^{13}C_2$-acetate (Figure 1 A). Proportions were essentially the same for ^{13}C-acetate treatments with acetylene, i.e., 78.3 ± 2.0 % and 13.6 ± 7.9.0 % for $^{13}C_2$- (m/z=61; fully labeled) and $^{13}C_1$-acetate (m/z=60), respectively (Figure 1 C). Such proportions were the same at day 11 when $^{13}C_2$- and $^{13}C_1$-acetate was spot checked. At the end of incubation, acetate values were near the detection limit. Thus, the majority of data for isotopic composition of acetate in ^{13}C-acetate treatments were not obtained. One replicate, however, showed an $^{13}C_2$-acetate proportion of 99.6%. The high $^{13}C_2$- and $^{13}C_1$-acetate proportions of total acetate essentially suggest the absence of significant $^{12}C_2$-acetate production from endogenous peat organic carbon. Volatile organic acids other than acetate representing typical fermentations products like propionate and butyrate were negligible (< 1 µM) during and after incubation, hence ^{13}C-labelled carbon in organic acids was not detectable. $^{12}C_2$-acetate (m/z = 59; unlabeled) proportions of total acetate in ^{12}C-acetate

291 treatments after initial supplementation was 98.7 ± 1.9 %, representing the natural proportion
292 of the ^{13}C-isotope. Acetate consumption and CO_2 production in ^{12}C-acetate treatments were
293 highly similar to those in ^{13}C-acetate treatments (Figure 6).

294 $^{13}CO_2$ was spot checked at days 11 and 16 (Figure 1 B, D). CO_2 of ^{13}C-acetate treatments
295 without and with acetylene had mean ^{13}C-proportions of 54.7 ± 1.4 %, and 31.1 ± 0.4 %,
296 respectively, at day 11. At the end of incubation at day 16, the mean ^{13}C-CO_2 abundance in
297 ^{13}C-acetate treatments without acetylene was 69.4 ± 3.4 %. For ^{13}C-acetate treatments with
298 acetylene, only one replicate was measured with an ^{13}C-proportion of 66.2 %. Such data
299 suggest that two thirds of CO_2 were derived from supplemented ^{13}C-labelled acetate in $^{13}C_2$-
300 acetate treatments and one third originated from endogenous peat carbon. Thus, data suggest
301 that substantially more acetate carbon was assimilated than indicated by the total carbon mass
302 balance alone (Table 2).

303

304 **Acetate-driven electron flow and pH**

305 Acetate consumption was concomitant to consumption of endogenous nitrate without apparent
306 delay (Figure 1). Endogenous nitrate was depleted within 6 days of incubation, which was
307 similar in all treatments (phase I; Figures 1 and SXY, Table 2). Nitrate was the most abundant
308 endogenous inorganic electron acceptor detected in cryoturbated peat sediments and
309 approximated 300 µM (Figure 1 E, G). Sulfate and Fe^{2+} were almost always below the detection
310 limit in all treatments, suggesting that sulfate and iron respiration were negligible for electron
311 flow. Initial nitrate consumption was concomitant to the production of minor amounts of N_2O in
312 the absence of acetylene in phase I when the pH was stable at *in situ* levels of 4.4 (Figure 1
313 E, F). Large quantities of N_2O were produced in the presence of acetylene, suggesting high
314 denitrification activities in cryoturbated peat sediments yielding primarily N_2 (Figure 1 E-H).
315 Minor amounts of NH_4^+ were likewise produced during phase I, suggesting nitrate
316 ammonification (Figure 7). Recovery of nitrate N in N_2-N was approximately 3- and 8-fold
317 higher than in N_2O and ammonium, respectively (Table 2). When nitrate was refed upon first
318 depletion to concentrations reflecting endogenous levels (phase II), N_2O was consumed
319 together with nitrate and N_2 was the primary reduced end product (Figure 1 E-H, Table 2).
320 Recoveries of electrons released during acetate oxidation to CO_2 in N_2 were 72 and 93% after
321 phase I and II, respectively. The pH increased from 4.4 to 5.5 during phase II, which was
322 concomitant to an increase in N_2O production and consumption in treatments with and without
323 acetylene, respectively (Figure 1 F, H). Indeed, the pH was significantly different from the start
324 of incubation from day 11 and 14 on in treatments without and with acetylene, respectively (p
325 (p = 0.05, ANOVA). Nitrate concentrations in all treatments were similar during the incubation
326 period (p = 0.05, ANOVA). A total of about 1.5 mM NO_3^- was consumed during the incubation
327 time (Tables 1 and 2).

328

Stable isotope probing of bacterial and archaeal 16S rRNA

16S rRNA-SIP was applied to trace and identify bacterial and archaeal nitrate reducers and nitrous oxide consumers which are capable of ^{13}C-acetate assimilation in acidic peat circle sediments. DGGE of heavy and light fractions showed visible differences in banding patterns, suggesting distinct community composition and successful ^{13}C-labeling of ^{13}C-acetate assimilating bacteria during incubation (Figure 8).

Bacterial communities characterized in triplicates had an average of 26,152 ± 9,481 operational taxonomic units (OTUs) among all samples and a mean coverage of 95.64 ± 0.9 %. Alpha diversity measured by the Inverse Simpson index that covers both richness and evenness were highest for samples before incubation (Table 3). Archaeal communities characterized in triplicates (except for t_0 heavy fraction, from which only two samples were obtained) had an average of 12,590 ± 6,669 OTUs among all samples and a mean coverage of 96.4 ± 0.6 %. Inverse Simpson indices of Archaea were lower than those of Bacteria for all samples (Table 3). The phyla Actinobacteriota (24.0 ± 2.4 % in heavy and 30.2 ± 1.3 % in light fraction) and Proteobacteria (30.8 ± 2.0 % in heavy and 23.0 ± 2.1 % in light fraction) were most prominent prior to incubation (Figure 2). The classes Acidimicrobiia (8.6 ± 0.4 % in heavy, 15.5 ± 0.3 % in light fraction), Actinobacteria (7.7 ± 1.6 % in heavy, 6.7 ± 0.3 % in light fraction), Thermoleophilia (7.6 ± 0.6 % in heavy and 8.0 ± 1.2 % in light fraction), Alpha- and Gammaproteobacteria (19.1 ± 1.6 % and 11.7 ± 0.9 % in heavy and 19.9 ± 2.0 % 3.2 ± 0.1 % in light fraction respectively) of the two dominant phyla were prevalent (Figure 9, Figure 11). Archael communities consisted almost exclusively of Crenarchaeota (former Thaumarchaeota), class Nitrososphaeria (Figure 10).

Bacterial community structure changed during incubation, which was reflected in the dominant phyla retrieved after incubation. Bacterial communities were dominated by Proteobacteria primarily consisting of Gammaproteobacteria, with relative abundances of 31.5 ± 3.9 and 38.4 ± 4.7 in heavy and light fractions of ^{12}C-acetate treatments, respectively, and relative abundances of 66.4 ± 2.7 and 14.7 ± 0.4 in heavy and light fractions of ^{13}C-acetate treatments, respectively. OTU 1, associated with the *Burkholderia-Caballeronia-Paraburkholderia* (*Burkholderiaceae*; Burkholderiales; former Betaproteobacteriales) dominated in both labeled and unlabeled treatments with relative abundances making up to 91 % of all Gammaproteobacteria after incubation, compared to a relative abundance of only about 1 % of all Gammaproteobacteria before incubation (Figure 11).Such data suggest a strong enrichment of Gammaproteobacteria during incubation. Archaeal communities were dominated by the class Nitrososphaeria (unclassified *Nitrososphaeraceae*, *Nitrosocosmicus* and Group 1.1c; Figure 10), within the phylum Crenarchaeota (former Thaumarchaeota), with a relative abundance of 92.8 ± 1.9 %. With a relative abundance of 99.99 ± 0.01 % across all treatments, Nitrososphaeria dominated the overall archaeal community. Interestingly, Euryarchaeota (including putative methanogens) were not detected.

Density resolved bacterial communities after and prior to the incubation differed, while the replicates showed high similarities (Figure 3, Figure 9). Differences in bacterial β-diversity evaluated by ANOVA did not differ significantly by replicates ($P = 0.4871$) but were different by treatment and fraction ($P < 0.001$). This was supported by Principal coordinate analysis (PCoA), which revealed clustering by replicate and fraction of treatments based on Bray-Curtis dissimilarity (Figure 3). The PCoA plot shows a clear separation on axis 1 explaining 68.1 % of variability, and distinctly separates ^{13}C-acetate heavy fractions from the associated light fractions, as well as from ^{12}C-acetate treatments and t_0 samples obtained prior to incubation. Heavy and light fractions of ^{12}C-acetate control treatments clustered together with light fractions of ^{13}C-acetate treatments. Most communities retrieved prior to incubation (t_0) formed a distinct cluster (Figure 3 B). Heavy fractions from ^{13}C-acetate treatments likewise showed a distinct clustering pattern, suggesting successful labeling. PCoA did not show clear trends for Archaea (Figure 12) with neither treatments, nor fractions clustering together. ANOVA revealed no significant difference between replicates ($P = 1$) or treatments and fractions ($P > 0.091 – 0.714$).

Differential abundance values (Log2Fold change; $P < 0.05$) were computed between heavy and light fractions of each treatment and compared across all treatments (Figure 4). The only OTU that was significantly more abundant in the heavy fractions of ^{13}C-acetate treatments compared to those of t_0 samples and those of ^{12}C-acetate treatments was OTU 1 of the Burkhlderiales, *Burkholderiaceaea* (2.15). The relative abundances of this OTU in heavy and light fractions of either ^{12}C-treatments or t_0 samples were similar. Archaeal log2Fold change values, which showed significances between heavy and light fractions within treatments, did not show differences between treatments. DESeq2 analysis for the archaeal community did not show any significant difference in the heavy fractions of ^{13}C-acetate treatment.

Discussion

Nitrate reduction and denitrification in sediment microcosms

This is the first study characterizing acetate assimilators under nitrate-reducing conditions in acidic peat circle sediment via stable isotope probing, to our knowledge. Cryoturbated peat circles of the subarctic tundra with *in situ* pH 4 emit high amounts of N_20, in the range of heavy fertilized agricultural and tropical rainforest soils (Potter et al., 1996; Repo et al., 2009; Werner et al., 2007) .

Potential sources of N_2O emitted from soil and sediment are nitrification, chemodenitrification, dissimilatory reduction of nitrate to ammonium (DNRA) and denitrification. Nitrification, more precisely ammonia oxidizing archaea (AOA), are well known to directly contribute to N_2O production in arctic soils, though under (micro-) oxic conditions (Siljanen et al., 2019). At low pH and under oxygen limited conditions chemodenitrification might occur, though main

404 products of this abiotic process are NO and NO_2 and the process will most certainly be
405 outcompeted by the faster microbial denitrification (Kresovic et al., 2009; van Cleemput, 1998).
406 Under anoxic and NO_3^-- limiting conditions DNRA can also contribute to the release of N_2O
407 whilst NO_3^- is reduced to NH_4^+. Since NO_3^- was not a limiting factor and NH_4^+ was only detected
408 in negligible quantities during incubation, the suggested main process leading to N_2O emission
409 in the experiment is microbial denitrification. Endogenous NO_3^- was depleted from the start of
410 incubation. Compared to *in vivo* field studies and *in situ* incubations, where peat circle sediment
411 emits high amounts of N_2O (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009),
412 in this study with microcosms, no N_2O could be detected, except in the presence of the N_2O-
413 reductase inhibitor acetylene. Hence, subsequently produced N_2O must have been further
414 reduced to N_2 in microcosms incubated without acetylene. Supplemented ^{13}C-acetate was
415 consumed without delay in microcosms and recovery in ^{13}C-CO_2 approximated 50 % and 61
416 % in ^{13}C-acetate treatments with and without acetylene, respectively. Soluble organic acids
417 were under detection limit before incubation and near or under detection limit during incubation,
418 indicating a low fermentation potential of peat circle sediment. These findings are congruent
419 with findings from Palmer et al., 2012, which showed the capacity of peat circle denitrifiers to
420 consume acetate and concurret occurrence of low fermentation of peat circle sediment
421 compared to the surrounding peat plateau soil. Based on these results, between 40 and 60 %
422 of labelled consumed acetate could have been used by microorganisms for assimilation and
423 therefore labeling for SIP.

424 Current findings indicate the potential of peat circle sediment microorganisms being capable
425 of complete denitrification at acidic pH, whilst using acetate as substrate.

426

427 **Archaeal community structure**

428 The archaeal community consisted of mainly Crenarchaeota (former Thaumarchaeota),
429 especially members of the class Nitrososphaeria/ family *Nitrososphaeraceae*.
430 Thaumarchaeota (recently re-integrated into the phylum Crenarchaeota; Parks et al., 2018)
431 are known ammonia oxidizers (AOA) that prefer ammonia at low concentration (De La Torre
432 et al., 2008; Lehtovirta-Morley et al., 2011), moreover, some AOA seem to even prefer a pH <
433 5.5 (Gubry-Rangin et al., 2010; Prosser and Nicol, 2008). AOA in investigated permafrost-
434 affected soils showed a high β-diversity of Thaumarchaeota, with niche differentiation of AOA
435 clades following soils moisture and nitrogen content of investigated soils (Alves et al., 2013).
436 N_2O fluxes from unvegetated (sub)arctic peat soil surfaces in Finland and Siberia were in the
437 range or even higher (76.8 µg N_2O-N m^{-2} h^{-1}) than from managed peatland soils from northern
438 countries and showed a positive correlation with nitrate concentration of soils and *amoA* gene
439 abundance (Siljanen et al., 2019). These findings highlight the importance of AOA as source
440 of N_2O from northern peatlands and triggers further investigations in this direction. Even though
441 the community structure of Archaea was not influenced by incubation conditions and N_2O was

not detected in the absence of acetylene in this study, it cannot be ruled out that AOA were not involved in N_2O production which was then further reduced to N_2 by bacterial denitrifiers. Unfortunately, due to the use of two distinct primer systems and separate sequencing of bacterial and archaeal 16S rRNA genes, no statement regarding the ratio of Archaea and Bacteria is possible, as well as a comparison on their relative abundances.

Bacterial community structure of potential nitrate reducers and denitrifiers

Numerous studies focus on denitrification due to the obligatory intermediate N_2O of this process. N_2O is a strong greenhouse gas with an approximately 300 times higher global warming potential than CO_2 (Stocker et al., 2018). More than 60 genera, within archaea, bacteria, and fungi, are known to be capable of denitrification, displaying a broad phylogenetic and functional variability (Philippot et al., 2007; Zumft, 1997). The release of N_2O from soil and sediment depends on both the microbial community as well as environmental factors. Arctic ecosystems were thought to be of minor importance concerning N_2O emission, due to slow mineralization of organic matter (Shaver et al., 1992) and a general limitation of nitrogen (Jonasson et al., 1999). However, permafrost-affected cryoturbated peat circles with bare surface of the arctic tundra harbor high amounts of NO_3^- and emit N_2O in substantial amounts at *in situ* pH 4 (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009).

Investigated bacterial community from the microcosm experiment revealed high relative abundances of Actinobacteria and Alphaproteobacteria before supplementation and incubation of peat circle sediment. This is congruent with results from Palmer et al., 2012, who could find a high relative abundance of *narG* sequences affiliated with Actinobacteria, that clearly dominated the investigated community of nitrate reducers in this environment and accounted for up to 95 % of the overall relative sequence abundance. Nitrite reducers, harboring the *nirK* gene were only minor abundant and dominated by an OTU affiliated with Alphaproteobacteria that had a relative abundance of 97 % of total *nirK* sequences, whilst over 99 % of all *nirK* sequences were affiliated with Alphaproteobacteria. Nitrate reducers harboring the *nirS* genes were more abundant than those harboring *nirK* and were mainly associated with Betaproteobacteria. Findings indicate that *nirS*- rather than *nirK*-type denitrifiers are associated with denitrification in acidic peat circle sediment. N_2O reducers, harboring the *nosZ* gene, were dominated by an OTU affiliated with Alphaproteobacteria as well, but overall copy numbers of the typical *nosZ I* gene were low, accounting for only 0.002 % of 16S rRNA gene copy numbers (Palmer et al., 2012). Furthermore, RNA transcripts of genes involved in denitrification of the functionally active microbial community from the High Arctic included different Alphaproteobacteria, supporting the findings of the current study (Altshuler et al., 2019)

During incubation, the bacterial community structure shifted towards the Gammaproteobacteria becoming the most abundant class, with sequences affiliated with the

genus *Burkholderia-Caballeronia-Paraburkholderia*. Closely related *nosZ* sequences retrieved from a comparable acidic palsa peat site in Finish Lapland were affiliated with *Burkholderia pseudomallei* (Palmer and Horn, 2012), supporting *Burkholderia-Caballeronia-Paraburkholderia* as candidates for the reduction of N_2O to N_2 in peat circle sediment. Additionally, the log2fold change value of 2.15 for the highly abundant OTU 1 in heavy fractions of the ^{13}C-acetate treatment compared to heavy fractions from samples before incubation and heavy fractions from the ^{12}C-acetate treatments is another indicator of the role of *Burkholderia* in denitrification under the given conditions. Only recently, genomes of two new *Caballeronia* (Burkholderiales) species originating from the same sampling site have been published (Hetz et al., 2020), which possess multiple nitrate and nitrite reductases, as well as the nitric oxide reductase *norV*, though the ability of complete denitrification has yet to be determined for both isolates. Genome sequences of other *Burkholderia* representatives revealed the presence of *nosZ* as well as *nirK* genes (Sanford et al., 2012). Isolates from *Sphagnum* tissue, that covers a Finnish acidic mire, included isolates from *Burkholderia* sp. that have their optimum pH at around 5 and emit N_2O, when NO_3^- was supplemented during incubation. Thereby the presence or absence of acetylene did not change the amount of N_2O produced. All isolates possessed the *narG* gene, for nitrate reduction, but amplification of either typical or atypical *nosZ* gene could not be detected, suggesting these *Burkholderia* sp. as incomplete denitrifiers. Sequencing of 16S rRNA targeted DGGE-cutting bands from overall microbial community incubations of the *Sphagnum* tissue, retrieved from incubations at 15 °C, that showed N_2O production, revealed *Burkholderia* sp. as major representatives of the bacterial community (Nie et al., 2015).

N_2O reduction potential at acidic pH

The aim of this study was to determine the ability of peat circle denitrifiers to reduce N_2O to N_2 in the presence of acetylene and to identify new acid tolerant key players involved in nitrate reduction and denitrification by SIP analysis. The capability of N_2O reduction under acidic conditions was shown before in peat circle sediment, when initially produced N_2O derived from endogenous N-sources was consumed at pH 4 (Palmer et al., 2012). Furthermore, the genetic potential of acidic soils for the reduction of N_2O, i.e. the abundance of the *nosZ* gene, was tested positive (Palmer et al., 2010, 2012). However, in contrast to the detected potential for complete denitrification, peat circle sediment emits high amounts of N_2O, both in field studies and in *in situ* experiments (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009). Interestingly, in the current study, there was only formation of N_2O, when the final step of denitrification was inhibited by acetylene. In their study from 2012 Palmer et al. investigated apparent Michaelis-Menten kinetics of nitrate-dependent denitrification in anoxic microcosms and found out that peat circle denitrifiers were saturated with less than half of the NO_3^- concentrations occurring *in situ* and suggested a limitation of electron donor availability that restricts denitrification in cryoturbated peat circles (Palmer et al., 2012). Another study

519 investigated the production of N_2O by the bacterial denitrifier *Alcaligenes faecalis* in a steady
520 state culture in response to changes in the availability of an electron donor. After pulses of
521 acetate, the *A. faecalis* culture immediately reduced accumulated NO_2^- and N_2 production was
522 increased, whereas N_2O production was not impaired by the addition of the electron donor
523 (Schalk-Otte et al., 2000). Combined results might therefore suggest an electron donor
524 limitation in cryoturbated peat circle sediment that favors the production of N_2O, despite the
525 genetic potential of the microbial community for complete denitrification. Hence, incubation
526 conditions of the current study might overcome the limitation of an electron donor since acetate
527 was supplemented for SIP analysis.

528 Nevertheless, N_2O reduction might not only be impaired by carbon limitation, but might also be
529 affected by pH, as known from neutrophilic model organisms like *Paracoccus denitrificans*, that
530 accumulate N_2O when pH drops below 7 (Bergaust et al., 2010). Findings from extracted
531 bacterial cells, derived from peat soil with pH 4, 6.1 and 8.0, showed no impairment of *nosZ*
532 gene transcription and thus no evidence for low N_2O reduction activity in acidic soils (Liu et al.,
533 2010). Other than expected, cells from acidic soils with pH 4 were not able to reduce N_2O to
534 N_2, not even if transferred to more neutral pH. Despite significant transcription of the *nosZ*
535 gene, cells from neutral soils were also unable to produce a functional nitrous-oxide reductase
536 at pH values below 6.1, but if *nosZ* was expressed at pH7, it was functional throughout the
537 tested pH range of 5.7 to 7.6. These findings are in favor of the hypothesis that the main cause
538 preventing N_2O reduction in soils with low pH is the preclusion of a successful assembly of a
539 functional N_2O reductase (Liu et al., 2014). An explanation attempt, in favor of complete
540 denitrification in acidic soils, might be the formation of microsites by clustering of active
541 denitrifiers when a high density is given, similar to biofilm formation. Within these microsites
542 the pH might be more neutral and the assembly of functional nitrous-oxide reductases might
543 be achieved (Liu et al., 2014). This, together with the availability of an electron donor, might
544 account for the reduction of N_2O to N_2 in the current study.

545

546 **Conclusion and limitations**

547 Microorganisms from permafrost-affected cryoturbated peat circle sediment of the Arctic
548 tundra are capable of complete denitrification at pH 4 in the presence of acetate as electron
549 donor. SIP analysis coupled to 16S rRNA Illumina MiSeq sequencing revealed taxa with the
550 highest relative abundances under applied incubation conditions and possible key denitrifiers
551 were identified by DESeq2 analysis. The high abundance together with a DESeq2 value of 2.7
552 suggests representatives of the genus *Burkholderia* are prime suspects involved in complete
553 denitrification. Depending on the species, *Burkholderia* are known to harbor the enzymatic
554 capacity of several steps involved in denitrification, ranging from nitrate to nitrous-oxide
555 reduction (Nie et al., 2015; Sanford et al., 2012). Nevertheless, transcripts of marker genes
556 for denitrification would be helpful to clearly identify microorganisms harboring genes for *narG*

and *nosZ*. Furthermore, treatments without electron donor would help to understand if a carbon limitation could lead to an increased N_2O production.

Data availability

Datasets of bacterial and archaeal 16S rRNA gene sequences derived from amplicon sequencing were deposited at the NCBI sequence read archive under BioSample accession numbers SAMN14211851 to SAMN14211856 and SAMN14210576 to SAMN14210581, respectively, in BioProject PRJNA608855.

Author Contributions

SH and MH designed the SIP experiments, wrote the original manuscript, and interpreted data. SH set up microcosms and performed all laboratory work, if not stated otherwise. TK performed DGGE analysis. MH conceived the original idea and oversaw all laboratory work.

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Tables

Table 1: Cumulated supplementation (acetate and nitrate) and production (carbon dioxide and nitrous oxide) during incubations in treatments with and without acetylene. Mean values of technical replicates with standard deviation.

	w/o acetylene		with acetylene	
Compound [mM]	**^{12}C-acetate**	**^{13}C-acetate**	**^{12}C-acetate**	**^{13}C-acetate**
CO_2	1.01 ± 0.03	0.92 ± 0.09	0.73 ± 0.02	0.72 ± 0.02
Acetate	0.66 ± 0.06	0.62 ± 0.16	0.46 ± 0.16	0.54 ± 0.03
NO_3^-	1.59 ± 0.10	1.62 ± 0.21	1.54 ± 0.04	1.61 ± 0.03
N_2O	0.00 ± 0.00	0.00 ± 0.00	0.32 ± 0.04	0.31 ± 0.05

Table 2: Mass- and electron balances during anoxic incubations of crytoturbated peat circle sediments in the presence of supplemental acetate and nitrate (see Figure 1). Values for acetate, CO_2, CH_4, and NH_4^+ represent means ± SD of all incubations. Values for N_2O are based on treatments without acetylene, and N_2 values were calculated by subtracting N_2O from treatments without acetylene from those with acetylene. Electron (e^-) release was calculated based on CO_2 production (i.e., dissimilation assuming complete acetate oxidation) rather than acetate consumption that represents assimilation and dissimilation.

Phase I (days 0 – 6)						Phase II (days 6-15)					
				Recovery (%)						Recovery (%)	
Consumption (mean ± SD µM)		Production (mean ± SD µM)		C- or N-mol	e^-	Consumption (mean ± SD µM)		Production (mean ± SD µM)		C- or N-mol	e^-
Δ Acetate	111 ± 19	Δ CO_2	124 ± 13	56	n.a.	Δ Acetate	405 ± 172	Δ CO_2-C	669 ± 186	83	n.a.
		Δ CH_4	2 ± 3	0.8	1.5			Δ CH_4-C	0 ± 2	0	0
Δ NO_3^-	215 ± 47	Δ N_2O-N	25 ± 10	12	21	Δ NO_3^--N	1148 ± 186	Δ N_2-N	498 ± 90	42	93
		Δ N_2-N	71 ± 11	33	72	Δ N_2O-N	40 ± 15			n.a.	1.5
		Δ NH_4^+-N	8 ± 5	4	13	Δ NH_4^+-N	7 ± 7			n.a.	n.a.
				C_{tot} [a] 57	e^-_{tot} [b]					C_{tot} 83	e^-_{tot}
				N_{tot} [c] 50	107					N_{tot} 42	95

[a] C_{tot}, total recovery of acetate carbon in CO_2 and CH_4

[b] e^-_{tot}, total recovery of electrons released during dissimilation of acetate, i.e. oxidation to CO_2, in reduced N-species

[c] N_{tot}, total recovery of nitrate nitrogen in N_2O, N_2, and NH_4^+

Table 3: Inverse Simpson values for bacterial 16S rRNA amplicon sequences. Mean values of biological replicates with standard deviation.

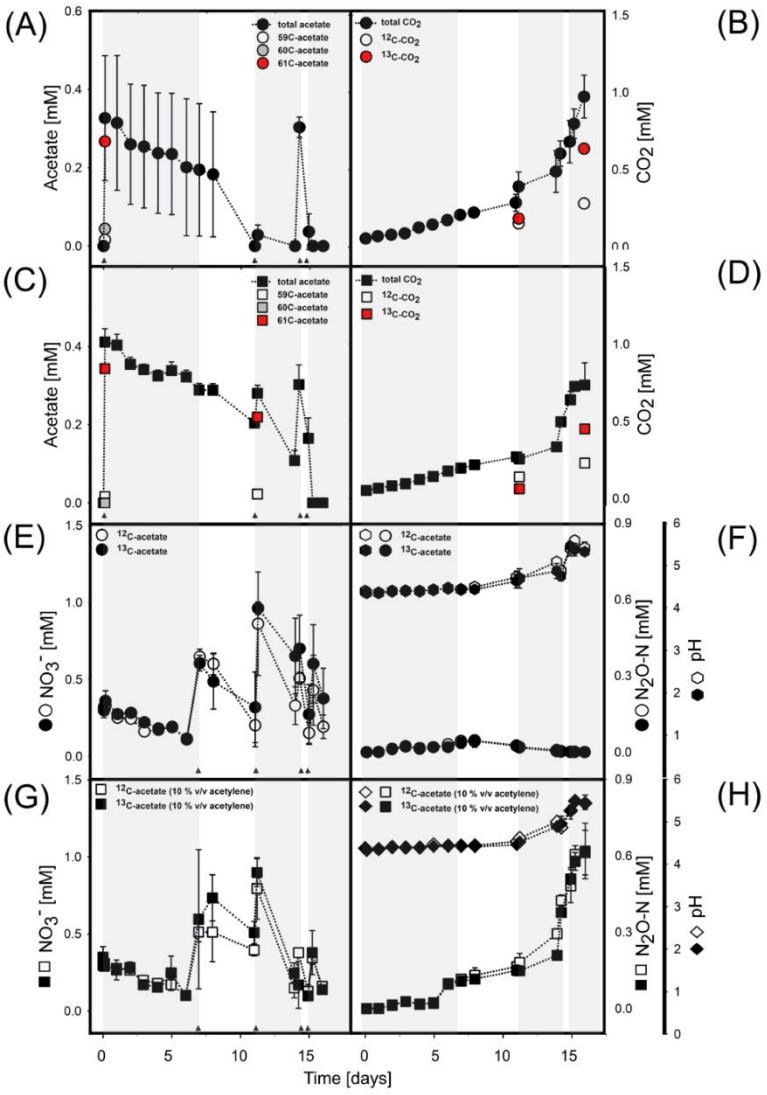
Treatment	Fraction	Inv Simpson [a]	LCI [b]	HCI [b]
t_0 [c]	Heavy	134.7 ± 7.6	129.0 ± 7.3	140.9 ± 7.9
t_0	Light	100.2 ± 6.4	96.0 ± 6.4	104.8 ± 6.4
^{12}C-acetate	Heavy	12.9 ± 3.4	12.3 ± 3.2	13.6 ± 3.7
^{12}C-acetate	Light	7.5 ± 2.1	7.2 ± 2.0	7.8 ± 2.2
^{13}C-acetate	Heavy	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.1
^{13}C-acetate	Light	48.1 ± 3.1	45.6 ± 2.9	50.8 ± 3.3

[a] Larger values indicate higher α-diversity.

[b] LCI and HCI indicate the 95 % low- end and high-end confidence intervals, respectively.

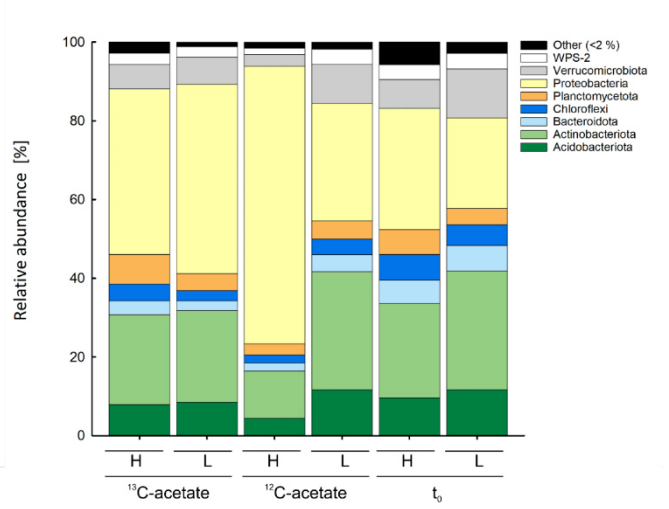
[c] t_0 – before incubation, all other samples at end of incubation

833 **Figures**



834

835 Figure 1: Acetate and NO_3^- consumption, CO_2 and N_2O-N production of ^{13}C-acetate treatments
836 with and without acetylene during incubation period with unraveled isotopes. Arrows indicate
837 supplementation of either acetate and NO_3^-. (A)/(C) Total acetate consumption in direct
838 comparison with non-labeled (59-C acetate), half-labeled (60C-acetate; one heavy C-atom),
839 and fully labeled (61C-acetate, 3 heavy C-atoms) acetate. (B)/(D) Total CO_2 production in
840 direct comparison to non-labeled (^{12}C-CO_2) and labeled (^{13}C-CO2) CO_2. (E)/(G) NO_3^-
841 consumption. (F)/(H) N_2O-N production.



842

843 Figure 2: Mean relative abundance of bacterial phyla more abundant than 2 % in at least on
844 sample. Others: phyla abundance smaller than two percent in all samples. Mean values of
845 triplicate incubations. Heavy (H) and Light (L) indicating the fractions after isopycnic
846 centrifugation.

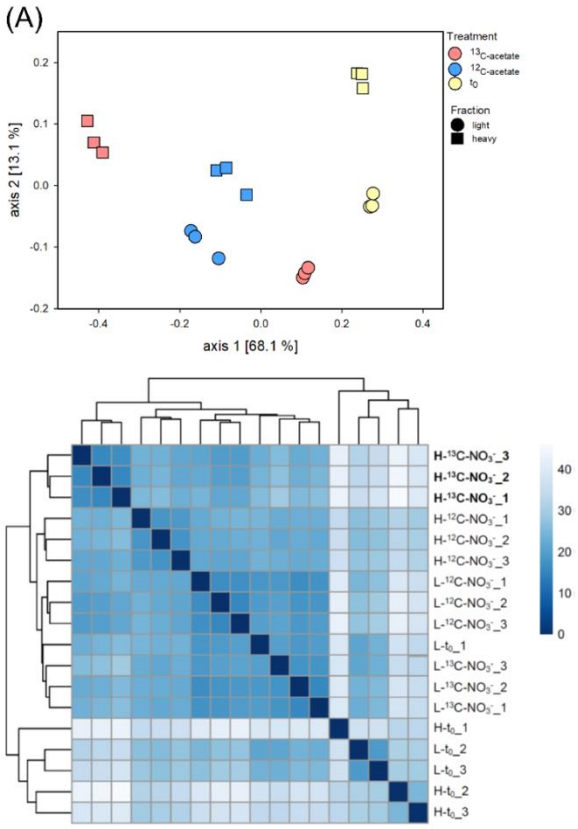
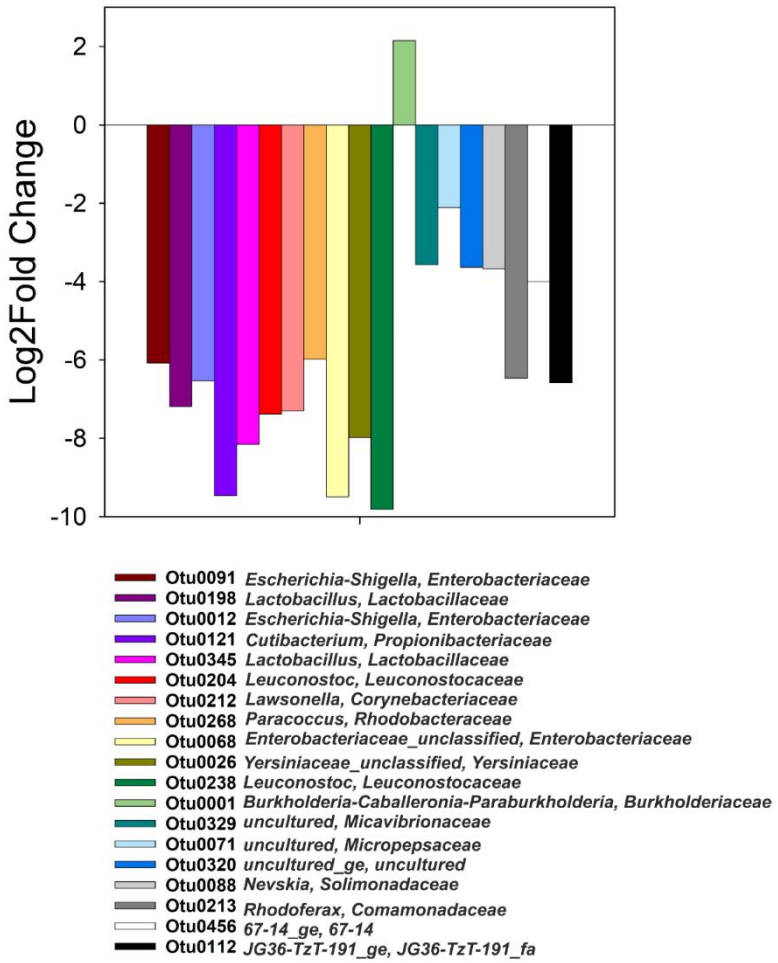


Figure 3: (A) Principal Coordinates Analysis (PCoA) plot of the relative abundance of bacterial OTUs on species level based on Bray-Curtis dissimilarity. Comparison between treatments and fractions from the beginning and end of incubation. (B) Heat map of bacterial OTUs.



853

854 Figure 4: Log2Fold change values ($p<0.05$) of heavy vs light fractions of ^{13}C-acetate
855 treatments. Values after subtraction of pre-supplemented samples and ^{12}C-acetate
856 treatments.

857

Supplement

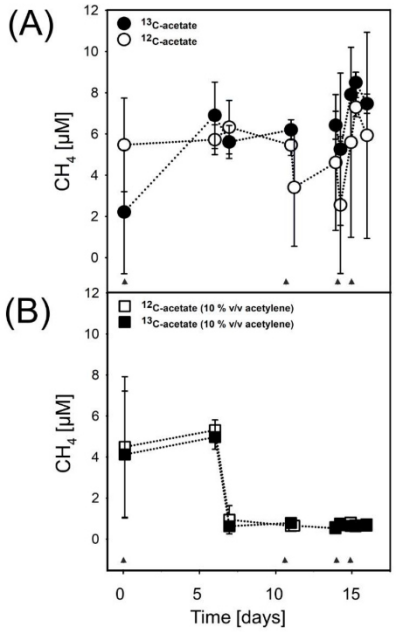


Figure 5: CH_4 production during incubation period. Mean values of triplicates with standard deviation. (A) Treatments incubated without acetylene. (B) Treatments incubated with acetylene. Arrows indicate acetate supplementation.

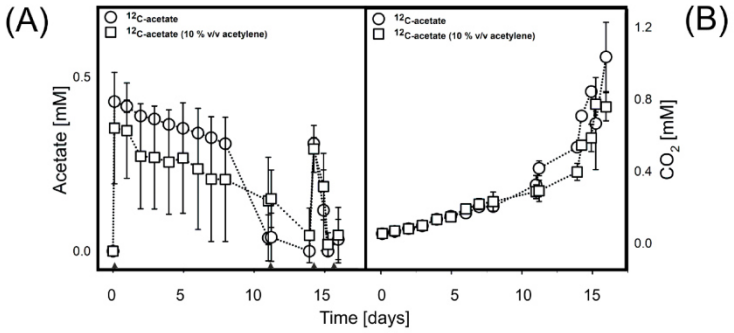
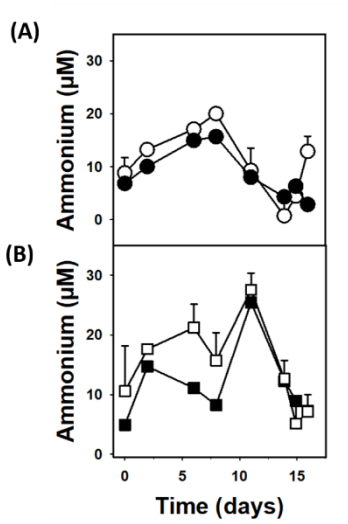


Figure 6: Acetate consumption (A) and CO2 production (B) during incubation period of 12C supplemented treatments. Mean values of triplicates with standard deviation. Arrows indicate acetate supplementation.



868

869 Figure 7: NH_4^+ production and consumption during incubation period. Mean values of
870 triplicates with standard deviation. (A) Treatments incubated without acetylene, (B)
871 Treatments incubated with acetylene. Arrows indicate acetate supplementation.

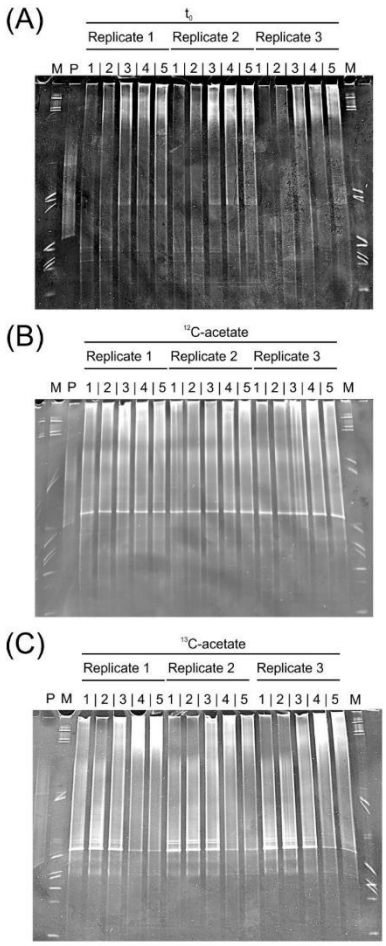
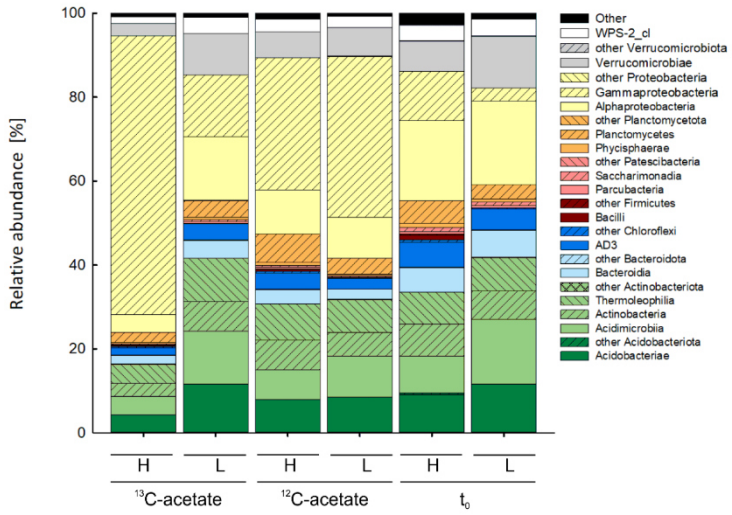
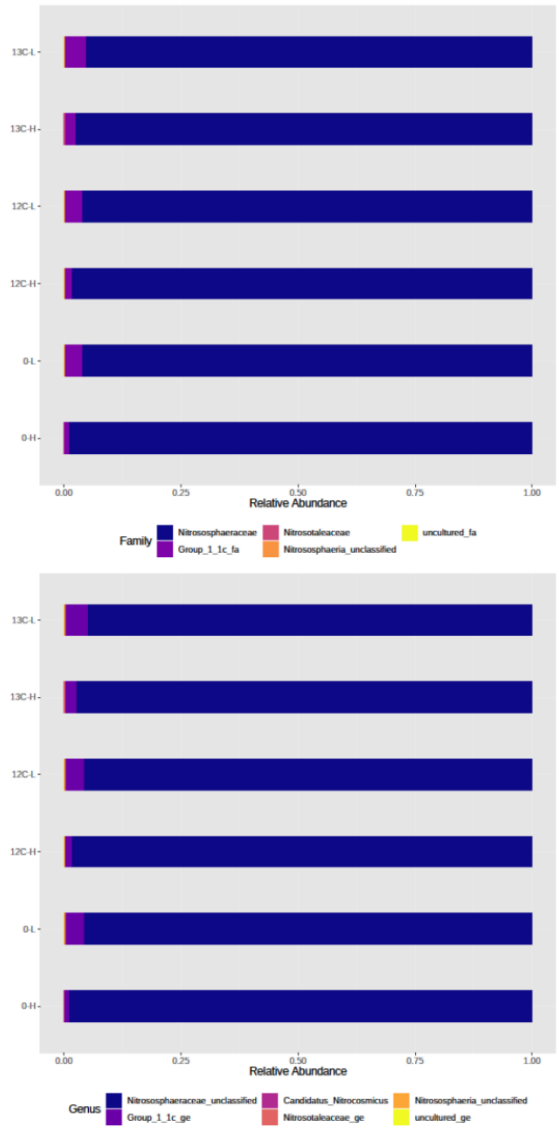


Figure 8 DGGE gels of heavy and light fractions of all replicates (1-3) and treatments. 1 – heaviest fraction; 5 – lightest fraction; M – marker; P – positive control. A: Heavy and light fractions from all three replicates of t0 samples. B: Heavy and light fractions from all three replicates of ^{12}C-acetate treatments. C: Heavy and light fractions from all three replicates of ^{13}C-acetate treatments.



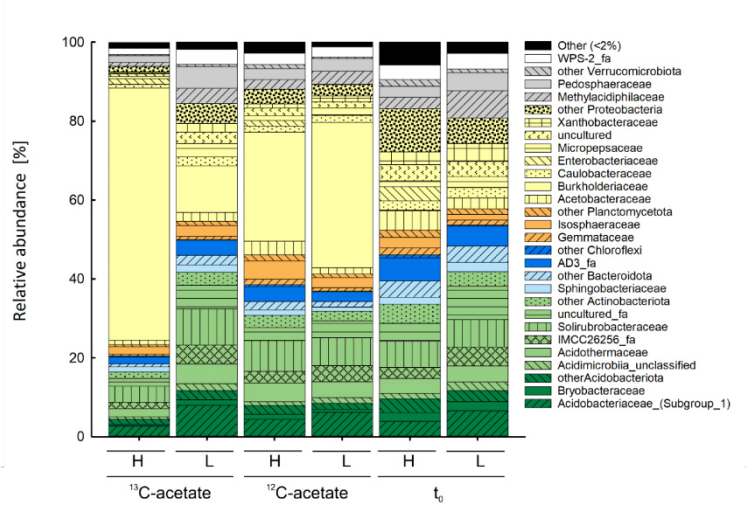
878

879 Figure 9. Relative abundance of bacterial classes within phyla more abundant than 2 % in at
880 least on sample. Others: phyla abundance smaller than two percent in all samples. Mean
881 values of triplicate incubations. Heavy (H) and Light (L) indicating the fractions after isopycnic
882 centrifugation.



883

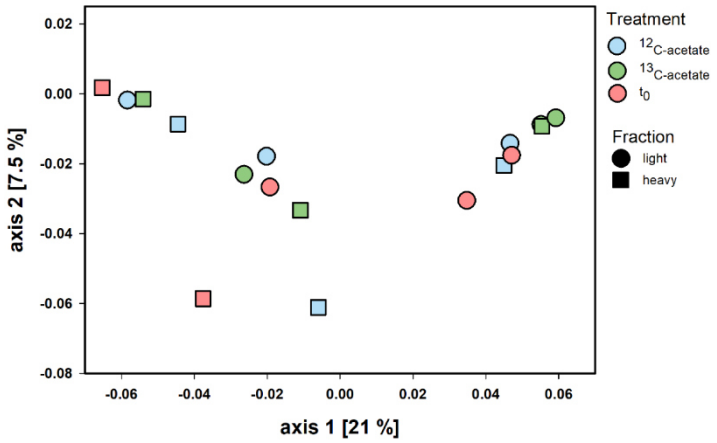
884 Figure 10: Relative abundance of archaeal families (upper) and genera (lower) in samples.
885 Mean values of triplicate incubations. Heavy (H) and Light (L) indicating the fractions after
886 isopycnic centrifugation, t_0 - pre-supplementation.



887

888 Figure 11. Mean relative abundance of bacterial families within phyla more abundant than 2 % in at
889 least on sample. Others: phyla abundance smaller than two percent in all samples. Mean values of
890 triplicate incubations. Heavy (H) and Light (L) indicating the fractions after isopycnic centrifugation.

891



892

893 Figure 12: Principal Coordinates Analysis (PCoA) plot of the relative abundance of archaeal
894 OTUs on species level based on Bray-Curtis dissimilarity. Comparison between treatments
895 and fractions from the beginning and end of incubation.

5.3.2 Key denitrifiers and acetate assimilators in permafrost affected acidic peatlands under nitrate reducing conditions

Key denitrifiers and acetate assimilators in permafrost affected acidic peatlands under nitrate reducing conditions

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Abstract

Nitrous oxide (N_2O) is a potent greenhouse gas and ozone depleting substance. The biggest source of N_2O is denitrification, the microbial reduction of nitrate to molecular nitrogen, that occurs under anoxic conditions. The process exists in many truncated forms, in which N_2O might be released as the end product. Major factors influencing denitrification are the availability of oxygen, carbon and nitrogen, the C/N ratio, and the pH. A pH below 6 is known to hinder the N_2O-reductase in neutrophilic model organisms, leading to an increased release of N_2O to the atmosphere. However, subarctic peat circles, located in the discontinuous permafrost zone with an *in situ* pH 4, were shown to emit high amounts of N_2O in the range of heavily fertilized agricultural and tropical rainforest soils. The aim of this study was to identify key nitrate reducers and denitrifiers of these cryoturbated peat circles, as well as a possible carbon limitation via SIP analysis. Anoxic microcosms with endogenous nitrate and ± acetylene at an in situ near pH 4 were used to test the effect of acetate on denitrification and N_2O production. Other than expected, in the absence of acetylene, N_2O was not detectable, suggesting complete denitrification at pH 4. Stable isotope probing coupled to 16S rRNA analysis via Illumina MiSeq amplicon sequencing identified nitrate reducing key players belonging to the family *Burkholderiaceae*.

Introduction

An estimated 16-25% of the global soil surface area is covered with permafrost, including large peatland areas, which make up to 80% of the surface area in West Siberia (Anisimov, 2007). Conservative estimates assume 67 Pg of Nitrogen (N) being stored in the upper 3 m of these peatland areas (Harden et al., 2012) . Nevertheless, only a small fraction of this N is bioavailable, making N the major limiting factor in Arctic soils (Nordin et al., 2004; Shaver and Chapin III, 1980). Along with global warming, permafrost thaw will likely increase N mobilization and thus decrease N limitation in permafrost soils (Anisimov, 2007; Marushchak et al., 2011). *In situ* measurements of increased N_2O

emissions upon warming and permafrost thaw undermine the importance of permafrost affected soils for climate change (Voigt et al., 2017b, 2017a; Yang et al., 2018). Between 1750 and 2005 the atmospheric concentration of N_2O increased from 270 to 319 ppb, this is critical, since the global warming potential of N_2O is 300 times higher than that of carbon dioxide (CO_2) (Forster et al., 2007; Spahni et al., 2005). N_2O is the third most important greenhouse gas after CO_2 and methane (CH_4) and a major ozone depleting gas (Forster et al., 2007; Ravishankara et al., 2009). The major source, with 60-70% of the global annual emissions of N_2O, are soils, including agricultural and tropical soils (Behrendt et al., 2019; Christensen, 2009; Conrad, 1996; Denman et al., 2007; Marushchak et al., 2011; Mosier et al., 1998; Repo et al., 2009). Meanwhile, the importance of Arctic peatlands and permafrost affected soils as sources of N_2O emissions are just emerging (e.g. (Behrendt et al., 2019; Christensen, 2009; Denman et al., 2007; Marushchak et al., 2011; Repo et al., 2009)), as different studies showed that Arctic soils produce (Abbott and Jones, 2015; Elberling et al., 2010) and release (Marushchak et al., 2011; Repo et al., 2009) amounts of N_2O in the range of heavily fertilized agricultural soils (Marushchak et al., 2011; Repo et al., 2009). 'Hot spots' of N_2O emissions in the East European tundra are so-called cryoturbated peat circles, which emit exceptionally high rates of N_2O throughout the growing season (1.9-32 mg N_2O $m^{-2}d^{-1}$) (Repo et al., 2009). These peat circles have an *in situ* pH around 4 and harbor high nitrate (NO_3^-) concentrations, as a possible result of coupled ammonification-nitrification reactions at the oxic/anoxic interface. Due to the absence of vegetation, peat circles lack the typical competition for N between plants and microorganisms and the NO_3^- is readily available for denitrification, a main source of N_2O under anoxic conditions (Palmer et al., 2012; Repo et al., 2009). Complete denitrification comprises the reduction of NO_3^- to molecular dinitrogen (N_2), but as many truncated forms of denitrification exist, e.g. microorganisms missing one or more associated genes, N_2O is not only an obligate intermediate, it can also be the end product of denitrification (Cofman Anderson and Levine, 1986; Stein and Klotz, 2016). Depending on the denitrifying community and *in situ* factors (e.g. pH, C/N ratio, substrate availability) denitrification can act as both source and sink of N_2O (Bergaust et al., 2010; Bru et al., 2011; Dorsch et al., 2002; Enwall et al., 2005; Holtan-Hartwig et al., 2000; van Cleemput, 1998). A low pH (< 5) impairs denitrification, likely through post-transcriptional effects of low pH on the assembly of N_2O reductase(Liu et al., 2010), and leads to an increase in the product ratio of N_2O to N_2 in certain systems (Cuhel et al., 2010; Simek et al., 2002).

The ability to denitrify is widespread and shows a high phylogenetic and functional variability by including over 60 genera within Bacteria, Archaea and Eukarya (Philippot et al., 2007; Zumft, 1997). Four reactions catalyzed by seven enzymes are involved during complete denitrification (Zumft, 1997). Thereof, the first step, the reduction of NO_3^- to nitrite (NO_2^-), is common to denitrification and nitrate ammonification, and includes either a membrane (*narG*) bound or a periplasmic (*napA*) nitrate reductase (Zumft, 1997). The key step of denitrification, the reduction of NO_2^- to the gaseous product nitric oxide (NO), is catalyzed by either a Cu-containing (*nirK*) or cytochrome cd_1 (*nirS*) nitrite reductase (Zumft, 1997). Despite differing in their catalytic site and structure, both enzymes have identical

functions (Jones et al., 2008). Just recently, an organism harboring both *nirS* and *nirK* genes was discovered (Graf et al., 2014). The formation of N_2O happens through the reduction of NO by one of two nitric oxide reductases, which can also play a role in NO detoxification in non-denitrifying organisms (Zumft, 2005). The final step of denitrification is the reduction of N_2O to N2, which is catalyzed by the only known enzyme to be capable of this reaction, the copper-containing nitrous oxide reductase (*nosZ*) (Jones et al., 2008; Zumft, 1997). Two distinct groups of Nos are known to date, clade I (Tat-dependent) is comprised mostly of canonical denitrifiers and clade II (Sec-dependent) of non-denitrifying N_2O reducers (Sanford et al., 2012).

In former studies with cryoturbated peat circle soil, *narG* outnumbered *nosZ*, revealing a quantitative imbalance between the genetic potential for dissimilatory nitrate reduction relative to N_2O reduction, which might contribute to the high N_2O emissions from peat circles (Palmer et al., 2012). Furthermore, the majority (60%) of retrieved *nosZ* genes were only distantly related to *nosZ* of cultured microorganism, indicating a new, acid-tolerant community of N_2O-reducers in this ecosystem (Palmer et al., 2012). Additionally, to the acidic pH, an electron donor limitation might favor the production and release of N_2O, since NO_3^- is not limiting factor (Palmer et al., 2012; Schalk-Otte et al., 2000).

It is hypothesized that the major microbial process contributing to the emission of N_2O from acidic peat circles is denitrification and that N_2O emissions do not only depend on the *in situ* pH but are dependent on readily available carbon. Therefore, the main objectives of this study were 1) to identify (new) nitrate reducers and denitrifiers by stable isotope probing (SIP), and 2) to test, whether carbon might limit the denitrification potentials in cryoturbated peat circles.

Materials and Methods

Sampling site

Samples were taken from cryoturbated peat circles from the study site in the Northeastern European Tundra in Russia, located in the discontinuous permafrost zone (67°03'N, 62°57'E, 100 m a.s.l.) with a mean annual air temperature of -5.6°C (Marushchak et al., 2011). Cryoturbated peat circles were described previously (Biasi et al., 2014; Hugelius et al., 2011; Repo et al., 2009). Generally, during growing season high amounts of N_2O are emitted (1.9-32 mg N_2O m^{-2} d^{-1}) (Repo et al., 2009) and the carbon to nitrogen (C/N) ratio is low (23 ± 2) in cryoturbated peat circles (Repo et al., 2009; Supplementary Methods). In summer 2014 soil from the upper 10 cm of three different peat circles was sampled and stored at 4°C until further processing. Within three months after sampling experiments were conducted. Soil moisture content was determined via weighing soil samples before and after drying at 60°C for one week and accounted for 74%.

Incubations under nitrate-reducing conditions with ^{13}C-acetate

Root particles were removed and soil from the sampled peat circles was pooled and homogenized. In 500 ml veral bottles, one part soil was mixed with twelve parts deionized water to a final volume of 300 ml with an *in situ* pH 4, and the bottles were sealed with an airtight rubber stopper. For each treatment soil slurry microcosms were prepared in triplicates and incubated in the dark at 15°C. Prior to supplementation, soil slurries were preincubated under microoxic conditions for nine days to remove excess endogenous nitrate and easily available carbon compounds. Afterwards, the gas phase was exchanged with 100% nitrogen. Microcosms with and without acetylene (10% v/v headspace) were prepared in order to differentiate between complete and incomplete denitrification. Acetylene is a known inhibitor of the N_2O reductase and hinders the further reduction of N_2O to N_2 (Yoshinari et al., 1977). At the incubation start 1 mM nitrate was supplemented to support denitrification. In order to achieve isotopic labeling of microorganisms ^{13}C-acetate (99% atoms, Sigma-Aldrich, MO, USA) was added to a final concentration of 300 µM and refed three times to achieve sufficient labeling. Control incubations included pure soil, nitrate supplemented microcosms with and without acetylene, and $^{12/13}$C-acetate supplemented microcosms. Throughout the incubation period nitrate, acetate, CH_4 and N_2O production was monitored. Gas chromatography (GC), equipped with an electron capture, thermal conductivity, and flame ionization detector (Hunger et al., 2011; Palmer et al., 2010; Supplementary Materials and Method) was used to measure the gaseous products CO_2 and N_2O. GC combustion-isotope ratio mass spectrometry (GC-C-IRMS; BayCEER – Laboratories for Isotopic-Biogeochemistry, University of Bayreuth, GER) was applied to determine $^{13}C/^{12}C$-isotope ratios of CO_2. Liquid samples and ^{13}C labeled soluble compounds were analyzed via high performance liquid chromatography (HPLC) (Palmer et al., 2010; Supplementary Materials and Method) and HPLC-ESI-MS (BayCEER – Atmospheric Chemistry, University of Bayreuth, GER), respectively. Nitrate, nitrite,

ammonium, iron(II), and sulfate were measured by colorimetric assays (Cataldo et al., 1975; Gadkari, 1984; Harrigan and McCance, 1966; Tabatai, 1992; Tamura et al., 1974). OriginPro 2020 version (OriginalLab Corporation, Northhampton, MA, USA) was used to perform statistical analyses. Basic data analyses, including visual inspection of all measured variables coupled with the Shapiro–Wilk normality test, were performed prior to statistical tests. To test for the treatment effect, i.e. differences between controls and supplemented microcosms, analysis of variance (ANOVA) was used. Soil slurry microcosms were sampled at the start and the end of incubation (13-day time span) for microbial community analysis by immediately suspending 20 ml slurry sample in 2.5 ml RNA stabilization buffer (100 mM sodium acetate, 100 mM EDTA, pH 5.2) together with 1 ml 20% SDS, 64 µl mercaptoethanol, and 2 ml equilibrated phenol. Samples were shock frosted in liquid nitrogen and store at -80°C to avoid decomposition of nucleic acids until further processing.

Nucleic acid extraction and isopycnic centrifugation

All solutions and glassware were made RNAse free by either treatment with DEPC or heat sterilization (180°C, 8 h), respectively, prior to nucleic acid extraction, and only certified DNase- and RNase-free plasticware was used. Samples from t_0 (before incubation) and after incubation of microcosms without acetylene were analyzed and nucleic acids extracted in triplicates. Prior to extraction of nucleic acids, a wash step modified after Placella (Placella et al., 2012) was implemented to remove the highly present humic substances in the samples. The protocol of Griffiths (Griffiths et al., 2000) was followed for coextraction of DNA and RNA. By treating nucleic acids extracts with DNase I (RNase free, New England Biolabs, MA, USA) pure RNA was retrieved, digestion success was verified via 16S rRNA gene amplification and visualization on agarose gel. RNA was quantified via RiboGreen (Thermo Fisher Scientific, MA, USA), DNA was quantified via spectrophotometry (DeNovix DS-11 FX, DeNovix Inc., DE, USA). 500 ng of RNA and 2.5 µg of DNA per sample were loaded onto a CsTFA gradient medium (Whiteley et al., 2007) or CsCl gradient medium centrifuged (Neufeld et al., 2007), respectively, and centrifuged. After isopycnic centrifugation for 67 h at 20°C at 130 000 g_{av} (RNA) or 40 h at 20°C at 177 000 g_{av} (DNA), 10 fractions of each sample were collected, nucleic acids precipitated for subsequent community analyses and the buoyant density (BD) of each fraction was determined.

Denaturing Gradient Gel Electrophoresis (DGGE) analysis of density-resolved rRNA

Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting was used to detect and compare differences in heavy and light fractions recovered from CsTFA gradients ahead of amplicon sequencing. For amplification of the 16S rRNA gene for DGGE primers Bact340F (TAC GGG AGG CAG CAG) and 907R (CCG TCA ATT CMT TTG AGT TT) were used, with primer Bact340F containing a G+C rich sequence at the 5' end (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC; clamp; Muyzer et al.,

1993). 40 µl PCR reactions containing 1x SensiMix™ SYBR® & Fluorescein (Bioline, London, UK), 500 nm of each primer and 4 µl of template cDNA were prepared. The initial denaturation was performed at 94°C for 8 min. Denaturation, annealing and elongation were at 94°C for 30 s, 55°C for 30 s and 72°C for 60 s, respectively, with a total of 35 cycle, followed by a final elongation at 72°C for 5 min. After amplicon length was checked on a 1% agarose gel, amplicons were resolved on a 35 – 65% DGGE gradient gel (63 V, 60°C, 16.5 h) and imaged after the run was complete (Appendix, Figure 9).

16S rRNA gene amplification and sequencing

For the paired end Illumina MiSeq amplicon sequencing of the archaeal and bacterial 16S rRNA gene of the RNA-SIP, reverse transcribed DNA (cDNA) of fractions was prepared (SuperScript IV, Thermo Fisher Scientific, MA, USA). Primer pairs A519F (CAG CMG CCG CGG TAA; Wang and Qian, 2009)/Arch1017R (GGC CAT GCA CCW CCT CTC; Yoshida et al., 2005) and 341F (CCT ACG GGN GGC WGC AG; Herlemann et al., 2011)/805R (GAC TAC HVG GGT ATC TAA TCC; Herlemann et al., 2011) were used for amplification, respectively. The 5' end of both forward and reverse primers was prolonged with an adapter sequence (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G, respectively). Each amplification reaction contained 1x SensiMix™ SYBR® & Fluorescein (Bioline, London, UK), 500 nM of each primer and 4 µl of template cDNA at a final volume of 40 µl. Initial denaturation was performed at 94°C for 8 min. Denaturation, annealing and elongation were at 94°C for 40 s, 53°C for 40 s and 72°C for 50 s, respectively, for amplification of bacterial 16S rRNA, with a total of 30 cycles. Amplification of archaeal 16S rRNA occurred for denaturation, annealing and elongation at 94°C for 30 s, 55.5°C for 30 s and 72°C for 30 s, respectively, with a total of 30 cycles. For both protocols, terminal elongation occurred at 72°C for 5 min. Heavy and light fractions from the DNA-SIP were amplified with the universal primer pair 515F(P) (GTG YCA GCM GCC GCG GTA A; Parada et al., 2016)/806R(A) (GGA 0CTA CNV GGG TWT CTA AT; Apprill et al., 2015), targeting both archaea and bacteria. Both forward and reverse primers were prolonged with the same adapter sequences at their 5' end as used for the separate targeting of archaea and bacteria. For each sample 40 µl reactions were performed, containing 1x Reaction Buffer BD, 2 U Crystal Taq-DNA-Polymerase (Biolabproducts GmbH, Bebensee, GER), 0.01 µg BSA, 200 µM dNTPs, 2.5 mM $MgCl_2$, 500 nM of each primer, and 3 µl of template DNA. Initial denaturation was performed at 94°C for 8 min. Denaturation, annealing and elongation were at 94°C for 30 s, 50°C for 35 s and 72°C for 35 s with a total of 35 cycles. Terminal elongation was at 72°C for 10 min. Right amplicon length was checked on a 1% agarose gel und subsequently purified with the GeneRead Size Selection Kit (Qiagen, Venlo, NL), before sequencing on the Illumina MiSeq platform at the University of Göttingen (Genomic and Applied Microbiology, University of Göttingen, GER), which supplied sequence data as .fastq files.

Sequence processing

Mothur v.1.39.5 (Schloss et al., 2009) and a modified standard operational protocol for MiSeq data (Kozich et al., 2013) were used to perform sequence analysis. First, sequence reads were paired-end joined and filtered by amplicon length, and sequences with ambiguous bases as well as duplicate sequences were removed. Afterwards sequences were aligned to the Silva database (Silva database v138; https://www.arb-silva.de/download/arb-files/) (Quast et al., 2012; Yilmaz et al., 2014), formatted to be compatible with mothur (© 2019 Patrick D. Schloss, PhD, https://mothur.org/blog/2020/SILVA-v138-reference-files/#legalese), with a maximum homopolymer length ≤ 8. Pre-clustering of sequences allowed one difference every 100 bp and with VSEARCH (v2.6.0; Rognes et al., 2016) chimeras were identified and removed. Non-specific amplified sequences (e.g. fragments of mitochondria, eukaryota) were removed after classification against the Silva database (Silva database v138) (Quast et al., 2012; Yilmaz et al., 2014). The OptiClust algorithm was used to assign operational taxonomic units (OTUs) at 97% similarity level. The Inverse Simpson diversity index (Simpson, 1949) was used to calculate coverage and α-diversity indices, for comparison of β-diversity Bray-Curtis dissimilarity matrices were used (Bray and Curtis, 1957). Differential analysis of count data (DESeq2 package; Love et al., 2014) was used for tests of quantification and statistical inference of systematic changes between conditions.

Nucleotide sequence accession numbers

Bacterial and archaeal 16S rRNA gene sequences derived from amplicon sequencing were deposited at the NCBI sequence read archive under BioSample accession numbers SAMN15397987 to SAMN15397996 and SAMN15398124 to SAMN15398133 and SRA numbers SRR12119076 to SRR12119105 and SRR12119019 to SRR12119043, respectively, in BioProject PRJNA642728. 16S rRNA Sequences derived from amplicon sequencing with amplicons from universal primers were deposited under BioSample accession numbers SAMN15398175 to SAMN15398182 and SRA numbers SRR12119044 to SRR12119067 within the same BioProject as bacterial and archaeal 16S rRNA sequences.

Results

Physiological processes during incubation

In unsupplemented control microcosms and microcosms supplemented with either $^{12/13}$C-acetate or nitrate ± acetylene pH was stable at around 4.3-4.5 during the 13-day incubation period (Appendix, Figure 7). From day 6 on the pH in microcosms supplemented with both $^{12/13}$C-acetate and nitrate ± acetylene began to rise from 4.3 to approximately 5.6 until the end of incubation (Figure 2) and was, in contrast to the controls, significantly different to the start of incubation from day 7 on, but did not differ significantly between treatments ± acetylene in the headspace.

Acetate could only be detected when it was supplemented, otherwise being below the detection limit in unsupplemented control microcosms and microcosms supplemented with nitrate ± acetylene only. After the initial supplementation (~400 µM) acetate was consumed without delay in all microcosms. In treatments were both acetate and nitrate were present acetate was depleted after day five and therefore refed, this was also the case after days six and eleven (Figure 1). Microcosms supplemented with acetate only were refed once after day six, since depletion of acetate was considerably slower than in the other treatments. By the end of the incubation period 0.40 ± 0.10 to 1.25 ± 0.06 mM acetate were supplemented in total (Table 1, Table 2). Acetate concentrations in treatments supplemented with NO_3^- ± acetylene did not differ significantly during the incubation period. Samples measured via HPLC-ESI-MS included one replicate of each treatment before supplementation and right after supplementation. After seven days of incubation two replicates of each treatment were analyzed, and after 13 days, at the end of incubation, every replicate was analyzed. Before acetate was supplemented, acetate could not be detected with either HPLC with or without ESI-MS in soil slurries. Mean isotopic abundance of 59C-acetate (unlabeled) of ^{12}C-acetate treatments after initial supplementation with acetate were 99.9 ± 0.1%, mean 61C-acetate (fully labeled) abundance in ^{13}C-acetate treatments at this timepoint was 83.1 ± 1.2% and mean 60C-abundance was 13.4 ± 0.8%, in contrast (Figure 1). At day seven ^{12}C-treatments showed 98.7 ± 1.3% 59C-acetate abundance. Data from the ^{13}C-acetate plus NO_3^- treatments with and without acetylene are missing for day eleven, since supplemented acetate was fully depleted at this timepoint. ^{13}C-acetate treatments without nitrate had mean abundances of 79.8 ± 1.1% and 14.1 ± 0.6% of 61C- and 59C-acetate, respectively. At the end of incubation in all ^{12}C-acetate supplemented treatments only 59C-acetate was detected. ^{13}C-acetate treatments incubated without NO_3^- had abundances of 78.4 ±1.8% and 12.7 ± 1.2% of 61C- and 60C-acetate, respectively. Acetate values of ^{13}C-acetate plus NO_3^- treatments were again under detection limit, but treatments incubated with acetylene had abundances of 80.2 ±1.3% and 9.5 ± 2.0% of 61C- and 60C-acetate, respectively.

During the incubation period no significantly different amount of CO_2 was produced in slurries containing only soil or NO_3^-, and in controls containing acetylene and NO_3^- only at the end of incubation CO_2 was significantly different from the start. In controls containing ^{12}C- and ^{13}C-acetate significant

amounts of CO_2 were detected after days 11 and 13, respectively. Acetate only lead to an increase of CO_2 of approximately 30% during incubation. Incubations containing both acetate and NO_3^- ± acetylene already showed CO_2 concentrations significantly different from the start of incubation after days 6 and 7. CO_2 production compared to pure soil increased by about 200%, and 130% compared to acetate only controls. Samples measured with IRMS-GC included all three replicates from pure soil slurries from days 2, 7 and 13, as well as all three replicates of each slurry supplemented with ^{13}C-acetate at days 7 and 13 (Figure 1). In pure soil slurries ^{13}C-CO_2 accounted for 1.1 ± 0.0% of total CO_2 at all timepoints measured. In treatments supplemented with only ^{13}C-acetate ^{13}C-CO_2 accounted for 32.9 ± 2.8% and 43.8 ± 2.9% of total CO_2 at days 7 and 13, respectively. Mean values of ^{13}C-CO_2 accounting for total CO_2 of treatments supplemented with ^{13}C-acetate and NO_3^- reached 52.2 ± 2.0% and 68.5 ± 0.6% at days 7 and 13, respectively. Organic acids, as well as CH_4 (Figure 8) were negligible at all times of incubation. All data taken together, with an assumed mean value of 80.44% for 61C-aceate across ^{13}C-acetate treatments ± acetylene at all timepoints, suggest a ^{13}C-acetate recovery in ^{13}C-CO_2 of approximately 32% in ^{13}C-acetate supplemented treatments and 29% in treatments supplanted with both ^{13}C-acetate and NO_3^- ± acetylene, at the end of incubation.

In control incubations with only soil, a background of NO_3^- was detected, but no formation of N_2O (Figure 7). This extends to the treatments supplemented with either ^{12}C- or ^{13}C-acetate. In controls supplemented with NO_3^- ± acetylene, NO_3^- was consumed after an initial lag-phase of about two days and N_2O was above detection limit at day 8 in both the presence and absence of acetylene, and up to 0.06 and 0.12 mM N_2O were detected, respectively. Similarly, no or only trace amounts of N_2O could be detected in microcosms supplemented with $^{12}C/^{13}$C-acetate and NO_3^-, when incubated without acetylene in the headspace (Figure 7). In contrast, when acetylene was present, N_2O could be detected after a lag-phase of approximately five days and was significantly different to the start of incubation from day 7 on (

Figure 2). At the end of the incubation up to 0.4 mM N_2O were detected. Since N_2O production was not affected in microcosms containing $^{12}C/^{13}$C-acetate and NO_3^- when the pH started to shift, N_2O production in microcosms not containing acetylene should not have been affected either. Recovery of supplemented NO_3^--N as N_2O-N approximated 30% and 46% in microcosms containing ^{12}C- and ^{13}C-acetate + NO_3^- + acetylene, respectively. When acetylene was absent only up to 0.1% of supplemented NO_3^--N could be recovered as N_2O-N. In controls slurries containing NO_3^- ± acetylene recovered N in N_2O-N approximated 14% and 23%, respectively. When only acetate was supplemented up to 9% of NO_3^--N were recovered as N_2O-N, though up to 17% was recovered as NH_4^+-N. In treatments supplemented with both acetate and NO_3^-, the recovery of NO_3^--N recovery as NH_4^+-N reached a maximum of 2%, similar as when only NO_3^- was supplemented (1.5 – 2-4 %). In pure soil slurry microcosms N-recovery in the form of NH_4^+ approximated 5.6%.

All data taken together, NO_3^- together with acetate stimulated NO_3^- consumption up to 200% compared to NO_3^- only supplemented controls. Since N_2O could only be detected in the presence of

298 acetylene, complete denitrification is probably taking place. Complete denitrification lead to further
299 reduction of N_2O to N_2 in the absence of acetylene, which could not be measured in microcosms due
300 to technical limitations. When both NO_3^- and acetate were supplemented, N_2O production was
301 stimulated and reached 300% compared to NO_3^- only controls.

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303 **Sample coverage and phylogenetic analysis of density-resolved microbial populations and**
304 **assignment to OTUs**

305 Bacterial communities characterized in triplicates had an average of 24,801 ± 8,474 sequences among
306 all samples and a mean coverage of 87.4 ± 2.5% was estimated among samples. A total of 36,951
307 operational taxonomic units (OTUs) was retrieved. Alpha diversity analyses by Inverse Simpson values,
308 which cover both richness and evenness, were highest for samples before incubation, except for light
309 fractions of ^{13}C-acetate treatments with NO_3^- (Table 4). Archaeal communities characterized in
310 triplicates (except for heavy fractions of ^{12}C-acetate + NO_3^- and t_0, from which only one sample and
311 could be analyzed, and two samples for the light fraction of treatment ^{12}C + NO_3^-) had an average of
312 6,535 ± 3,681 sequences among all samples with mean coverage of 100.0 ± 0.0 %. Only four archaeal
313 OTUs were retrieved. The mean Inverse Simpson value for all samples was 1.0 ± 0.0 and average 95%
314 low-end and high-end confident intervals of 1.0 ± 0.0 and 1.0 ± 0.0, respectively.

315 Bacterial communities were similar in triplicate samples (Data not shown) and means values were used
316 for further analyses. Before incubation, relative abundance of the classes Gammaproteobacteria (26.9
317 ± 13.1% in heavy and 8.4 ± 4.4% in light fractions) and Alphaproteobacteria (9.9 ± 3.7% in heavy and
318 16.1 ± 1.6% in light fractions), both Proteobacteria, as well as Verrucomicrobiae (Verrucomicrobiota;
319 10.5± 0.4% in heavy and 13.1 ± 1.0% in light fractions), were the highest. Followed by Acidimicrobiia
320 (Actinobacteriota) and Bacteroidia (Bacteroidota) (Figure 3). Relevant actinobacterial OTUs were OTUs
321 2, 6 (both *Enterobacteriaceae*), 3 and 4 (both *AD3*_fa) for both light and heavy fractions, additionally
322 OTUs 7 (*Xanthobacteraceae*) and 8 (*Cytophagaceae*) were prominent in light fractions. After
323 incubation, communities were still dominated by the phylum Proteobacteria, especially the class
324 Gammaproteobacteria, with highest relative abundances of 49.5 ± 7.5 and 52.5 ± 10.6 in heavy
325 fractions of ^{13}C-acetate treatments without and with NO_3^-, respectively, and 23.6 ± 15.1 and 30.9 ±
326 11.3 in heavy fractions of ^{12}C-acetate treatments without and with NO_3^-, respectively (Figure 3). OTU 1
327 (*Burkholderiaceae*) (Appendix, Figure 10) dominated in both labeled and unlabeled treatments.

328 Archaeal communities were comprised by only the phylum Crenarchaeota and dominated by the order
329 Nitrososphaerales (OTU1), with a relative abundance of 97.7 ± 6.2% across all treatments. The
330 remaining percent consisted of members of the order Group_1.1c.

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Phylogenetic analysis of the acetate-assimilating nitrate-reducing community

Differences in bacterial β-diversity evaluated by AMOVA did not differ significantly by replicates ($P = 1$; except for t0 light $p = 0.511$) but were different by treatment and fraction ($P < 0.001$). Principal coordinate analyses (PCoA) based on Bray-Curtis dissimilarity supports these findings, revealing clustering by treatments and fractions (Figure 4). Samples from the start of incubation clearly separated from heavy fractions of ^{13}C-acteate + NO_3^-, which clustered not too far from ^{13}C-acetate treatments without NO_3^-. AMOVA of archaeal sequences revealed no significant difference between replicates or treatments and PCoA analysis did not show any differences in clustering by treatments or fractions (Data not shown). Log2Fold change values ($P < 0.05$) were computed between heavy and light fractions of each treatment and compared across all treatments. Bacterial OTU 1 differed significantly in between heavy and light fractions of the ^{13}C-acetate supplemented treatments (5.2) , but not in the presence of NO_3^-. When the *P value* was adjusted to near-significant ($p < 0.1$) OTU with the highest value in the heavy fractions of ^{13}C-acetate + NO_3^- treatments compared to the light fractions was OTU 166 with a log2Fold Change value of 3.9. No significant values for this OTU occurred in the controls or other treatments. This OTU is taxonomically assigned to the genus *Mucilaginibacter* within the order Sphingobacteriales. When heavy fractions between ^{13}C-acetate ± NO_3^- were compared OTU 238 with a value of 3.7 showed up, as well as when heavy and light fractions together of ^{13}C-acetate ± NO_3^- were compared having a value of 2.9. This OTU could as well be taxonomically assigned to the genus *Mucilaginibacter* within the order Sphingobacteriales. DESeq2 analysis for the archaeal community showed significant values in the heavy fraction of ^{13}C-acetate + NO_3^- treatment for OTU 1, but also for ^{12}C- and ^{13}C-acetate treatments and in the same range. Data for t_0 and ^{12}C-acetate + NO_3^- comparing heavy and light fraction are missing, due to missing sequence data. Comparison between heavy fractions of treatments ± NO_3^- did not show any tendencies as well.

Archaeal and bacterial nitrate reducing community as resolved by DNA-SIP

Characterized archaeal and bacterial community by DNA-SIP had an average of 66,054 ± 14,678 sequences among all samples and a mean coverage of 99.0 ± 0.5% was estimated among samples. A total of 8,483 OTUs was retrieved. Bacteria dominated in all treatments with mean relative abundances of 94.0 ± 5.4%. Nevertheless, maximum relative abundance of Archaea reached 7.2%. Alpha diversity analyses by Inverse Simpson values, which cover both richness and evenness, were higher for samples supplemented with only ^{12}/^{13}C-acetate, than for those supplemented with both acetate and NO_3^- (Data not shown). Communities were similar in triplicate samples (Data not shown) and mean values were further analyzed. Treatments supplemented with acetate only had balanced relative abundances of Alpha- and Gammaproteobacteria, while in treatments additionally supplemented with NO_3^- Gammaproteobacteria dominated with up to 60% (Figure 5). Again, one OTU, belonging to the *Burkholderiaceae* (Appendix, Figure 11) was dominant throughout treatments and fractions. PCoA

based on Bray-Curtis dissimilarity showed a separation by treatment and fractions, particularly separating treatments supplemented with NO_3^- from those supplemented with only acetate on axis 1 (Figure 6). Furthermore, heavy and light fractions from treatments containing NO_3^- were distinct from each other. Log2Fold change values ($P < 0.05$) were computed between heavy and light fractions of each treatment and compared across all treatments. In treatments supplemented with ^{13}C-acetate and NO_3^- the highest value (7.3) was obtained for OTU 573 (Actinobacteriota, class Thermoleophilia), when comparing heavy and light fractions, followed by OTUs 449 (5.5; Alphaproteobacteria), 407 (5.4; class Gemmatimonadetes), 332 (5.0; class Alphaproteobacteria), 410 (4.6; class Alphaproteobacteria) and 349 (4.5; class Thermoleophilia). For these OTUs no significant differences occurred in other treatments. When heavy fractions between treatments were compared, OTU 377 (class Gammaproteobacteria) was obtained with a value of 2.4. No other values were obtained for this OTU.

Discussion

Processes of the N-cycle in soil slurry microcosms

This study characterizes microbial acetate assimilators under nitrate-reducing conditions in soil of permafrost affected peat circles via SIP. These acidic peat circles of the Arctic tundra emit amounts of N_2O in the range of heavily fertilized agricultural and tropical rainforest soils (Potter et al., 1996; Repo et al., 2009; Werner et al., 2007). Biological processes leading to the emission of N_2O from soil and sediment include nitrification, dissimilatory reduction of nitrate to ammonium (DNRA) and denitrification. Well known for their contribution to N_2O production are ammonia oxidizing archaea, however not under anoxic, but (micro-) oxic conditions (Siljanen et al., 2019). In the absence of oxygen and under NO_3^- limiting conditions, DNRA can contribute to the release of N_2O from soil and sediment by the reduction of NO_3^- to ammonium (NH_4^+). This was not the case for treatments supplemented with both acetate and NO_3^-, since only negligible amounts of NH_4^+ were detected and excess of NO_3 was present during incubation. In contrast, NH_4^+ production could be detected in microcosms supplemented with only acetate, suggesting DRNA as the source of it, fueled by background NO_3^- still present after the preincubation as seen in the unsupplemented controls. It is known that DNRA is favored over denitrification when the C/N ratio is high, which was the case in these control microcosms (Rütting et al., 2011; Tiedje et al., 1983; Yoon et al., 2015). Another process able to contribute to N_2O production is chemodenitrification, an abiotic process that occurs at low pH and under oxygen limited conditions. Though this process will most certainly be outcompeted by the faster microbial denitrification and its main product is NO, not N_2O (Kresovic et al., 2009; van Cleemput, 1998). Therefore, the main process suggested to contribute to N_2O production in the experiment is microbial denitrification. Additionally, since N_2O could only be detected when acetylene was present, a known inhibitor of the N_2O-reductase (Yoshinari et al., 1977), the end product was rather N_2 than N_2O. Hence, in microcosms without acetylene in the headspace, produced N_2O must have been further reduced to N_2. This is in contrast to *in situ* field studies and incubations results, which report high amounts of N_2O being released from peat circle soil (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009). These findings also included that peat circles denitrifiers have the capacity to consume acetate, which was supported by this study when supplemented ^{13}C-acetate was consumed from the beginning of incubation without delay and approximately 30% of it could be recovered in ^{13}C-CO_2. Before and during incubation soluble organic acids were under or near detection limit, indicating a low fermentation potential of peat circle soil. Again, these findings are congruent with previous studies, that reported a low fermentation potential of peat circle soil compared to surrounding peat plateau (Palmer et al., 2012). Taking all data together and considering previous findings, during incubation up to 60% of consumed labeled acetate could have been used by microorganisms for assimilation and therefore labeling of these microorganisms for SIP. Thus, it is suggested that microorganisms from peat circle soil are capable of complete denitrification at acidic pH, using acetate as substrate.

Archaeal community structure

The archaeal community consisted of only the orders Nitrososphaerales, which dominated by over 97% mean relative abundance across all treatments, and Group_1.1c, both belonging to the Crenarchaeota. These orders were previously ranked within the phylum Thaumarchaeota, which was recently re-integrated into the phylum Crenarchaeota, and harbor known ammonia oxidizers (AOA) that might prefer an acidic pH below 5.5 (Gubry-Rangin et al., 2010; Prosser and Nicol, 2008) and prefer ammonia at low concentrations (De La Torre et al., 2008; Lehtovirta-Morley et al., 2011). Soil moisture and nitrogen content of investigated Arctic soils shaped AOA clade niche differentiation and had a high β-diversity of Thaumarchaeota (Alves et al., 2013). Other permafrost affected peat soil surfaces from Finland and Siberia that emit N_2O in the range of or even higher (76.8 µg N_2O-N m^{-2} h^{-1}) than managed peatland soils from northern countries showed a positive correlation between NO_3^- concentration of soils and *amoA* gene abundance (Siljanen et al., 2019). Even though the community structure was not influenced by the different incubation conditions in this study, the importance of AOA as *in situ* source of N_2O from northern peatlands has to be considered and further investigations should be triggered this way. Relative archaeal abundances of 0% to > 10% can be found in soils of all ecosystem and climate types (Bates et al., 2011). With relative abundances of up to 7% of all 16S gene sequences recovered by DNA-SIP, the relative abundances of Archaea are as one could expect.

Bacterial community structure of potential nitrate reducers and denitrifiers

The ability to denitrify is widespread, displaying a broad phylogenetic and functional variability. More than 60 genera within the Archaea, Bacteria, and Eukarya, are known to be able to denitrify (Philippot et al., 2007; Zumft, 1997). Due to the obligate intermediate N_2O, denitrification is the focus on numerous studies. N_2O, also known as laughing gas, is a strong greenhouse gas with a 300-fold stronger global warming potential than CO_2 (Stocker et al., 2018). Both biotic and abiotic processes can lead to the release of N_2O from soils and sediments, denitrification being one of them. Considering a general limitation of nitrogen (Jonasson et al., 1999) and slow mineralization of organic matter (Shaver et al., 1992), Arctic ecosystems have been regarded of minor importance concerning N_2O emissions. Despite this assumption, a decade ago, permafrost affected peat circles of the Arctic tundra with bare surface were discovered to emit substantial amounts of N_2O at an *in situ* pH 4 (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009).

Investigated bacterial community from the microcosm experiment revealed high relative abundances of Proteobacteria (Gamma- and Alphaproteobacteria), followed by Verrucomicrobiota, Actinobacteriota, and Bacteroidota, before supplementation and incubation of peat circle soil. A study conducted by Palmer et al., 2012 with soil from the same study site lead to congruent results. The inherent soil denitrifier community, as analyzed via amplicon pyrosequencing of the structural genes *narG*, *nirS, nirK*, and *nosZ*, revealed a high abundance of *narG* sequences affiliated with Actinobacteria,

that accounted for up to 95% of the overall sequence abundance of nitrate reducers. Though, sequences affiliated with Alpha- and Gammaproteobacteria were retrieved as well. Nitrite reducers harboring the copper-dependent nitrite reductase NirK were affiliated with Alphaproteobacteria accounting for over 90% of total *nirK* sequences. However, organisms harboring the cytochrome cd_1 dependent nitrite reductase NirS were more abundant in the investigated soil than those harboring NirK and the majority affiliated with Betaproteobacteria, now re-integrated into the Gammaproteobacteria. Bacteria harboring the *nosZ* gene were dominated by Alphaproteobacteria as well, though the overall copy numbers of *nosZ* were low and accounted for only 0.002% of 16S rRNA copy numbers (Palmer et al., 2012). Findings suggest the denitrifier community in peat circle soil is inherently dominated by Alphaproteobacteria. RNA transcripts of structural denitrification genes of the functional active microbial community from the High Arctic included as well members of the Alpha- and Gammaproteobacteria (Altshuler et al., 2019).

At the end of incubation, the bacterial community structure was shifted towards the Gammaproteobacteria, with sequences taxonomically affiliated with the family *Burkholderiaceae* being most abundant. Closely related N_2O reductase sequences retrieved from a comparable acidic palsa peat site in Finnish Lapland were affiliated with *Burkholderia pseudomallei* (Palmer and Horn, 2012), supporting *Burkholderiaceae* as candidates for the reduction of N_2O to N_2 in peat circle soil. This together with the high relative abundance of bacterial OTU1 at the end of incubation lead to suggest that it is one or even the key player of denitrification in acidic peat circle soil of this study. However, log2Fold change values were not significant between heavy and light fractions in the presence of nitrate. Though, when only acetate was supplemented, a log2Fold change value of 5.2 was calculated when heavy and light fractions of the ^{13}C-acetate treatments were compared. This, together with the recovery of background NO_3^- -N as NH_4^+ of 17%, suggests that OTU is not a key player of (complete) denitrification, but rather a key nitrate reducer, possibly being capable of DNRA. Diverse obligate and facultative anaerobic bacteria are known to perform DNRA within the Gamma-, Delta, and Epsilonproteobacteria as well as within the Bacteroidetes (Mohan et al., 2004; Smith et al., 2007; Tiedje, 1988). Thus OTU1, which is taxonomically affiliated with the *Burkholderiaceae*, is not a typical suspect for DNRA (formerly classified as Betaproteobacteria, now integrated into the Gammaproteobacteria). Metagenomic data from an Alaskan permafrost affected boreal forest soil suggested a low genetic potential for dissimilatory reduction of nitrate to ammonium in terms of *nrfA* gene abundances (Taş et al., 2014). Similar findings were obtained for arctic polygons (Taş et al., 2018). Average nitrate ammonification rates (0.23 ± 0.05 µmol N m^{-2} h^{-1}) measured from sediment in Alaska via isotope pairing technique were one to two orders of magnitude smaller than denitrification rates, suggesting that DNRA contributes only little to the overall sediment NH_4^+ turnover (McTigue et al., 2016). Generally, few studies on DNRA in permafrost systems are available, though genetic potential for this process was previously detected by metagenomics (Taş et al., 2018).

The screening of bacterial genomes, including sequences of *Burkholderiaceae* representatives, revealed the presence of *nosZ* as well as *nirK* genes in diverse *Burkholderiaceae* (Sanford et al., 2012). Isolated *Burkholderia* sp. from *Sphagnum* tissue covering a Finnish acid mire, were reported to have their pH optimum at approximately 5 and produce N_2O after NO_3^- supplementation during incubation, independent of the presence or absence of acetylene. Every isolate harbored the gene for the nitrate reductase NarG, while neither typical nor atypical N_2O reductase genes could be amplified and detected. Suggesting these *Burkholderia* sp. as incomplete denitrifiers (Nie et al., 2015). Two recently isolated *Caballeronia* strains (Burkholderiaceae) from peat circle soil, representing a potential new species, were as well reported to encode diverse nitrate reductases of the *narG*, *napA*, and *nasA* type, but no genes encoding for *nosZ* were reported (Hetz et al., 2020). These data taken together emphasize on *Burkholderiaceae* being important nitrate reducers in acidic peat circle soil. When the overall microbial community of the *Sphagnum* tissue from the Finnish acid mire was sequenced targeting 16S rRNA DGGE-cutting bands from incubations which showed N_2O production, not only *Burkholderia* sp., but also *Mucilaginibacter*, were revealed as major representatives of the bacterial community (Nie et al., 2015). In the present study, log2Fold change value of OTU 166, affiliated with *Mucilaginibacter*, was 3.9 in the heavy fractions of ^{13}C-acetate + NO_3^- treatments compared light fractions and had no (near) significant change in samples before incubation, controls and ^{12}C-acetate treatments. Additionally, when heavy and light fractions together of ^{13}C-acetate ± NO_3^- were compared OTU 238 with a near significant log2Fold change value of 3.7 showed up, also affiliated with *Mucilaginibacter*. *Mucilaginibacter* have been found and isolated in acidic and or permafrost-affected soils, like an acidic *Sphagnum* peat bog in Siberia (Pankratov et al., 2007), the Arctic tundra of Finnish Lapland (Männistö et al., 2010), and the High Arctic tundra of Norway (Jiang et al., 2012). The role of *Mucilaginibacter* in nitrate reduction and denitrification in these soils has yet to be determined, though current results suggest participation in nitrate reduction and denitrification of this genus in investigated peat circle soil. The overall relative abundances of both OTU 166 and OTU 238 were low, especially when compared to OTU 1, and their relative abundance accounted for only 0.5% and 0.7% in heavy fractions of ^{13}C-acetate + NO_3^- treatments, respectively, and less in all other controls and treatments. Leaving the question of the overall importance of these microorganisms in the denitrifier community.

Known for their ability to reduce N_2O to N_2 under acidic conditions are members of the genus *Rhodanobacter* (Van Den Heuvel et al., 2010). *NirS* sequences closely related to *Rhodanobacter* sp. were previously retrieved from an acidic (pH ~4) Finnish palsa peat, with relative abundances of OTUs in amplicon libraries of 5.0% in the upper 20 cm of soil (Palmer and Horn, 2012). Relative abundances of *Rhodanobacter* in the current study were highest in light fractions before supplementation (0.4%) and were slightly lower after incubation without significant differences between treatments, when 16S rRNA is compared. Relative abundances in the DNA-SIP were higher with up to 1.9% of total OTUs. Again, this leaves the question of the overall importance of these microorganisms in the denitrifier community of investigated peat circles.

N_2O reduction potential at acidic pH

This study aimed on identifying new acid tolerant nitrate and nitrous oxide reducers and key denitrifiers of peat circle soil by SIP analysis. The addition of easily available labeled and unlabeled carbon in form of acetate to microcosm incubations together with control microcosms were supposed to reveal a possible carbon limitation in cryoturbated peat circles.

The genetic potential of N_2O reduction, i.e. the abundance of *nosZ* genes, under acidic conditions was tested positive in previous studies (Palmer et al., 2010, 2012), as well as when initially produced N_2O derived from endogenous N-sources was consumed at pH 4 in laboratory incubations with peat circle soil (Palmer et al., 2012). Though, in contrast to the detected potential for complete denitrification, peat circles emit high amounts of N_2O in both field and *in situ* experiments (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009).

To our surprise, in the current study, N_2O could only be detected in the presence of acetylene, when the final step of denitrification, the reduction of N_2O to N_2, was inhibited. One explanation might be that there was no electron donor limitation, when acetate was supplied. In their study from 2012 Palmer et al. investigated apparent Michaelis-Menten kinetics of nitrate-dependent denitrification in anoxic microcosms, revealing that peat circle denitrifiers were saturated with less than half of the NO_3^- concentrations occurring *in situ*, therefore suggested a limitation of electron donor availability that restricts denitrification in cryoturbated peat circles (Palmer et al., 2012). With the addition of acetate to the slurry incubations, electron donor limitations were overcome and denitrification no longer restricted, leading to complete denitrification. This is supported by a study conducted with the bacterial denitrifier *Alcaligenes faecalis* in a steady state culture. The production of N_2O was impaired by the addition of the electron donor acetate, but after pulses of acetate, the *A. faecalis* culture immediately reduced accumulated NO_2^- and N_2 production was increased (Schalk-Otte et al., 2000). Electron donor limitation in cryoturbated peat circle soil might therefore favor the emission of N_2O, despite the molecular potential of the microbial community for the further reduction of N_2O to N_2. Thus, conditions in microcosm incubations of the current study, i.e. the supplementation of acetate for SIP analysis, might have overcome this limitation.

Another well-known factor influencing denitrification is pH. Neutrophilic model organisms like *Paracoccus denitrificans* accumulate and release N_2O when pH drops below 7 (Bergaust et al., 2010). In a study with extracted bacterial cells, originally derived from peat soil with different pH (4, 6 and 8), *nosZ* gene transcription was not impaired by pH, leaving the reason for low N_2O reduction activity in acidic soils further on in the dark (Liu et al., 2014). Interestingly, cells derived from soil with pH 4 were not able to reduce N_2O to N_2, not even when transferred into more pH neutral medium. Cells derived from more neutral soil that showed significant transcription of the *nosZ* gene, were nonetheless unable to produce a functional N_2O-reductase when the pH was below 6. Only if *nosZ* was expressed at pH 7, it was as well functional at a lower pH range tested (5.7 – 7.6). These findings are in favor of the

hypothesis that the main cause preventing N_2O reduction in soils with low pH is the preclusion of a successful assembly of a functional N_2O reductase (Liu et al., 2014). But how is it possible that several studies complete denitrification was measured at acidic pH? The explanation might me microsites. When a high density of active denitrifiers is given they might cluster together, similar to biofilm formation, resulting in a higher pH within these microsites and enabling the assembly of a functional N_2O-reductase (Liu et al., 2014). In conclusion, microsites together with an easily bioavailable electron donor might account for the reduction of N_2O to N_2 in the current study of peat circle soil.

Conclusions

Permafrost affected cryoturbated peat circle soil from the Arctic tundra host acid tolerant microorganisms capable of complete denitrification at pH 4 with acetate as carbon source and electron donor. Taxa with highest relative abundances under applied incubation conditions and possible key players of denitrification were identified via SIP analyses coupled to 16S rRNA Illumina MiSeq amplicon sequencing and DESeq2 analysis. The collective data indicate that 1) *Burkholderia-Paraburkholderia-Caballeronia* are major nitrate reducers, 2) the denitrifier community in acidic cryoturbated peat circles is capable of complete denitrification at pH 4 with acetate as electron donor and, 3) cryoturbated peat circle soil is carbon limited, leading to the *in situ* emission of N_2O rather than N_2

Author Contributions

SH and MH designed the SIP experiments, wrote the original manuscript, and interpreted data. SH set up microcosms and performed all laboratory work, if not stated otherwise. MH conceived the original idea and oversaw all laboratory work.

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Tables

Table 1: Compound consumption (acetate and nitrate) and production (carbon dioxide and nitrous oxide) during incubations in treatments with and without acetylene. Mean values of technical replicates with standard deviation.

	w/o acetylene		with acetylene	
Compound [mM]	**^{12}C-acetate, NO_3^-**	**^{13}C-acetate, NO_3^-**	**^{12}C-acetate, NO_3^-**	**^{13}C-acetate, NO_3^-**
CO_2	1.11 ± 0.18	1.05 ± 0.11	1.12 ± 0.20	1.03 ± 0.13
Acetate	1.18 ± 0.10	1.25 ± 0.06	1.06 ± 0.01	1.20 ± 0.05
CH_4	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.00
NO_3^-	1.98 ± 0.09	2.21 ± 0.04	2.35 ± 0.23	1.81 ± 0.17
N_2O	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.07	0.41 ± 0.05
NH_4^+	0.03 ± 0.00	0.03 ± 0.01	0 03 ± 0.01	0.04 ± 0.01

Table 2: Compound consumption (acetate and nitrate) and production (carbon dioxide and nitrous oxide) during incubations in controls with and without acetylene. Mean values of technical replicates with standard deviation.

Compound [mM]	**soil**	**NO_3^-**	**NO_3^- (10 % v/v acetylene)**	**^{12}C-acetate**	**^{13}C-acetate**
CO_2	0.35 ± 0.03	0.33 ± 0.02	0.39 ± 0.04	0.46 ± 0.05	0.44 ± 0.07
Acetate	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.48 ± 0.16	0.40 ± 0.10
CH_4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NO_3^-	0.23 ± 0.02	0.90 ± 0.19	1.08 ± 0.12	0.26 ± 0.10	0.23 ± 0.02
N_2O	0.01 ± 0.01	0.06 ± 0.00	0.12 ± 0.01	0.03 ± 0.01	0.0 ± 0.00
NH_4^+	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.0

Table 3: Net N-recovery from supplemented nitrate as N_2O-N and NH_4^+ during incubations in treatments with and without acetylene. Mean values of technical replicates with standard deviation.

	w/o acetylene		with acetylene	
N-recovery [%]	**^{12}C-acetate, NO_3^-**	**^{13}C-acetate, NO_3^-**	**^{12}C-acetate, NO_3^-**	**^{13}C-acetate, NO_3^-**
N_2O-N	-12.22 ± 1.27	-7.87 ± 0.22	37.47 ± 13.16	75.81 ± 29.74
NH_4^+	-0.44 ± 0.53	- 0.53 ± 0.43	-0.05 ± 0.86	0.96 ± 1.54
Total N-recovery	-12.66 ± 0.85	-8.40 ± 0.22	37.42 ± 13.35	76.78 ± 31.13

Table 4: Inverse Simpson values for bacterial 16S rRNA amplicon sequences. Mean values of biological replicates with standard deviation. t_0 – pre-supplementation, all other samples at end of incubation.

Sample	Fraction	Inv Simpson	LCI	HCI
^{12}C-acetate, NO_3^-	Heavy	20.3 ± 5.1	18.5 ± 4.5	22.5 ± 5.8
^{12}C-acetate, NO_3^-	Light	17.6 ± 6.4	16.2 ± 6.0	19.2 ± 6.8
^{13}C-acetate, NO_3^-	Heavy	4.7 ± 2.4	4.4 ± 2.2	5.0 ± 2.6
^{13}C-acetate, NO_3^-	Light	26.6 ± 5.8	24.1 ± 2.2	29.7 ± 6.7
^{12}C-acetate	Heavy	42.5 ± 27.4	38.4 ± 24.5	47.6 ± 31.0
^{12}C-acetate	Light	32.0 ± 14.4	29.0 ± 12.9	36.6 ± 16.3
^{13}C-acetate	Heavy	5.4 ± 1.8	5.0 ± 1.7	5.8 ± 2.0
^{13}C-acetate	Light	135.3 ± 5.7	126.1 ± 5.6	146.0 ± 5.7
t_0	Heavy	57.3 ± 75.3	52.6 ± 69.0	62.9 ± 82.7
t_0	Light	97.6 ± 21.9	89.9 ± 20.3	106.6 ± 23.8

Larger values indicate higher α-diversity. LCI and HCI indicate the 95 % low- end and high-end confidence intervals, respectively.

Table 5: Inverse Simpson values for bacterial and archaeal 16S rRNA amplicon sequences generated with universal primers after the incubation period. Mean values of biological replicates with standard deviation.

Sample	Fraction	Inv Simpson	LCI	HCI
^{12}C-acetate, NO_3^-	Heavy	2.8 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
^{12}C-acetate, NO_3^-	Light	7.2 ± 0.5	6.9 ± 0.5	7.5 ± 0.5
^{13}C-acetate, NO_3^-	Heavy	3.7 ± 0.9	3.6 ± 0.9	3.8 ± 1.0
^{13}C-acetate, NO_3^-	Light	2.8 ± 0.4	2.8 ± 0.3	2.9 ± 0.4
^{12}C-acetate	Heavy	23.2 ± 6.7	22.1 ± 6.4	24.4 ± 7.1
^{12}C-acetate	Light	23.7 ± 4.5	22.6 ± 4.4	24.8 ± 4.6
^{13}C-acetate	Heavy	56.3 ± 69.3	54.0 ± 66.8	58.8 ± 72.0
^{13}C-acetate	Light	31.8 ± 34.7	30.4 ± 33.2	33.2 ± 36.3

Larger values indicate higher α-diversity. LCI and HCI indicate the 95 % low- end and high-end confidence intervals, respectively.

Figures

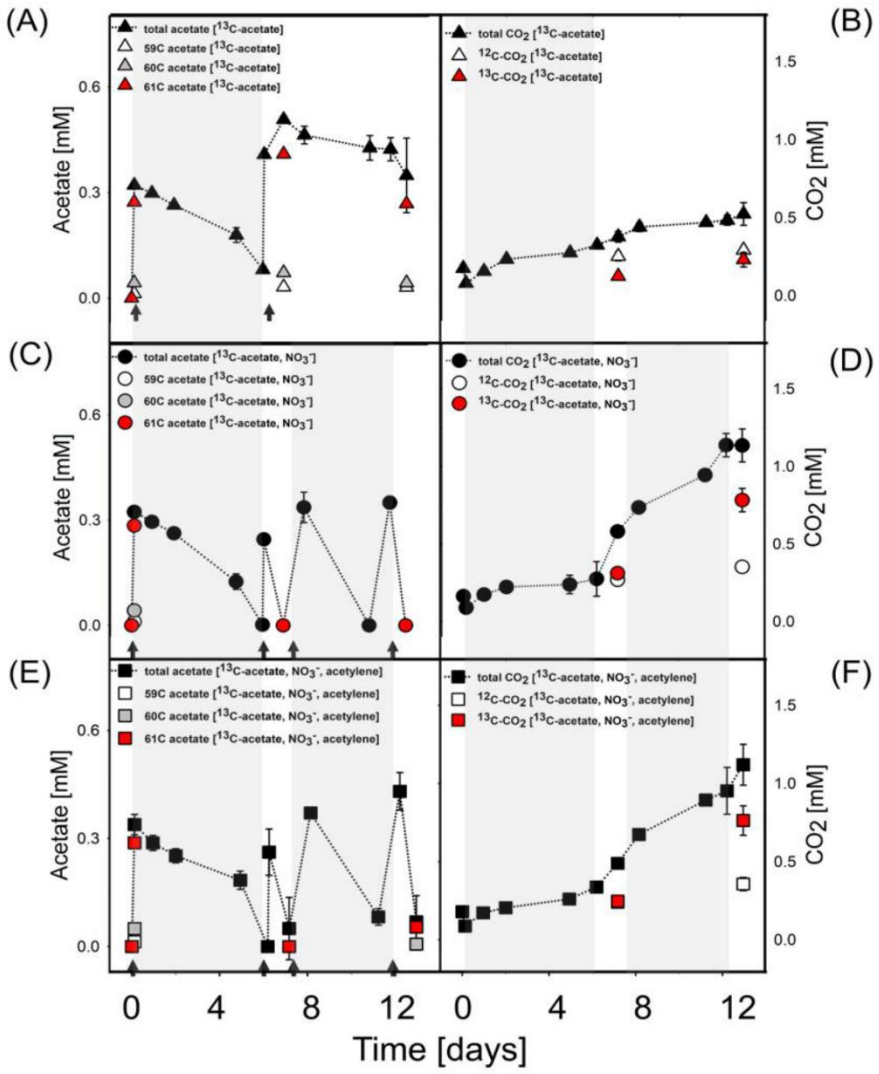
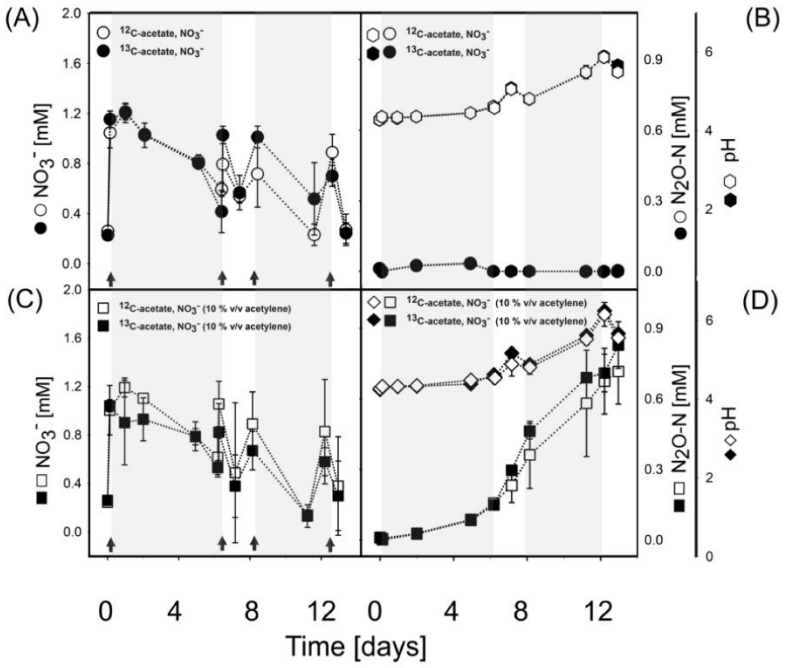


Figure 1: Acetate consumption and CO_2 production of ^{13}C-acetate treatments with and without acetylene during incubation period with unraveled isotopes. Arrows indicate acetate supplementation. (A)/(C)/(E) Total acetate consumption in direct comparison with non-labeled (59C-acetate), half-labeled (60C-acetate, one heavy C-atom) and fully-labeled (61C-acetate, 2 heavy C-atoms) acetate. (B)/(D)/(F) Total CO_2 production in direct comparison to non-labeled (^{12}C-CO2) and labelled (^{13}C-CO_2) CO_2. Upper pictures of treatments incubated without acetate only; middle pictures of treatments incubated with acetate and NO3- without acetylene; lower pictures of treatments incubated with acetate, nitrate, and acetylene. Arrows indicate acetate supplementation.



831

832 Figure 2: *Nitrate consumption (A)/(C) and nitrous-oxide-N production with pH behavior (B)/(D) during incubation period. Mean*
833 *values of triplicates with standard deviation. Upper pictures of treatments incubated without acetylene; lower pictures of*
834 *treatments incubated with acetylene. Arrows indicate nitrate supplementation.*

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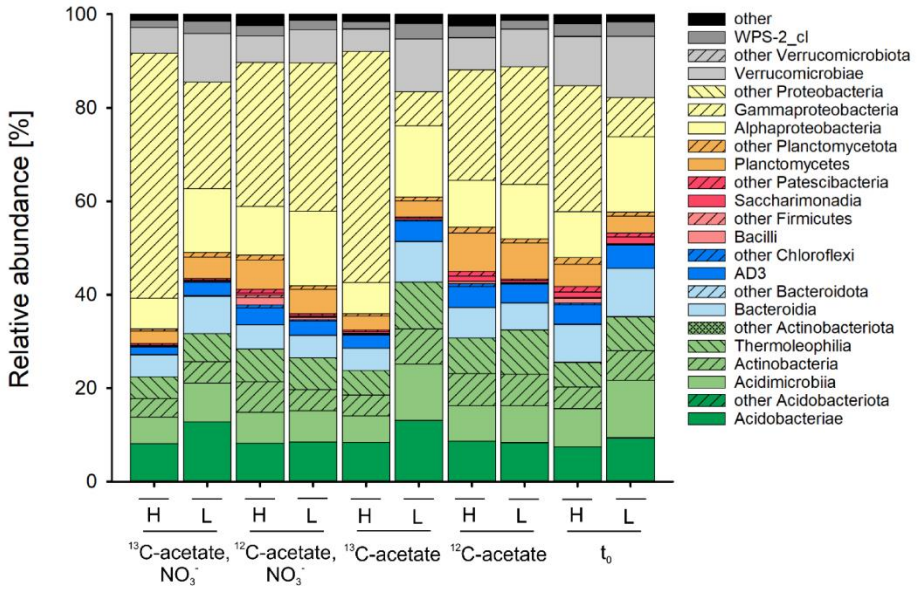

836

837 *Figure 3:* Relative abundance of bacterial classes within phyla more abundant than 2 % in at least on sample of RNA-SIP.
838 Other: phyla abundance smaller than two percent in all samples. Mean values of triplicate incubations. Heavy (H) and Light
839 (L) indicating the fractions after isopycnic centrifugation; t_0 – pre-supplementation.

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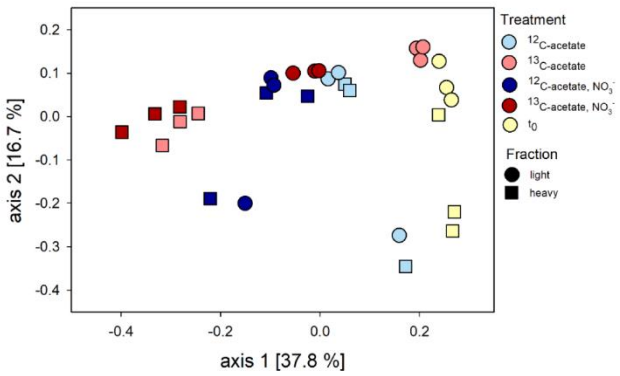
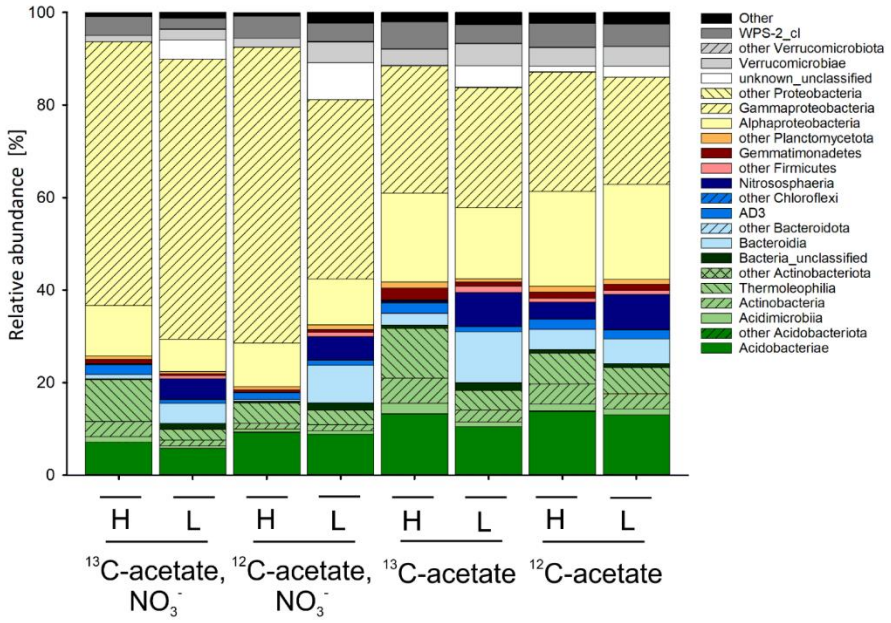


841

842 *Figure 4:* PCoA plot based on Bray-Curtis dissimilarity. Dissimilarity plot of the relative abundance of bacterial OTUs between
843 treatments and fractions at the beginning and the end of incubation on species level of the RNA-SIP.

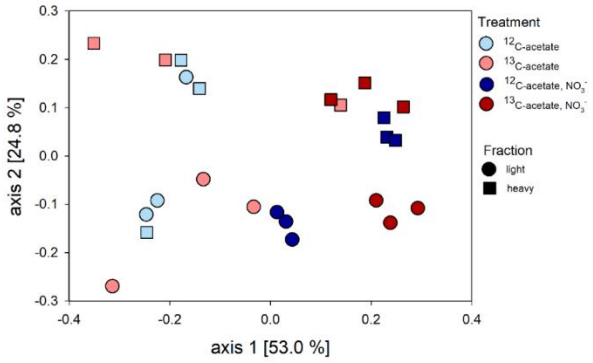
844



845

846 *Figure 5:* Relative abundance of archaeal and bacterial classes within phyla more abundant than 2 % in at least on sample.
847 Other: phyla abundance smaller than two percent in all samples. Mean values of triplicate incubations. Heavy (H) and Light
848 (L) indicating the fractions after isopycnic centrifugation.

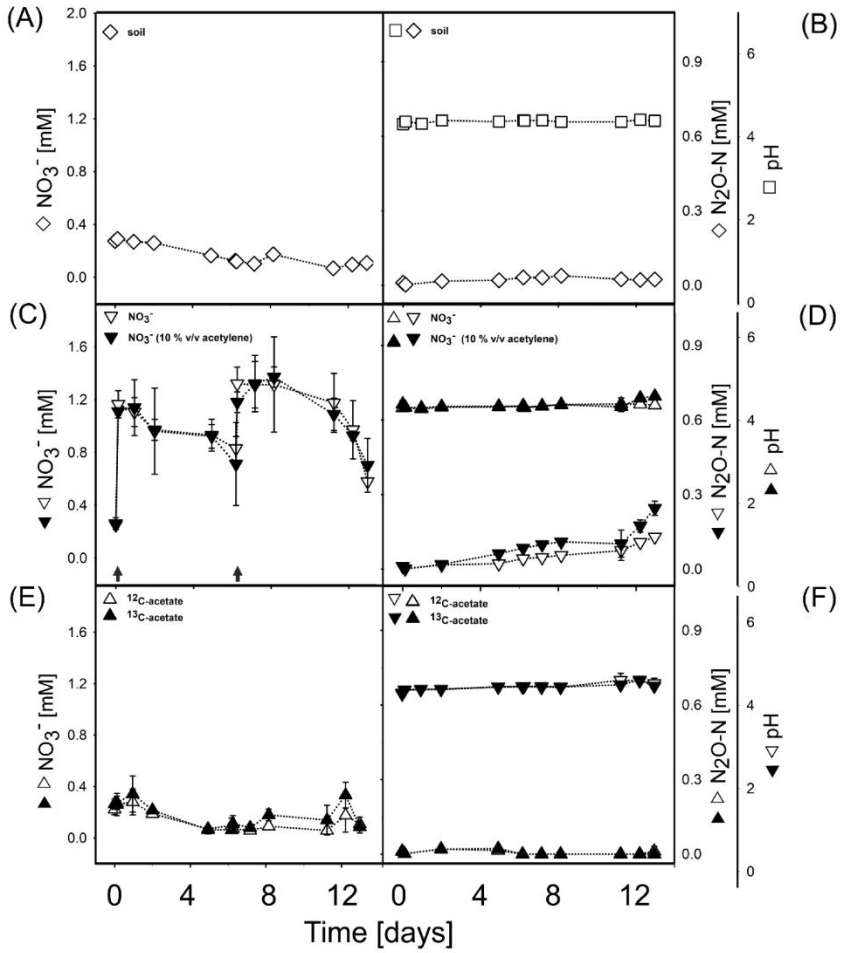
849



850

851 *Figure 6:* PCoA plot based on Bray-Curtis dissimilarity. Dissimilarity plot of the relative abundance of archaeal and bacterial
852 OTUs between treatments and fractions at the end of incubation on species level of the DNA-SIP.

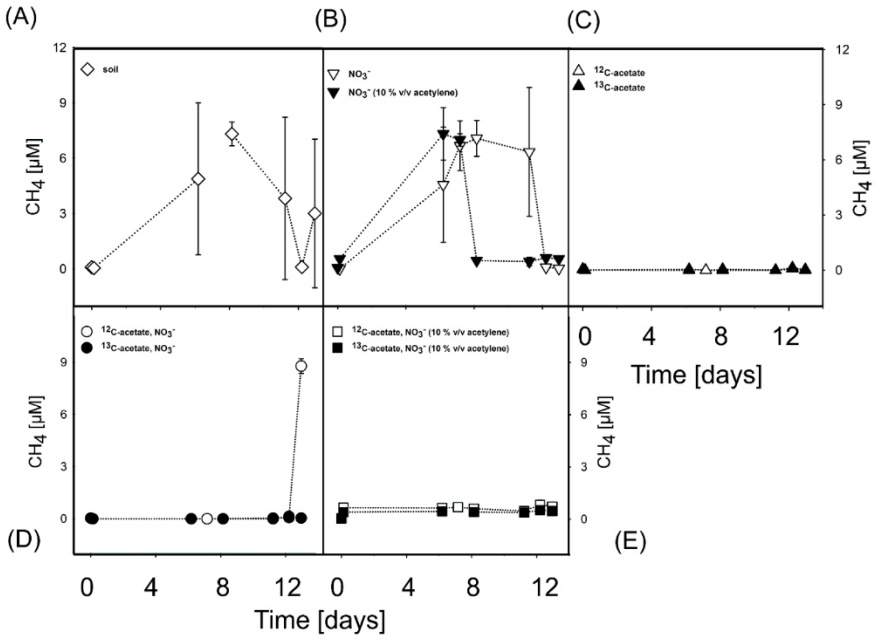
853 **Appendix**



854

855 Figure 7: Nitrate concentration and consumption (A)/(C)/(E) and nitrous-oxide-N production with pH behavior (B)/(D)/(F) of
856 control incubations. Mean values of triplicates with standard deviation. Upper pictures of pure soil incubations; middle
857 pictures of controls with nitrate with or without acetylene; lower pictures of incubations with acetate. Arrows indicate nitrate
858 supplementation.

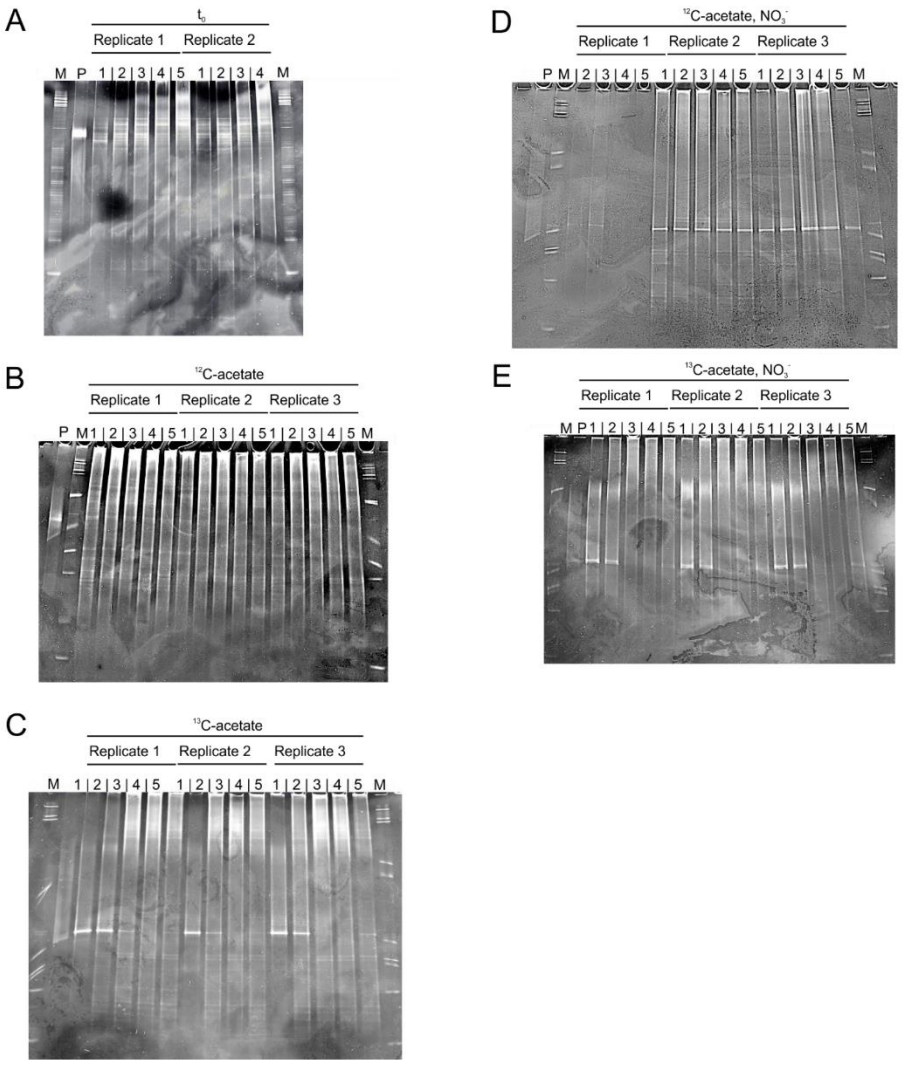
859



860

861 Figure 8: Methane production during incubation period. Mean values of triplicates with standard deviation. (A) Pure soil
862 incubations, (B) treatments incubated with nitrate with and without acetylene, (C) incubations with acetate, (D) treatments
863 incubated with acetate and nitrate, (E) treatments incubated with acetate, nitrate, and acetylene.

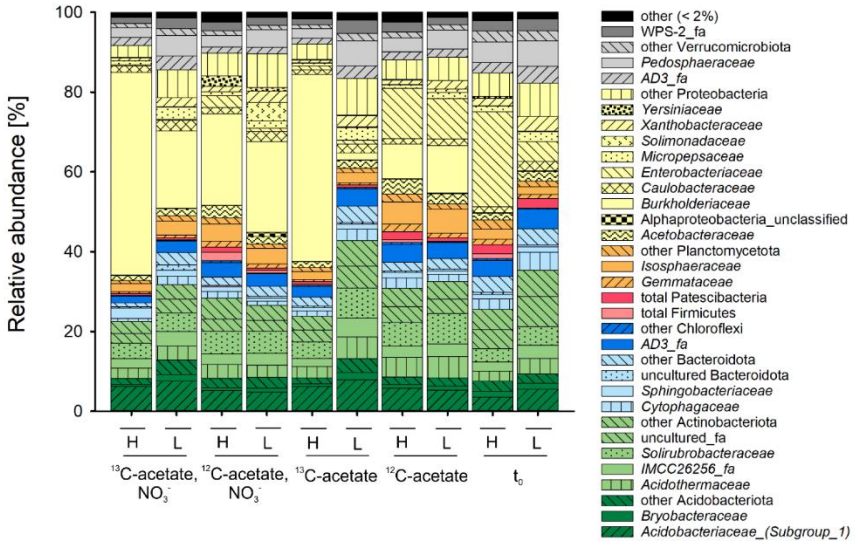
864



865

866 Figure 9: DGGE gels of heavy and light fractions of all replicates (1-3) and treatments. 1 – heaviest fraction; 5 – lightest
867 fraction; M – marker; P – positive control. **A**: Heavy and light fractions from all three replicates of t_0 (pre-supplementation)
868 samples. **B:** Heavy and light fractions from all three replicates of ^{12}C-acetate treatments. **C:** Heavy and light fractions from all
869 three replicates of ^{13}C-acetate treatments. **D:** Heavy and light fractions from all three replicates of ^{12}C-acetate + NO_3^-
870 treatments. **E:** Heavy and light fractions from all three replicates of ^{13}C-acetate + NO_3^- treatments.

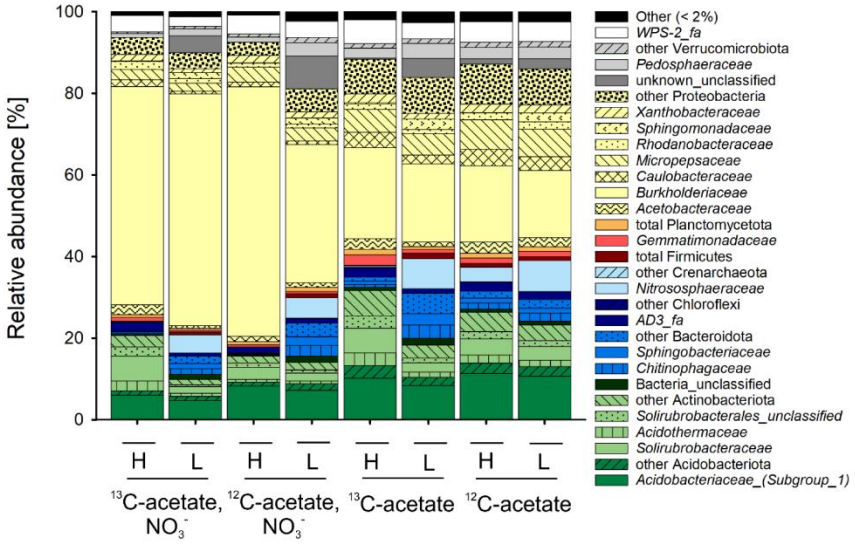
871



872

873 Figure 10: Relative abundance of bacterial families within phyla more abundant than 2 % in at least on sample of RNA-SIP.
874 Other: phyla abundance smaller than two percent in all samples. Mean values of triplicate incubations. Heavy (H) and Light
875 (L) indicating the fractions after isopycnic centrifugation; t_0 – pre-supplementation.

876



877

878 Figure 11: Relative abundance of bacterial and archaeal families within phyla more abundant than 2 % in at least on sample
879 of DNA-SIP. Other: phyla abundance smaller than two percent in all samples. Mean values of triplicate incubations. Heavy (H)
880 and Light (L) indicating the fractions after isopycnic centrifugation.

5.3.3 Nitrate fuels pH dependent complete denitrification in acidic permafrost affected peatlands

Nitrate fuels pH dependent complete denitrification in acidic permafrost affected peatland

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Abstract

Acidic peat circles (pH 4) in the Eastern European Tundra harbor up to 2 mM pore water nitrate and emit the greenhouse gas N_2O like heavily fertilized agricultural soils in temperate regions. The main process yielding N_2O under oxygen limited conditions is denitrification, that is the sequential reduction of nitrate to N_2O and N_2. Crucial factors impacting denitrification and N_2O/N_2 ratios are organic carbon to nitrate ratios and pH. The assembly of a functional N_2O reductase of classical neutrophilic model denitrifiers is impaired at pH < 6, resulting in increased N_2O/N_2 ratios. In contrast to this, the surrounding vegetated peat plateau, with similar *in situ* pH, does not emit N_2O, though is rich in ammonium and organic carbon. Microcosm experiment revealed the potential for peat circle soil to reduce supplemented nitrate not only at pH 6, but also at *in situ* near pH 4, to N_2. Therefore, being a potential sink for N_2O emissions. Furthermore, nitrate supplemented peat plateau soil has the potential for complete denitrification as well, as shown by microcosms experiments, though only at pH 6. At more acidic pH the released end product of nitrate reduction is N_2O.

Introduction

Approximately 16-25 % of the global soil surface area is comprised of permafrost, including large peatland areas. These soils are estimated to store half of the global below ground organic carbon that can serve as potential electron donor for the generation of reactive Nitrogen (N) from less reactive N-species (Anisimov, 2007; Tarnocai et al., 2009). Permafrost soils are assumed to be large N reservoirs, since high organic carbon content is positively correlated with high organic N content in northern peatlands (Post et al., 1985). An estimated 67 Pg N are stored in the upper 3 m of these soils (Harden et al., 2012), leading to the conclusion that northern peatland soils alone store about 10 % of the global soil organic matter N (Limpens et al., 2008; Loisel et al., 2014). Processes involved in N-cycling and therefore the release of N-gases depend on the availability of reactive N-species. In remote, pristine permafrost-affected soils with low atmospheric deposition, the availability of reactive N is primarily controlled by ammonification, i.e. the mineralization of organic N, and N-fixation (Canfield et al., 2010).

Alongside these processes, four further N transformation processes altering the oxidation state of N (-3 to +5) exist: nitrification, nitrate dissimilation, anammox and assimilation. These processes can be put in two main categories. The first category being assimilation, i.e. the acquisition of N for incorporation into biomass, and the second category being dissimilation, i.e. processes associated with the conservation of energy in form of ATP (Thamdrup, 2012). However, only a small fraction of N that is stored in permafrost affected soils is bioavailable for (micro-)organisms, making N the major limiting nutrient in Arctic soils (Nordin et al., 2004; Shaver and Chapin III, 1980). Global warming, along with permafrost thaw, is expected to increase the N-availability, thus fueling processes of the N-cycle (Anisimov, 2007; Marushchak et al., 2011). This is in line with recent studies showing an increase in nitrous oxide (N_2O) emissions following warming and permafrost thaw in *in situ* experiments (Voigt et al., 2017b, 2017a; Yang et al., 2018). N_2O is an important greenhouse gas and ozone depleting substance, with a long atmospheric lifetime (Forster et al., 2007; Ravishankara et al., 2009). On a 100-year basis, its global warming potential is about 300-fold higher than that of carbon dioxide (CO_2) (Forster et al., 2007; Spahni et al., 2005). The primary sources of N_2O emission are soils, i.e. agricultural and tropical rainforest soils, accounting for 60-70 % of annual N_2O emissions (Behrendt et al., 2019; Christensen, 2009; Conrad, 1996; Denman et al., 2007; Marushchak et al., 2011; Mosier et al., 1998; Repo et al., 2009). Due to slow mineralization of organic matter under cold, humid conditions (Shaver et al., 1992) and low atmospheric N deposition, biological processes are generally N limited in ecosystems at northern latitudes (Martikainen et al., 1993; Potter et al., 1996), resulting in a competition for available N between vegetation and microorganisms (Thamdrup and Dalsgaard, 2008) and therefore only emit low amounts of N_2O.

The importance of Arctic peatlands and permafrost affected soils as sources of N_2O are just emerging (e.g. Behrendt et al., 2019; Christensen, 2009; Denman et al., 2007; Marushchak et al., 2011; Repo et al., 2009), as different studies showed that Arctic soils produce (Abbott and Jones, 2015; Elberling et al., 2010) and release (Marushchak et al., 2011; Repo et al., 2009) amounts of N_2O in the range of heavily fertilized agricultural soils (Marushchak et al., 2011; Repo et al., 2009). So-called cryoturbated peat circles, located in the East European tundra, emit exceptionally high rates of N_2O throughout the growing season (1.9-32 mg N_2O $m^{-2}d^{-1}$), making them hot spots of N_2O emissions (Repo et al., 2009). Peat circles are round patches of bare peat, surrounded by vegetated peat plateau, with an *in situ* pH around 4 and pore water nitrate (NO_3^-) concentrations of up to 2 mM, which is readily available for denitrification, the major source of N_2O under oxygen limited conditions (Palmer et al., 2012; Repo et al., 2009). Denitrification is the reduction of NO_3^- or nitrite (NO_2^-) to molecular dinitrogen gas (N_2) via the intermediates nitric oxide (NO) and N_2O. Since microorganisms might miss one or more genes associated with the denitrification process, many truncated forms exist and N_2O is not only an obligate intermediate, it can also be the end product of denitrification (Cofman Anderson and Levine, 1986; Stein and Klotz, 2016). Not only the denitrifying community, but also abiotic factors have an influence on the denitrification process. Depending on, e.g. pH , the C/N ratio, oxygen content and substrate

availability, denitrification can act as source or sink of N_2O (Bergaust et al., 2010; Bru et al., 2011; Dorsch et al., 2002; Enwall et al., 2005; Holtan-Hartwig et al., 2000; van Cleemput, 1998). In certain systems the production ratio of N_2O to N_2 is increased when the pH is below 5 (Cuhel et al., 2010; Simek et al., 2002).

In contrast to cryoturbated peat circles, unturbated vegetated peat plateau soils from the same study site with the same acidic pH, do essentially not emit N_2O *in situ* (Marushchak et al., 2011; Repo et al., 2009). Likely accountable for this rather contrasting N_2O emissions, are differing denitrifier communities between the bare and vegetated peat soils, as shown in phylogenetic studies, that correlate with the denitrification potentials of both soil types (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009).

It is hypothesized that the major microbial process contributing to the emission of N_2O from acidic cryoturbated peat circles is denitrification and that N_2O emissions depend on the *in situ* pH. Therefore, the main objectives of this study were 1) to show the effect of pH on the N_2O emission potential, and 2) to test, whether pH impairs the denitrification potentials in cryoturbated peat circles.

Materials and Methods

Sampling site

Samples were taken from cryoturbated peat circles (PC) and surrounding unturbated peat plateau (PP) from the subarctic study site in the Northeastern European Tundra in Russia, located in the discontinuous permafrost zone (67°03'N, 62°57'E, 100 m a.s.l.) with a mean annual air temperature of -5.6 °C (Marushchak et al., 2011). Both PC and PP were described previously, (Biasi et al., 2014; Hugelius et al., 2011; Repo et al., 2009). Generally, during growing season high amounts of N_2O are emitted from PC (1.9-32 mg N_2O m^{-2} d^{-1}) whilst N_2O emissions from PP are negligible (Repo et al., 2009) and the carbon to nitrogen (C/N) ratio is low (23 ±W 2) in PC compared to PP (59 ± 10) soil (Repo et al., 2009; Supplementary Methods). Soil from the upper 10 cm of three different PC and PP sites was sampled in summer 2014 and stored at 4 °C until further processing. Within three months after sampling experiments were conducted. Soil moisture content of PC and PP was determined via weighing soil samples before and after drying at 60 °C for one week and accounted for 74 % and 85 %, respectively.

Assessment of pH dependent denitrification potentials in soil microcosms

Root particles were removed and soil from the sampled PC and PP was pooled and homogenized prior to incubations. All incubations were carried out in triplicates at both *in situ* pH ~4 and more neutral pH 6. Approximately 40 g of soil homogenate was mixed with 6.5 parts sterile deionized water for each microcosm and placed into 500 ml veral bottles that were sealed with airtight rubber stoppers. Prior to supplementation PC soil slurries were preincubated for 10 days under microoxic conditions to remove excess endogenous NO_3^-. PP microcosms were not preincubated, since endogenous NO_3^- was negligible. At the start of the experiment the gas phase was exchanged with 100 % N_2 or 100 % synthetic air (20% O_2, rest N_2; Linde GmbH, Pullach, Germany) for anoxic and oxic treatments, respectively. To differentiate between incomplete and complete denitrification, microcosms with and without the N_2O reductase inhibitor acetylene (10 % v/v headspace) were prepared. Acetylene hinders the reduction of N_2O to N_2, which leads to N_2O being the measurable end product of denitrification (Yoshinari et al., 1977). Microcosms were incubated at 15 °C in the dark on an overhead-shaker. At the incubation start ~0.7 mM NO_3^- was supplemented to support denitrification. Supplementation occurred once, if not stated otherwise. Control incubations included unsupplemented soil under oxic and anoxic conditions, as well as NO_3^- supplemented incubations under oxic conditions. Throughout the incubation period pH, nitrate, O_2, as well as CO_2 and N_2O production were monitored.

Analytical procedures

pH was measured repeatedly during incubation and was determined with a pH electrode (InLab 422; Mettler Toledo GmbH, Gießen, Germany). Gas chromatography (GC), equipped with an electron capture and thermal conductivity detector (Palmer et al., 2010; Supplementary Materials and Method) was used to measure the gaseous products N_2O, CO_2, and O_2. For quantification of CH_4 GC equipped with a flame ionization detector was used (Wüst et al., 2009). Nitrate, as well as nitrite and ammonium were measured by colorimetric assays (Cataldo et al., 1975; Gadkari, 1984; Harrigan and McCance, 1966). Liquid samples were analyzed for soluble organic compounds like organic acids via high performance liquid chromatography (Palmer et al., 2010; Supplementary Materials and Method).

Statistical analysis

Statistical analyses were performed in OriginPro 2020 version (OriginalLab Corporation, Northhampton, MA, USA). Basic data analyses performed prior to statistical tests included visual inspection of all measured variables coupled with the Shapiro–Wilk normality test. To test for the treatment effect, i.e. differences between controls and supplemented microcosms at each pH and soil, analysis of variance (ANOVA) was used.

Results

Effect of pH and oxygen availability on the denitrification potential of PC soil

Consumption of NO_3^- occurred right after supplementation without delay and was monitored until it was depleted. Supplemented NO_3^- was almost depleted after 2 days at pH 6 and took about 3 times longer at pH4 (Figure 1). During incubation at pH 4 0.69 ± 0.07 mM and 0.49 ± 0.07 mM NO_3^- in the presence and absence of acetylene were consumed, respectively, after subtracting values from controls (Table 1). At pH 6 0.88 ± 0.10 mM and 0.71 ± 0.06 mM NO_3^- in the presence and absence of acetylene were consumed, respectively. Microcosms supplemented with NO_3^- incubated under oxic conditions produced only minor amounts of N_2O, which accounted for 1.4 % and 3.1 % of recovered supplemented NO_3^- at pH 4 and pH 6, respectively, and NH_4^+ was under the detection limit. Microcosms incubated under anoxic conditions supplemented with NO_3^- only produced considerable amounts of N_2O when acetylene was present, independent of pH. N_2O could first be detected after day 1 and day 2 of incubation at pH 4 and pH 6, respectively. At the end of incubation, 64 % and 49 % of supplemented NO_3^- were recovered as N_2O at pH4 and pH6, respectively. In the absence of acetylene, the recovery was < 1 %, and NH_4^+ was under the detection limit. Produced N_2O in the presence of acetylene at pH 4 differed significantly from incubations without acetylene from day 4 on and from day 2 on at pH 6. Therefore, acetylene stimulated the accumulation of N_2O under these incubation conditions, indicating complete denitrification at both pH 4 and pH 6. Furthermore, N_2O production was not in a plateau phase when NO_3^- was depleted, indicating recovery as N_2O-N might even be higher if incubations were conducted longer.

CO_2 production was not significantly different between anoxic incubations with and without NO_3^- at pH 4 and CO_2 production was stimulated by 40 – 50 % in the presence of NO_3^-. At pH 6, CO_2 production was stimulated by NO_3^- as well, increasing by 50 %, and being significantly different from day 1 on at pH 6. Anoxic incubations with and without acetylene showed no significant differences in CO_2 production at same time points during incubation at both pH values, as indicated by Tukey-Kramer test, and at the end of incubation about 0.54 - 0.66 mM CO_2 were produced (Table 1). Oxic treatments with NO_3^- produced up to 1 mM CO_2 during the incubation period with NO_3^- stimulating the production compared to oxic controls by 43 % and 26 % at pH4 and pH6, respectively. CO_2 production between oxic incubation with and without NO_3^- differed significantly from shortly after incubation at pH6 (Appendix, Figure 3). Due to damage of one oxic control for pH 4, no statistical statement is possible, though there is no visible trend for a significant difference.

Effect of pH and oxygen availability on the denitrification potential of peat plateau (PP) sediment

Supplemented NO_3^- was consumed with a delay and depleted after 49 days at pH 4 and 12 days at pH6, respectively (Figure 2). During incubation at pH 4 0.38 ± 0.08 mM and 0.36 ± 0.08 mM NO_3^- in the

presence and absence of acetylene were consumed, respectively, after subtracting values from controls (Table 2). At pH 6 0.53 ± 0.05 mM and 0.30 ± 0.07 mM NO_3^- in the presence and absence of acetylene were consumed, respectively. Microcosms supplemented with NO_3^- incubated under oxic conditions produced negligible amounts of N_2O, which accounted for 1.4 % of recovered supplemented NO_3^- at pH 4 and < 1 % at pH 6, respectively. NH_4^+ accounted for only 1.5 % of recovered supplemented NO_3^- at pH6, but for 20.5 % at pH 4. Produced CO_2 was in the range of incubated controls (90-103 %). Microcosms incubated under anoxic conditions supplemented with NO_3^- produced N_2O independent of acetylene, which could be observed from day 6 on, that accounted for around 70 % at pH 4 at the end of incubation. NH_4^+ was under the detection limit. At pH 6 N_2O production was detectable from day 6 on as well. Without acetylene the recovery of NO_3^- as N_2O reached about 12 %, when acetylene was present about 82 % of supplemented NO_3^- were recovered as N_2O. Here, acetylene stimulated the accumulation of N_2O under these incubation conditions at pH 6, indicating complete denitrification. However, at pH 4, N_2O production was not stimulated, indicating incomplete denitrification, terminating with the emission of N_2O as end product. Furthermore, N_2O production did not seem to in a plateau phase when NO_3^- was depleted, indicating recovery as N_2 or N_2O-N might even be higher if incubations were conducted longer.

CO_2 production was not significantly different between anoxic incubations with and without NO_3^- at pH 4, but CO_2 production was stimulated by NO_3^- supplementation and approximately doubled compared to controls. At pH 6, CO_2 production showed no significant difference and was not stimulated by NO_3^- addition. Anoxic incubations with and without acetylene showed no significant differences in CO_2 production during incubation at both pH values, as indicated by Tukey-Kramer test, and at the end of incubation about 1.50 – 2.77 mM CO_2 were produced (Table 2). Oxic treatments with NO_3^- produced up to 13 mM CO_2 during the incubation period at pH 4 and showed no stimulated production compared to oxic controls, though CO_2 values were significantly different at same time points of the incubation period. At pH 6 up to 4 mM CO_2 were produced, with a decrease of about 7 % compared to oxic controls. CO_2 production was not significantly different between oxic treatments with and without NO_3^- during incubation (Appendix, Figure 4).

Anaerobic fermentation activities and trophic links to denitrifiers

Organic acids were only detected sporadically in PC incubations and in negligible amounts near detection limit at both pH values, e.g. detected lactate at the beginning of incubation was in the range of 20-50 µM and still in the same range after incubation, showing no significant difference (Appendix, Table 3). Lactate detected in PP microcosms was in the same range at the beginning and the end of incubation as PC incubations and did not change significantly between time or treatments as well, while trace amounts of succinate (< 50 µM) were detected sporadically in microcosms (Appendix, Table 4). At both pH values in anoxic microcosms with acetylene initial butyrate (~ 80 µM at pH4, ~ 100 µM

206 at pH 6) was depleted at the end of incubation ($p > 0.05$), whereas in treatments ± NO_3^- without
207 acetylene butyrate (50 – 100 µM) was detected at the end of incubation without initial detection of it
208 ($p > 0.05$). At the *in situ* near pH 4 acetate, formate and propionate were under the detection limit,
209 while at pH 6 formate (~ 100 µM) and acetate (~ 500 µM) were detected at the end of incubation in
210 anoxic microcosms, independent of NO_3^- supplementation or acetylene. Such data suggest higher
211 fermentation potentials in PP than in PC sediment.

Discussion

Arctic permafrost regions were considered insignificant in terms of N_2O emissions, due to slow mineralization of organic matter (Shaver et al., 1992) and a general limitation of nitrogen (Jonasson et al., 1999), until approximately a decade ago, when cryoturbated acidic peat circles were reported as hot spots of N_2O emissions (Denman et al., 2007; Marushchak et al., 2011; Repo et al., 2009; Siljanen et al., 2019). These soils in the subarctic tundra with *in situ* near pH 4 emit N_2O in the range of heavy fertilized agricultural and tropical rainforest soils (Potter et al., 1996; Repo et al., 2009; Werner et al., 2007). N_2O is a strong greenhouse gas with an approximately 300 times higher global warming potential than CO_2 (Stocker et al., 2018) and both the microbial community and environmental factors influence the release of N_2O from soil and sediment.

Denitrification in PC soil as potential N_2O sink

In contrast to field studies and *in* situ incubations (Marushchak et al., 2011; Palmer et al., 2012; Repo et al., 2009; Siljanen et al., 2019), the acetylene inhibition assay indicated N_2 as the end product of denitrification in PC microcosms incubated under anoxic conditions supplemented with NO_3^- at pH 4 and pH 6. Subsequently produced N_2O must have been further reduced to N_2 in microcosms incubated without acetylene. These findings indicate the potential of PC soil microorganisms being capable of complete denitrification at acidic pH.

Acidic wetlands, including vegetated and unvegetated peat, are known temporary sinks of atmospheric N_2O, as reported by *in situ* measurements and microcosm experiments (Kolb and Horn, 2012; Marushchak et al., 2011; Palmer et al., 2010; Palmer and Horn, 2012), even though the relative proportion of N_2O to total N gases is generally higher in acidic soils in contrast to more pH-neutral ones (Simek and Cooper, 2002). This extends to the phylogenetic diversity, as the soil bacterial community structure is affected by pH (Fierer and Jackson, 2006; Lauber et al., 2009). Acid-tolerant bacteria capable of complete denitrification of the *Rhodanobacter* sp., e.g. *Rhodanobacter denitrificans*, have been found in acidic subsurface environments associated with denitrification (Green et al., 2010; Van Den Heuvel et al., 2010). A major controlling factor of N_2O emission, besides pH, is the C/N ratio. Denitrifiers harboring the *nosZ* gene, approximately two-thirds of cultured denitrifiers (Jones et al., 2008), can utilize N_2O as terminal electron acceptor when NO_3^- is limited under anoxic conditions (Zumft, 1997; Zumft and Kroneck, 2007). Collected data from the current study as well as from a previous study (Palmer et al., 2012) indicate that N_2O will be used as terminal electron acceptor by acid-tolerant denitrifiers of PC sediment, therefore PC sediment can act as temporary sink for N_2O as seen the present microcosm study.

Denitrification potential of PP soil

Vegetated unturbated PP has the same acidic pH as bare cryoturbated PC soil, but does not emit N_2O in *situ* (Marushchak et al., 2011; Repo et al., 2009; Siljanen et al., 2019). Surprisingly, the major end product of NO_3^- reduction in PP microcosms incubated under anoxic conditions at pH 6, as indicated by the acetylene inhibition technique, was N_2, suggesting complete reduction of NO_3^- to N_2 by denitrifiers. In contrast to PC soil, soil from PP is generally low in NO_3^- and was negligible before supplementation (< 0.01 mM), which is mutual to previous studies (Marushchak et al., 2011; Repo et al., 2009). Data suggest that supplemented NO_3^- stimulated the indigenous denitrifier community. Analysis of structural marker genes in PP soil revealed the genetic potential of the indigenous microbial community for complete denitrification (Palmer et al., 2012), supporting current findings for complete denitrification in PP soil under given incubation conditions. However, denitrifier communities from PP and PC soil phylogenetically differ as well as the abundances of detected marker genes for denitrification (Palmer et al., 2012).

Under oxygen limited conditions denitrifiers not only compete for NO_3^- with plants but as well with microorganisms capable of dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje et al., 1983). Important regulators for the regulation of differential electron flow toward denitrification and DNRA are the NO_3^-/NO_2^- concentrations, pH, and the C/N ratio. With DNRA being favored under NO_3^- limited conditions (Bleakley and Tiedje, 1982; Fazzolari et al., 1998). Unturbated vegetated PP has a high NH_4^+ to NO_3^- ratio restricting nitrification activity, hence NO_3^- availability for denitrifiers (Marushchak et al., 2011). Therefore, DNRA might outcompete denitrification for NO_3^- under field conditions. When the energy yields of DNRA and denitrification are compared, denitrification provides more potential energy per electron donor, but if energy yields are compared by the yield per mole NO_3^-, DNRA has a slight advantage to denitrification (Tiedje et al., 1983). Organisms capable of both DRNA and denitrification, e.g. the model organism *Shewanella loihica*, carry out DNRA when NO_3^- concentrations are low (Yoon et al., 2015a, 2015b), underlining the hypothesis of DNRA as main process in oxygen depleted PP soil.

In PP microcosms incubated under anoxic conditions with NO_3^- at *in situ* near pH 4 N_2O production was not stimulated in the presence of acetylene. Potential processes leading to the emission of N_2O from soils and sediment are nitrification, chemodenitrification, and DNRA. Ammonia oxidizing archaea are well known to directly contribute to N_2O production during nitrification in arctic soils, though under (micro-)oxic conditions (Siljanen et al., 2019). Under anoxic and NO_3^- limited conditions DNRA is favored, but NO_3^- was not a limiting factor and NH_4^+ was not detected during incubation. Another process able to contribute to N_2O production is chemodenitrification, an abiotic process that occurs at low pH and under oxygen limited conditions. Though this process will most certainly be outcompeted by the faster microbial denitrification and its main product is NO, not N_2O (Kresovic et al., 2009; van Cleemput, 1998). Thus, the suggested main process leading to N_2O emission in the experiment is microbial denitrification.

283 The differences in the recovery of NO_3^- as N_2O-N or N_2 at the two pH values, despite the supposedly
284 identical genetic potential for complete denitrification, might be explained by preclusion of a
285 successful assembly of a functional N_2O reductase, the main cause preventing N_2O reduction in soils
286 with low pH (Liu et al., 2014).

287

288 **Contrasting denitrifiers in PC and PP sediment**

289 Contrasting N_2O emissions from cryoturbated PC and unturbated PP soils (Marushchak et al., 2011;
290 Palmer et al., 2012; Repo et al., 2009; Siljanen et al., 2019) are the result of low NO_3^- concentrations of
291 the vegetated PP soil and the dissimilar denitrifier communities, rather than pH, even though pH has
292 a significant effect on denitrifier communities in temperate soils (Bru et al., 2011). Cryoturbated PC
293 soil has a low NH_4^+ to NO_3^- ratio which is indicative for nitrification-derived NO_3^- that is readily available
294 for denitrifiers, while unturbated vegetated PP tundra displays high NH_4^+ to NO_3^- ratios that restrict
295 nitrification activity and hence denitrification (Marushchak et al., 2011). Different reactions to
296 supplemented NO_3^- and NO_2^- in microcosm experiments of indigenous denitrifiers further support the
297 hypothesis of distinct denitrifier communities impacting regulation and hence possible prediction of
298 N_2O fluxes (Holtan-Hartwig et al., 2000; Ma et al., 2011; Palmer et al., 2012). In the current study
299 supplemented NO_3^- was consumed without delay in PC microcosms, whereas NO_3^- was consumed with
300 delay in PP microcosms. Furthermore, reactions to NO_3^- supplementation differed between the soils
301 at the same pH. While at pH 6 complete reduction of NO_3^- to N_2 could be detected from microcosms
302 independent of soil type, this was only the case for PC soil at *in situ* pH4, whereas in PP microcosms,
303 N_2O was the released end product.

304

305 **N_2O reduction potential at acidic pH**

306 The aim of this study was to determine the ability of peat circle and peat plateau soil to reduce N_2O to
307 N_2. The capability of N_2O reduction under acidic conditions was shown before in peat circle sediment,
308 when initially produced N_2O derived from endogenous N-sources was consumed at pH 4 (Palmer et al.,
309 2012) and proof for the genetic potential of acidic soils for the reduction of N_2O, i.e. the abundance of
310 the *nosZ* gene, was found (Palmer et al., 2010, 2012). Cryoturbated PC soil emits high amounts of N_2O,
311 both in field studies and in *in situ* experiments (Marushchak et al., 2011; Palmer et al., 2012; Repo et
312 al., 2009), contrasting to the potential for complete denitrification in the current study as detected by
313 the acetylene-inhibition technique at both *in situ* near pH 4 and more neutral pH 6. Denitrification is
314 the suggested main source of N_2O emitted by PC soil, due to high NO_3^- concentrations, a low C/N ratio
315 and a water-filled pore space of >70 %, favoring the process (Marushchak et al., 2011; Palmer et al.,
316 2010; Pihlatie et al., 2004; Repo et al., 2009). This, together with relatively high net N mineralization
317 rates, results most likely in high N_2O emissions (Diáková et al., 2016). It is hypothesized that internal

N-cycling fuels the large N_2O emissions in PC soil, since a large proportions of excess mineralized N is available for denitrifiers (Diáková et al., 2016). Therefore, NO_3^- will probably not be limited for PC denitrifiers and it is not mandatory for energy yield to utilize N_2O as terminal electron acceptor (Zumft, 1997; Zumft and Kroneck, 2007).

Permafrost-affected systems play an important role on global N_2O emissions and the N-cycling in general, as more and more evidence emerges (Green et al., 2010; Jones et al., 2008; Throbäck et al., 2004; Voigt et al., 2017a). Therefore, it is important to get a deeper understanding of microbial communities associated with the N-cycle and their potentials, as well as their ecophysiology and factors influencing them, in order to get a higher level of understanding of possible sources and sinks of N_2O in permafrost affected environments.

Author Contributions

SH and MH designed the microcosm incubation experiments, wrote the original manuscript, and interpreted data. SH set up microcosms and performed all laboratory work, if not stated otherwise. MH conceived the original idea and oversaw all laboratory work.

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496

Tables

Table 1: Net compound consumption and gross production during incubations in peat circle (PC) microcosms. Mean values of technical replicates with standard deviation. NO_3^- values after subtracted controls. Incubation duration: 6.5 and 2.5 days for pH 4 and pH 6, respectively.

	NO_3^- w/o acetylene				NO_3^- with acetylene	
	oxic		anoxic			
Compound [mM]	**pH4**	**pH6**	**pH4**	**pH6**	**pH4**	**pH6**
CO_2	0.99 ± 0.09	1.06 ± 0.54	0.54 ± 0.02	0.64 ± 0.06	0.59 ± 0.03	0.66 ± 0.03
NO_3^-	0.31 ± 0.05	0.14 ± 0.09	0.49 ± 0.07	0.71 ± 0.06	0.69 ± 0.07	0.88 ± 0.10
N_2O	< 0.01	< 0.01	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.03	0.21 ± 0.01
NH_4^+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

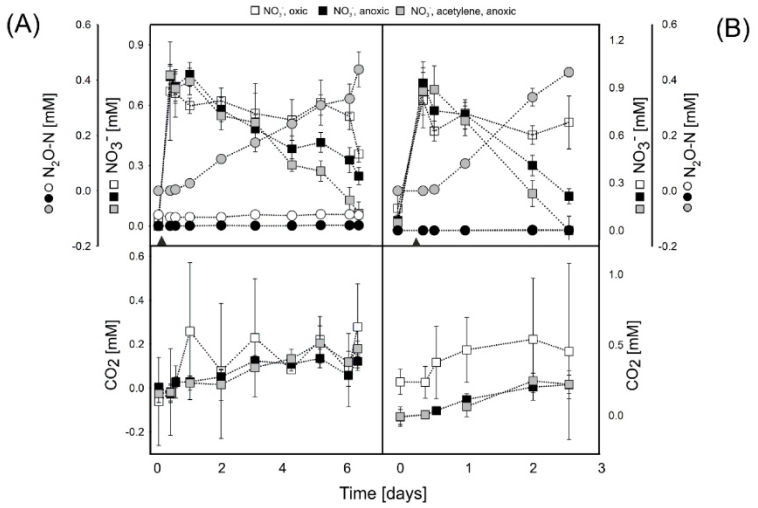
n.d. – not detected

Table 2: Net compound consumption and gross production during incubations in peat plateau (PP) microcosms. Mean values of technical replicates with standard deviation. NO_3^- values after subtracted controls. Incubation duration: 49 and 13 days for pH 4 and pH 6, respectively.

	NO_3^- w/o acetylene				NO_3^- with acetylene	
	oxic		anoxic			
Compound [mM]	**pH4**	**pH6**	**pH4**	**pH6**	**pH4**	**pH6**
CO_2	13.02 ± 2.28	4.00 ± 0.85	2.77 ± 0.34	1.50 ± 0.16	2.74 ± 0.44	1.91 ± 0.31
NO_3^-	0.32 ± 0.11	0.17 ± 0.16	0.36 ± 0.08	0.30 ± 0.07	0.38 ± 0.08	0.53 ± 0.05
N_2O	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.00	0.02 ± 0.01	0.13 ± 0.08	0.22 ± 0.10
NH_4^+	0.07 ± 0.03	0.00 ± 0.01	n.d.	0.00 ± 0.01	n.d.	0.00 ± 0.00

n.d. – not detected

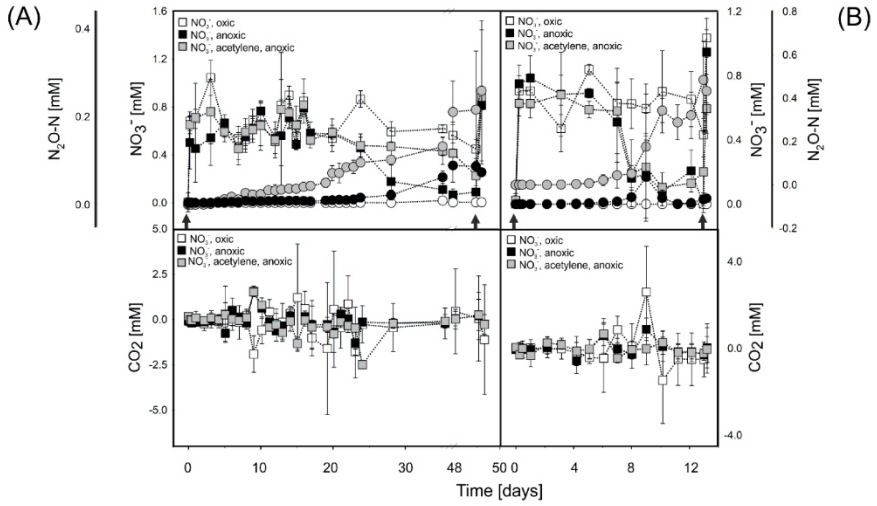
507 **Figures**



508

509 Figure 1: Net nitrate consumption, N_2O-N and CO_2 production during incubations in peat circle (PC) microcosms at **(A)** pH 4
510 and **(B)** pH 6. Mean values of technical replicates with standard deviation and subtracted controls. Arrow indicates NO_3^-
511 supplementation. Upper pictures of net NO_3^- consumption and N_2O-N production; lower pictures of net CO_2 production.

512

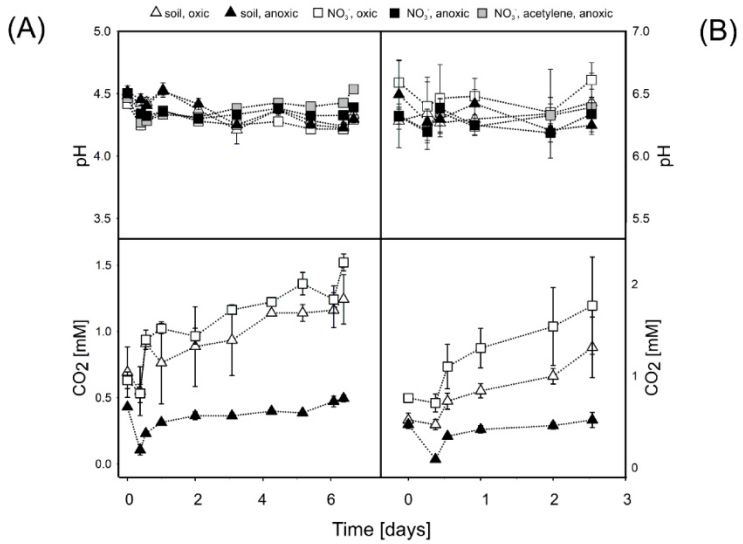


513

514 Figure 2: Net nitrate consumption, N_2O-N and CO_2 production during incubations in peat plateau (PP) microcosms at **(A)** pH
515 4 and **(B)** pH 6. Mean values of technical replicates with standard deviation and subtracted controls. Arrow indicates NO_3^-
516 supplementation. Upper pictures of net NO_3^- consumption and N_2O-N production; lower pictures of net CO_2 production.

517

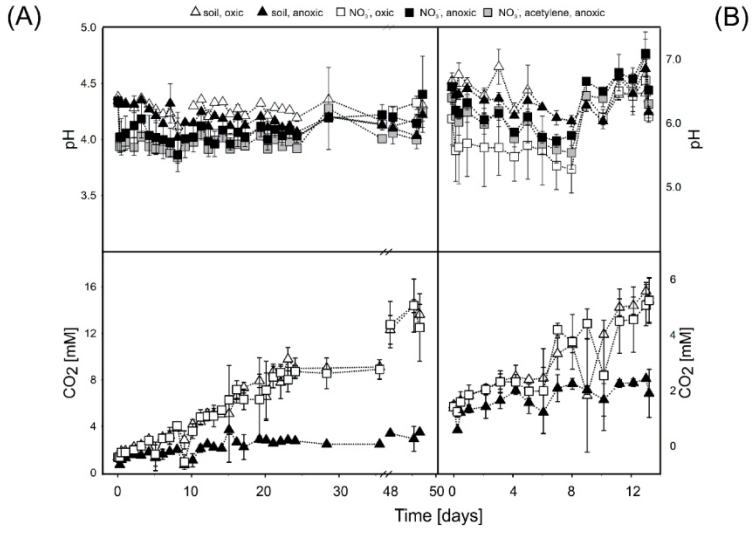
518 **Appendix**



519

520 Figure 3: pH during incubation period in control and treatment incubation and CO_2 production during incubations of controls
521 in peat circle (PC) microcosms at **(A)** pH 4 and **(B)** pH 6. Mean values of technical replicates with standard deviation. Upper
522 pictures of pH; lower pictures of CO_2 production

523



524

525 Figure 4: pH during incubation period in control and treatment incubation and CO_2 production during incubations of controls
526 in peat plateau (PP) microcosms at **(A)** pH 4 and **(B)** pH 6. Mean values of technical replicates with standard deviation. Upper
527 pictures of pH; lower pictures of CO_2 production.

Table 3: Organic acid consumption and production during incubations in peat circle (PC) microcosms. Mean values of technical replicates with standard deviation.

pH	Compound [µM]	Soil, oxic	Soil, anoxic	NO_3^-, oxic	NO_3^-, anoxic	NO_3^-, acetylene, anoxic
4	Succinate	0.78*	n.d.	-0.09*	32.70*	20.52*
	Lactate	2.26 ± 1.87	-0.11 ± 3.02	-12.72 ± 25.40	-5.11 ± 16.89	-0 07*
	Butyrate	n.d.	n.d.	n.d.	n.d.	n.d.
6	Succinate	41.64*	36.25*	34.64*	n.d.	n.d.
	Lactate	-8.19 ± 18.60	-2.81 ± 5.80	15.02*	-6.11 ± 7.01	-2.47 ± 15.74
	Butyrate	n.d.	n.d.	n.d.	n.d.	-29.31 ±5.96

n.d. – not detected, * - detected in only one replicate

Table 4: Organic acid consumption and production during incubations in peat plateau (PP) microcosms. Mean values of technical replicates with standard deviation.

pH	Compound [µM]	Soil, oxic	Soil, anoxic	NO_3^-, oxic	NO_3^-, anoxic	NO_3^-, acetylene, anoxic
4	Succinate	35.42*	-45.76*	-34.00*	n.d.	n.d.
	Lactate	4.61 ± 16.57	-7.30 ± 8.37	-0.88 ± 9.09	2.82 ± 11.60	4.50 ± 4.90
	Butyrate	n.d.	105.50*	n.d.	72.71*	-40.76 ± 30.97
6	Succinate	29.41*	51.52*	31.36*	-35.75*	-28.31*
	Lactate	3.11 ± 1.85	-18.46*	1.32 ± 13.15	-2.42 ± 29.44	11.72 ± 39.52
	Butyrate	n.d.	n.d.	n.d.	n.d.	-99.62 ± 54.45
	Formate	14.50 ± 7.65	100.95 ± 41.81	3.12*	22.36*	57.28 ± 65.48
	Propionate	n.d.	111.19*	n.d.	n.d.	n.d.
	Acetate	n.d.	436.79 ± 28.33	n.d.	-73.02*	309.85 ± 56.67

n.d. – not detected, * - detected in only one replicate

Curriculum vitae

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04/2021	Teilnahme am Workshop „GMP kompakt - Einführung in Good Manufacturing Practice und pharmazeutisches Qualitätsmanagement”
02/2020	Teilnahme am Workshop „Writing Scientific Papers and Theses in the Life Sciences”
2017-2019	Konzeption und Durchführung des 2-wöchigen Praktikums „Spezielle Methoden der Bodenmikrobiologie“ für den Masterstudiengang Molekulare Mikrobiologie
2017-2019	Durchführung des 2-wöchigen Praktikums „Spezielle Methoden der Umweltmikrobiologie“ für den Masterstudiengang Molekulare Mikrobiologie
2016-2019	Konzeption und Durchführung des 2-wöchigen Praktikums „Experimentelle Übung Mikrobiologie“ für den Masterstudiengang Molekulare Mikrobiologie
05/2018	Teilnahme am Workshop „Good Scientific Practice – Protecting Scientific Integrity”
2014-2020	Betreuung von Bachelor- und Masterstudenten
08/2009-09/2009	Wissenschaftszentrum Straubing 6-wöchiges Praktikum: Laborarbeit

Zuschüsse/Konferenzen

2019	1. Tagung der Molekularen Biowissenschaften am Campus Herrenhausen, Hannover (GER), Vortrag
2018	23rd ENC Meeting, Alicante (ESP), Vortrag und Erhalt des FEMS Early Career Scientists Meeting Grant
2018	VAAM, Wolfsburg (GER), Vortrag
2016	ISME 16, Montreal (CA), Posterbeitrag und Erhalt des The ISME16 Travel Grant
2016	XI. ICOP, Potsdam (GER), Posterbeitrag

Publizierte Abstracts auf Tagungen und Konferenzen

2018	Hetz, 2018. *Burkholderiaceae* are primary acetate assimilating denitrifiers in peat circles of the arctic tundra capable of complete denitrification at pH 4. 70th Annual Conference of the Association for General and Applied Microbiology (VAAM 2018).

2018	Hetz, Horn, 2018. Complete denitrification at pH 4 in peat circles of the arctic Tundra is primarily driven by acetate assimilating Burkholderiaceae. 17th International Symposium on Microbial Ecology (ISME-17).
2016	Hetz, Horn, 2016. Denitrifiers limited by available organic carbon drive nitrous oxide production in peat circles of the arctic Tundra despite their capability for nitrous oxide consumption. XI. International Conference on Permafrost (ICOP 2016).
2016	Hetz, Horn, 2016. Nitrous oxide production in peat circles of the arctic tundra is driven by available organic carbon limited denitrifiers. 16th International Symposium on Microbial Ecology (ISME-16).

Publikationen

Peer reviewed

2021	Hetz SA, Horn MA. *Burkholderiaceae* Are Key Acetate Assimilators During Complete Denitrification in Acidic Cryoturbated Peat Circles of the Arctic Tundra. Front Microbiol 2021;12:1–18.
2020	Hetz SA, Poehlein A, Horn MA. Whole-Genome Sequences of Two New *Caballeronia* Strains Isolated from Cryoturbated Peat Circles of the Permafrost-Affected Eastern European Tundra. Cameron Thrash J (ed.). *Microbiol Resour Announc* 2020;9:30–2.
2020	Kaupper T, Hetz S, Kolb S *et al.* Deforestation for oil palm: impact on microbially mediated methane and nitrous oxide emissions, and soil bacterial communities. *Biol Fertil Soils* 2020;**56**:287–98.
2014	Wieczorek AS, Hetz SA, Kolb S. Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries. *Biogeosciences* 2014;11:3339–52.

Buchkapitel

2021	Horn MA, Hetz SA. Microbial nitrogen cycling in permafrost soils: implications for atmospheric chemistry. In: Liebner S, Ganzert L (eds.). *Microbial Life in the Cryosphere and Its Feedback on Global Change*. Berlin, Boston: De Gruyter, 2021.