Variation in bacterial, archaeal and fungal community structure and abundance in High Arctic tundra soil

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Abstract

Arctic ecosystems are under pressure from climate change and atmospheric nitrogen (N) deposition. However, knowledge of the ecology of microbial communities and their responses to such challenges in Arctic tundra soil remain limited, despite the central role these organisms play for ecosystem functioning. We utilised a plot-scale experiment in High Arctic tundra on Svalbard to investigate short-term variation (9 days), following simulation of a N deposition event (4 kg N ha^{-1} yr^{-1}), in the structure and abundance of bacterial, archaeal and fungal communities between organic and mineral soil horizons. T-RFLP analysis showed significant differences between horizons in bacterial and archaeal community structure. Q-PCR analysis showed that fungal abundance did not differ significantly between soil horizons, whilst bacterial and archaeal abundance was significantly higher in mineral than in organic horizons, despite soil water and total C and N contents being significantly greater in the organic horizon. In the organic horizon, bacterial community structure and fungal abundance varied significantly over time. In the mineral horizon, there was significant variation over time in bacterial abundance, in archaeal community structure and in both fungal community structure and abundance. In contrast, N deposition did not lead to significant variation in either the structure or the abundance of microbial communities. This research demonstrates that microbial community structure and abundance can change rapidly (over only a few days) in Arctic tundra soils and also differently between soil horizons in response to different environmental drivers. Moreover, this variability in microbial community structure and abundance is soil horizon- and taxonomic domain-specific, highlighting the importance

of investigating microbial communities across all soil horizons and over short periods of time.

Introduction

The Arctic tundra comprises nearly 5 % of the land on Earth (Nemergut et al. 2005) and is characterised by long winters and short summers. Moreover, Arctic tundra soils are considered nutrient-poor with nutrient availability (e.g. nitrogen and phosphorus) limiting plant productivity and microbial communities (Shaver and Chapin 1980; Nordin et al. 2004; Rinnan et al. 2007). These characteristics contribute to the sensitivity of Arctic tundra to environmental change (Robinson et al. 1995; van Wijk et al. 2004), for which most climate models predict significant increases in temperature and precipitation across polar regions (Anisimov and Fitzharris 2001; IPCC 2007). Several studies have investigated the impact of temperature increases (Chapin et al. 1995; Van Wijk et al. 2004) or nutrient additions (Gordon et al. 2001; Tye et al. 2005) upon Arctic tundra ecosystems, demonstrating their clear sensitivity to environmental change. Whilst most studies have focused on above-ground responses within plant communities, a number of recent studies have considered the impacts of environmental changes such as warming and/or increase in nutrients on the bacterial (Deslippe et al. 2005; Rinnan et al. 2007; Walker et al. 2008; Lamb et al. 2011; Deslippe et al. 2012) and fungal (Robinson et al. 2004; Fujimura et al. 2007; Deslippe et al. 2011, 2012) communities in Arctic tundra soil, which play a key role in biogeochemical cycles. Furthermore, recent studies have shown that microbial diversity in Arctic soils is similar or even higher than that in other biomes such as boreal forests, tropical forests, temperate forests, grasslands, deserts or prairies,

highlighting the potential importance of microbial communities to Arctic tundra functioning (Neufeld and Mohn 2005; Fierer and Jackson 2006; Wallenstein et al. 2007; Lauber et al. 2009; Chu et al. 2010, 2011; Geml et al. 2012). Whilst such studies have improved our understanding of microbial diversity in Arctic tundra, there has been little research that has sought to understand how microbial community structure and abundance vary either spatially (including between different soil horizons) and/or temporally in this region despite the key role played by soil microbial communities in biogeochemical cycles.

Robinson et al. (2004) showed that the diversity of fungal communities, as assessed by culture-dependent approaches, was higher in the organic soil than in mineral soil in tundra near Ny-Ålesund, Svalbard (70°N), with this variation between soils probably related to the higher C and nutrient content in the organic soil. Deslippe et al. (2012) also found fungal community structure differed between organic and mineral soil horizons. Additionally, bacterial community structure and diversity have also been shown to differ between organic and mineral soil horizons in subarctic (Rinnan et al. 2007; Deslippe et al. 2012) and Low Arctic tundra (Wallenstein et al. 2007). Recently, Lee et al. (2013) showed that bacterial and archaeal community structure, and microbial enzyme activities differed between organic and mineral soil horizons within High Arctic tundra. Seasonal changes in both bacterial (McMahon et al. 2011) and archaeal (Høj et al. 2005) community structure have been reported in Arctic soils. Both methanogens and Group 1.3b *Crenarchaeaota* were found to increase in their relative abundance with increasing soil water content whilst lower temperatures selected for non-methanogenic archaea (Høj et al. 2006, 2008). In contrast, other studies have not shown any change in bacterial

community structure between early and late summer in different vegetation types in the Finnish subarctic (Ma¨nnisto¨ et al. 2007) or Alaska (Deslippe et al. 2012). Similarly, the fungal community assemblages and structure were found not to vary significantly over the summer season near Ny-Ålesund, Svalbard (Robinson et al. 2004), or in Alaskan tundra (Deslippe et al. 2012). Together, these earlier studies suggest that bacterial, archaeal and fungal communities within the Arctic tundra display contrasting variability between different soil horizons and/or over time and that members of the three different microbial domains are not influenced by the same environmental drivers. The latter point is of particular interest when seeking to understand the impact of anthropogenic pollution and global change, such as increases in temperature or atmospheric nitrogen deposition, upon Arctic ecosystems.

Hence, Arctic ecosystems are potentially vulnerable to the effects of acute atmospheric nitrogen deposition events that result from polluted air masses travelling from lower latitudes to the Arctic with minimal dispersal (Hodson et al. 2005, 2010; Ku¨hnel et al. 2011). Hodson et al. (2005, 2010) found that acute N deposition events can occur in which 40 % of the annual atmospheric N input can be deposited as acidic rainfall (~pH 4) in < 1 week. Whilst the impacts of these acute pollution events have not previously been studied, a prior investigation studying the effects of 15 years of nitrogen addition totalling 1250 kg N ha^{-1} within Swedish subarctic heathland found significant changes in bacterial community structure and increase in bacterial biomass within both organic and mineral soil horizons due to N addition (Rinnan et al. 2007). Schmidt et al. (2000) also found, at the same location as Rinnan et al. (2007), that bacterial community structure was affected after 4 years of NPK addition (total N addition 400 kg N ha^{-1}), but

bacterial biomass was not affected. In contrast, Lamb et al. (2011) did not find any effect on bacterial community structure despite 15 years of NPK addition at rates of 100 and 500 kg ha^{-1} yr^{-1} in the tundra in Alaska. Simulation of N deposition in the High Arctic showed increase in bacterial biomass and microbial activity after 2 years of low NH_4 addition rate (5 kg N ha^{-1} yr^{-1}; Stapleton et al. 2005).

In the context of increasing environmental threats to the Arctic environment, including global warming and atmospheric N deposition, it is important to understand how different environmental drivers affect soil microbial communities over time in different soil horizons, in order to then predict and study the responses of these communities to anthropogenically driven global change. Hence, in this study, a field experiment was used in High Arctic tundra in conjunction with molecular analysis to investigate spatial and temporal variation in the structure and abundance of soil microbial (bacterial, archaeal and fungal) communities. The first objective of this study was to simultaneously investigate changes in these microbial communities over a short period of time between organic and mineral soil horizons and to relate such variation to changes in soil chemistry. The second objective was to investigate the short-term responses of microbial communities to simulated acute nitrogen deposition.

Methods

Research location, soil sampling and characterisation

A field experiment consisting of 10 plots (each 1.5 x 1.5 m) was established in July 2009 on tundra soil at Leirhaugen (78º55’231”N, 11º49’819”E), 1.95 km to the South-East of Ny-Ålesund (West Spitsbergen, Svalbard). The soil consists of an organic horizon (1 – 5

136 cm) over a deeper mineral horizon (chemical characteristics for the plots and both soil
137 horizons prior to the start of the experiment are shown in Table 1). Plant cover comprised
138 mainly *Salix polaris* Wahlend (38%) and bryophytes (49%). An acute acidic N deposition
139 event ('N treatment') was simulated on five of the ten plots by application of NH_4NO_3 at
140 a rate of 4 kg N ha^{-1} yr^{-1} in solution with pH adjusted to 4 with HNO_3, to mimic the acute
141 N deposition event reported by Hodson et al. (2010) during which 0.4 kg N ha^{-1} was
142 deposited as acidic rainfall (~pH 4) in less than one week. The five remaining plots
143 ('Control') received only water (~pH 6). The pH 6 of the water used for the control was
144 different from the treatment in order to investigate simultaneously the effect of N
145 deposition and acid rain characteristic of the acute N deposition event being simulated. N
146 treatment and water applications were applied over 2 days (21st and 22nd of August 2009),
147 using an equal volume of 2.5 l $plot^{-1}$ day^{-1}. The total amount of N applied (i.e. 1.15 g of N
148 m^{-2}) was split equally between each application. Over the course of the field experiment
149 (i.e. from the 21st to the 29th of August) the ground was fully free of snow and *Salix*
150 *polaris* had leaves, the cumulative precipitation during the experiment was 5.7 mm, and
151 the average atmospheric temperature was 3.8 ± 1.8 °C. In summer 2009, the snow melt
152 occurred late in the season with the ground mostly free of snow by the end of the first
153 week of July, and the first snow fall occurring in the first week of September.

154 Soil samples were taken (5 × 5 cm) to a depth of 10 cm, randomly within each
155 plot (one soil sample per plot and per date of sampling), prior to the first N application
156 (Day-2) and, after 1 and 7 days post N application (Day+1 and Day+7, respectively).
157 Vegetated areas were selected because an additional study investigating the responses of
158 the plant cover to N additions was also conducted (Choudhary et al. in prep.). The size of

159 the soil samples was chosen to minimize the effect of soil sampling on each plot and
160 subsequently on plant and microbial communities because the plots were to be sampled
161 several times during the summer and also over subsequent years. Soil samples were
162 returned to the field laboratory, and divided into organic and mineral horizons and
163 homogenised separately. From each horizon, ~1.5 g soil samples were aseptically taken
164 and stored at -20 ºC for subsequent molecular analysis in the UK. Soil pH was
165 determined from 2 g of soil, 10 ml of deionized water was added, then the suspension
166 was vortexed for 10 s, and the pH reading was taken after 1 min of settlement in the
167 upper portion of the suspension (Robinson et al. 2004). Soil moisture was measured in 2
168 – 5 g of soil, weighed before and after drying at 50 ºC for 72 h. After measuring the soil
169 moisture, the soil samples were finely ground for C, N, C^{13} and N^{15} analysis, determined
170 using an isotope ratio mass spectrometer (ANCA GSL 20-20, PDZ Europa, Cheshire,
171 UK). The ^{13}C and ^{15}N content were also measured enabling comparison with a
172 subsequent study at the site using ^{15}N-labelled NH_4NO_3 to follow the fate of N within soil
173 horizons, in order to compare results between years. The ^{13}C and ^{15}N content can also
174 inform changes in organic matter and microbial activity, e.g. activity of mycorrhyzal
175 fungi were found to change with soil ^{15}N enrichment (Etcheverría et al. 2009).The soil
176 samples did not contain any inorganic C (i.e. presence of carbonate were tested by HCl
177 washing of the soil). Hence, the total C content was equal to the organic C content in the
178 soil. The C/N ratio was therefore calculated based on the total organic C content and the
179 total N content. Inorganic N (NH_4^+ and NO_3^-) was extracted from 2 g of dry soil by
180 vertical rotation shaking with 20 ml of KCl (2 M) for 1 h. The solution was filtered
181 (paper filter, Wathman 44, ø 110 cm, retention 3 µm) and analysed by continuous flow

182 colorimetry (Kamphake et al., 1967; Krom, 1980) with an auto-analyser (FIA-flow2
183 nutrients analyzer, Burkard Scientific Ltd, Uxbridge, UK).
184
185 **DNA extraction and PCR amplification**
186 DNA was extracted from 0.25 g soil samples using the PowerSoil® DNA isolation kit
187 (Mo-Bio laboratories, Carlsbad, CA, USA) following the manufacturer's instructions
188 except for the last step where the DNA was eluted with 100 µl of nuclease-free water
189 (Ambion, Warrington, UK). DNA extracts were stored at -20 °C. 16S rRNA gene were
190 amplified by PCR using primers: 63F (5'-CAGGCCTAACACATGCAAGTC-3') and
191 1389R (5'-ACGGGCGGTGTGTACAAG-3') (Marchesi et al. 1998; Osborn et al. 2000)
192 for bacteria and Arch109F (5'-ACKGCTCAGTAACACGT-3') and Arch958R
193 (YCCGGCGTTGAMTCCAATT) (DeLong 1992) for archaea. Fungal internal
194 transcribed spacers (ITS) were amplified by PCR using the primers ITS1F (5'-
195 CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS4 (5'-
196 TCCTCCGCTTATTGATATGC) (White et al. 1990). Each of the forward primers was
197 fluorescently labelled at the 5' end with 6'carboxyfluorescein (6-FAM). Bacterial and
198 archaeal 16S rRNA gene amplification reactions (50 µl) contained 0.15 µM of each
199 oligonucleotide primer, 100 µM of each dNTP, 1× reaction buffer, 20% (vol/vol) Q
200 solution and 1.25 U of *Taq* polymerase (Qiagen, Crawley, UK) and 1 µl of soil DNA.
201 Fungal ITS region reactions (50 µl) contained 0.2 µM of each oligonucleotide primer, 50
202 µM of each dNTP, 1× reaction buffer, 1 mM $MgCl_2$, and 2.5 U of *Taq* polymerase
203 (Bioline, London, UK) and 1 µl of soil DNA. Bacterial 16S rRNA genes were amplified
204 using an initial denaturation at 94 ºC for 2 min, followed by 30 cycles of 94 ºC for 30 s,

57 ºC for 45 s, and 72 ºC for 90 s, followed by a final extension at 72 ºC for 10 min. Archaeal 16S rRNA genes were amplified using denaturation at 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 45 s, 55 ºC for 60 s, and 72 ºC for 90 s, followed by a final extension at 72 ºC for 10 min. Fungal ITS regions were amplified using an initial denaturation at 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 55 ºC for 30 s, and 72 ºC for 60 s, followed by a final extension at 72 ºC for 10 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Crawley, UK) following the manufacturer's instructions and eluted in 50 µl of nuclease-free water (Ambion, Warrington, UK).

T-RFLP and ARISA analyses

Purified bacterial and archaeal 16S rRNA gene PCR products (10 µl) were digested with 10 U of the restriction enzyme AluI and 1× restriction enzyme buffer (Roche, Hertfordshire, UK) in a total volume of 15 µl at 37 ºC for 3 h. 5 µl of the digests were desalted via precipitation with 0.25 µl of glycogen (20 mg ml^{-1}) and 75 µl of 0.3 mM $MgSO_4.7H_2O$ in 70% ethanol, prior to resuspension in 5 µl of nuclease-free water (Ambion, Warrington, UK). Desalted products (0.5 or 1 µl) were mixed with formamide containing 0.5% ROX-labelled GS500 internal size standard (Applied Biosystems, Warrington, UK) in a total volume of 10 µl. Digested products were denatured at 94 ºC for 3 min, briefly transferred to ice, and electrophoresed for 20 min in POP-7 polymer on an ABI 3730 PRISM® Genetic Analyzer (Applied Biosystems, Warrington, UK). An initial injection voltage of 2 V and 5 or 10 s of injection time were used. 10 voltage ramps were performed with 20 s voltage intervals steps before reaching the final electrophoresis

228 run voltage of 15 V. Purified fungal ITS region PCR products (10 µl) were desalted as
229 described above. Desalted products (0.5 to 2 µl) were mixed with formamide containing
230 0.5% ROX-labelled GS2500 internal size standard (Applied Biosystems, Warrington,
231 UK) in a total volume of 10 µl and electrophoresed using the same conditions as for 16S
232 rRNA gene products, except that the run duration was 1 h.

233 T-RFLP and ARISA profiles were analysed initially using GeneMapper® v3.7
234 software (Applied Biosystems, Warrington, UK). Terminal restriction fragments (T-RFs)
235 between 50 and 500 bp and with peaks height ≥ 50 fluorescence units were included in
236 the analysis. Amplicons of between 200 and 1000 bp and with peak heights ≥ 100
237 fluorescence units were included for the ARISA analysis. Relative abundances of the
238 peak area of each individual T-RF (or ARISA amplicon) were calculated as a proportion
239 of the total peak area of all peaks present within an individual T-RFLP (or ARISA)
240 profile and aligned using the T-Align software with a confidence interval of 0.5 (Smith et
241 al. 2005). T-RFs and ARISA amplicons that had a relative abundance of < 0.5% of the
242 total percentage area were excluded from subsequent analysis.

243

244 **Quantitative-PCR (Q-PCR) analysis**

245 Variation in the abundance of bacterial and archaeal 16S rRNA genes, and fungal ITS
246 regions was assessed by Q-PCR. Q-PCR standards of each targeted amplicon were
247 generated from PCR products amplified separately from both organic and mineral
248 horizons of soil samples taken immediately external to the plots, prior to N application
249 (i.e. Day-2). 16S rRNA genes were amplified using primers Eub338 (5'-
250 ACTCCTACGGGAGGCAGCAG-3') (Lane 1991) and Eub518 (5'-

ATTACCGCGGCTGCTGG -3') (Muyzer et al. 1993) for bacteria and Parch519F (5'-CAGCMGCCGCGGTAA-3') (Øvreas et al. 1997) and Arch1060R (GGCCATGCACCWCCTCTC) for archaea (Reysenbach and Pace 1995). Fungal internal transcribed spacers (ITS) were amplified using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and 5.8S (5'-CGCTGCGTTCTTCATCG) (Vilgalys and Hester 1990). Amplification reactions were as described for T-RFLP analysis and ARISA except that Q-solution was not used. Bacterial 16S rRNA genes and fungal ITS regions were amplified using an initial denaturation at 94 ºC for 3 min, followed by 40 cycles of 94 ºC for 30 s, 50 ºC for 45 s, and 72 ºC for 30 s, followed by a final extension at 72 ºC for 7 min. Archaeal 16S rRNA genes were amplified using denaturation at 95 ºC for 3 min, followed by 40 cycles of 95 ºC for 30 s, 57 ºC for 45 s, and 72 ºC for 45 s, followed by a final extension at 72 ºC for 10 min. PCR products (i.e. 50 µl) were visualised by gel electrophoresis and bands were excised from the gels and extracted using the QIAquick® Gel Extraction Kit (Qiagen, Crawley, UK) following the manufacturer's instructions, prior to quantification of each individual PCR product using a NanoDrop spectrophotometer (NanoDrop 8000 Spectrophotometer Thermo Scientific, Wilmington, USA). For each target amplicon (bacterial and archaeal 16S rRNA genes, and fungal ITS amplicons), purified PCR products amplified from the organic and mineral horizons were pooled to generate one composite amplification standard for each target gene or amplicon, prior to calculation of target gene or amplicon numbers as described by Smith et al. (2006). For each standard curve, a tenfold dilution series was used ranging from 10^2 to 10^9 16S rRNA genes μl^{-1} for bacteria and archaea and from 10^2 to 10^8 amplicons μl^{-1} for fungi. Standard curve template DNA and the "no

template control" (NTC) were amplified in triplicate in the same plate as the environmental samples.

Q-PCR amplifications were performed in 25 µl volumes containing 12.5 µl of QuantiFast® SYBR® Green PCR MasterMix (Qiagen, Crawley, UK), 9 µl of nuclease-free water (Ambion, Warrington, UK), 1.25 µl of each primer (10 µM) and 1 µl of soil DNA using a CFX96™ Real-Time System (Bio-Rad, Hemel Hempstead, UK) and the same cycling conditions that were used to generate Q-PCR standards, except the final elongation step was removed, and the fluorescence was measured at the end of each synthesis step (i.e. 72 °C). Threshold cycle (C_t) values and amplicon numbers were determined automatically using the Bio-Rad CFX Manager™ software. The following standard curve descriptors are reported with the Q-PCR results (see Fig. 3): NTC C_t , linear regression coefficient (r^2), the y-intercept value and the amplification efficiency (*E*) (Smith et al. 2006). Specificity of the Q-PCR was assessed via a melting curve analysis (increase of temperature from 72 ºC to 95 ºC by 0.5 ºC for 0.05 s) at the end of each Q-PCR amplification (Ririe et al. 1997). The melting curve for the bacterial and archaeal 16S rRNA gene Q-PCR assay showed specificity for the amplified targeted genes. As expected, the melting curve of the Q-PCR for fungal ITS showed the amplification of products of different lengths, due to the variability in length of ITS regions between different fungal taxa (Manter and Vivanco 2007).

Statistical analysis

T-RFLP and ARISA relative abundance datasets (see above) were square-root transformed and similarity matrices constructed using the Bray-Curtis method (Clarke et

297 al. 2006). Similarities between samples were displayed using 2D non-metric multi-
298 dimensional scaling (nMDS) plots using the PRIMER software (v6, PRIMER-E Ltd,
299 Plymouth, UK). A non-parametric permutation-based test: ANOSIM (Analysis of
300 SIMilarity; 20,000 permutations available; PRIMER v6) was used to investigate potential
301 differences between microbial communities between soil horizons, over time and in
302 response to a simulated episodic nitrogen deposition event (Clarke and Green 1988).
303 Two-way ANOSIM was used to compare one factor against the other factors and one-
304 way ANOSIM to investigate the influence of individual factor. ANOSIM analysis yields
305 an R value, whereby ANOSIM values close to R = 1 indicate a high separation between
306 groups (e.g. between soil horizons, sampling time points, or N treatment), while
307 ANOSIM values close to R = 0 indicate a low group separation.

308 Relationships between microbial community structure, soil chemistry and
309 microbial gene abundance were investigated via correlation analysis from both soil
310 horizons simultaneously or for the organic and mineral soil horizons separately.
311 Correlation between microbial community structure versus soil chemistry and microbial
312 community structure or gene abundance were investigated using the RELATE test
313 (PRIMER v6) comparing Bray-Curtis similarity matrices derived from T-RFLP analysis
314 or ARISA profiles and Euclidean distance matrices (Clarke and Ainsworth 1993) of each
315 soil chemistry variable (i.e. soil water, soil pH, C, N, C/N ratio, ^{13}C, ^{15}N, N-NO_3^- and N-
316 NH_4^+) or microbial gene abundance, and between Bray-Curtis similarity matrices of
317 microbial structure (e.g. bacteria *versus* archaea). Prior to the construction of Euclidean
318 distance matrices, first N-NO_3^-, N-NH_4^+ and microbial gene abundance data were log
319 transformed (log(data + 0.1)), then all the data for the soil chemistry and microbial

abundance were normalised, to reduce the effect of outliers samples on the results and allow comparison between variables of different measurement scales (Clarke and Gorley 2006). RELATE is a permutation-based test (rank correlation method: Spearman, 999 permutations) yielding a Spearman coefficient ρ, ranging from 0 to 1, in which a ρ value close to one indicates a strong correlation between microbial community structure and the variable (ie. soil chemistry, microbial gene abundance and structure). To investigate correlation between microbial genes abundance and soil chemistry or microbial abundance (i.e. between microbial communities), Spearman rank correlation between microbial gene abundance (i.e. Q-PCR) data and soil chemistry or microbial gene abundance, were performed using R v3.0.0 (R Development Core Team 2013).

Variation in the microbial genes abundance and soil chemistry were analysed by ANOVA to investigate differences between soil horizons, over time and in response to simulated episodic nitrogen deposition events using R v3.0.0 (R Development Core Team 2013). When significant ($P < 0.05$) effects were found, the Tukey HSD test was used to reveal the detail of the effect. The normality of the models residuals and homoscedasticity of data were checked prior to statistical analysis and when one or both of these conditions were not met the data were log transformed before statistical analysis, except for $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ for which the data were square root-transformed.

Results

Soil chemistry

Soil water content of the organic horizon was significantly higher (~1.5x; $F = 53.10$, $P = 3.0\ 10^{-9}$) than in the mineral horizon across the treatments and between dates of sampling

(Online Resource Fig. S1). The soil pH of the mineral horizon (6.68 ± 0.18, n = 30) was significantly higher (F = 22.50, P = 1.99 10^{-5}) than in the organic horizon (6.49 ± 0.16, n = 30) (Online Resource Fig. S1). The addition of water (with or without low pH) to the different plots did not significantly increase the soil water content or reduce the pH of either soil horizons at any dates of sampling (P > 0.05). Total C and N soil contents were significantly higher (~2.7x F = 112.24, P = 4.80 10-^{14}, and ~2x F = 74.03, P = 3.24 10^{-11}, respectively) in the organic horizon than in the mineral horizon (Online Resource Fig. S2). ANOVA analysis showed that the C content of the organic horizon significantly (F = 6.77, P = 0.0049) decreased between dates of sampling, but the Tukey HSD test did not reveal any significant change between dates of sampling for a specific treatment. Total C and N did not change significantly between the control and 4 kg N ha^{-1} yr^{-1} treatments (P > 0.05; Online Resource Fig. S2). The C/N ratio was also significantly higher (~1.3x; F = 135.22, P = 1.97 10^{-15}) in the organic horizon than in the mineral horizon, and decreased significantly (P < 0.001) in both soil horizons by ~37% between Day-2 and subsequent sampling dates (Online Resource Fig. S2).

In contrast to the C and N content, the ^{13}C content was significantly (F = 40.01, P = 8.64 10^{-8}) higher in the mineral horizon (1.082 ± 0.0015, n = 30) than in the organic horizon (1.080 ± 0.0013, n = 30; Fig S3). In the organic horizon, the ^{13}C content decreased significantly (F = 40.01, P = 8.64 10^{-8}) from Day-2 for the treatments plots, while in the mineral horizon the decrease in ^{13}C was close to significant (F = 2.77, P = 0.083). Similarly, the ^{15}N content was significantly (F = 61.93, P = 3.99 10^{-10}) higher in the mineral (0.3668 ± 0.00091, n = 30) horizon than in the organic horizon (0.3659 ± 0.00094, n = 30), and the ^{15}N content decreased between Day-2 and the two other dates of

sampling in the organic (F = 35.91, $P = 8.41\ 10^{-8}$) and mineral (F = 40.01, $P = 8.64\ 10^{-8}$) horizons for both treatments (Online Resource Fig. S3). The ^{13}C and ^{15}N content did not change significantly between the control and 4 kg N ha^{-1} yr^{-1} treatments ($P > 0.05$; Online Resource Fig. S3). The N-NO_3^- and N-NH_4^+ content were significantly higher (2.4 x and 4.8 x, respectively) in the organic than in the mineral horizon (ANOVA: F = 7.28, P = 0.0098; F = 17.65, P = 0.00012, respectively), but the Tukey HSD test did not reveal significant differences for a specific treatment or date of sampling (Online Resource Fig. S4). The N-NO_3^- and N-NH_4^+ content did not change significantly between the control and 4 kg N ha^{-1} yr^{-1} treatments or between dates of sampling ($P > 0.05$).

Variation in bacterial, archaeal and fungal community structure

Complex T-RFLP and ARISA profiles were generated with, on average (after removing T-RFs based on their fluorescence and relative abundance), each bacterial and archaeal profile comprised of 37 ± 6 and 26 ± 7 T-RFs, respectively, and 23 ± 10 ARISA amplicons for each fungal profile. Variation in bacterial and archaeal community structures and fungal community structure were investigated by T-RFLP analysis and ARISA, respectively. Significant differences (P = 0.00005) between the bacterial communities present within the organic and mineral soil horizons (Fig. 1; Table 2) were revealed by nMDS analysis and ANOSIM (R = 0.55; Table 2). Archaeal 16S rRNA genes were successfully amplified from DNA extracted from the organic soil horizon from seven plots at Day-2, and from only three plots at Day+1 and could not be amplified from plots sampled on Day+7. However, archaeal 16S rRNA genes were amplified from DNA extracted from the mineral horizon from all plots and at every sampling time point.

389 Consequently, comparison of archaeal communities via nMDS analysis and ANOSIM
390 between organic and mineral horizons were performed only on Day-2 data (Fig. 1) and
391 revealed significant differences (P = 0.005) between organic and mineral horizon soils (R
392 = 0.38; Table 2). No significant differences (P = 0.08) were found, via nMDS analysis
393 and ANOSIM, between the fungal communities present within the organic and mineral
394 horizons (Fig. 1; Table 2). When ANOSIM was performed between soil horizons for each
395 date of sampling separately, the fungal community structure was nearly significantly
396 different at Day+7 (R = 0.16, P = 0.08).
397 Short-term temporal variation was observed in bacterial community structure
398 within the organic horizon but not in the mineral horizon (Table 2), which was not visible
399 on 2D nMDS (Fig. 2a) but only on 3D nMDS (Online Resource Fig. S5). Within the
400 organic horizon, bacterial community structure differed significantly (P = 0.046) between
401 Day-2 *vs.* Day+7 (R = 0.17; Table 2). Temporal variation in the structure of the archaeal
402 community could only be investigated within the mineral horizon, as 16S rRNA genes
403 were not always amplified from the organic horizon (see above). Temporal variation in
404 archaeal community structure within the mineral horizon was observed with significant
405 differences between the communities present on Day-2 *vs.* Day+1 (P = 0.03), and Day-2
406 *vs.* Day+7 (P = 0.007), but no significant difference was found between Day+1 *vs.* Day+7
407 (Fig. 2b, Table 2). The R value was higher between Day-2 *vs.* Day+7 than Day-2 *vs.*
408 Day+1 (R = 0.39 and R = 0.16, respectively). Similarly, temporal variation in fungal
409 community structure was also observed in the mineral horizon, differing significantly (R
410 = 0.29; P = 0.009) between Day-2 *vs.* Day+7, but with no significant differences between

411 other sampling dates (Fig. 2b, Table 2). However, fungal community structure within the
412 organic horizon did not change significantly over time ($P > 0.05$, Table 2).
413 The N treatment did not result in any significant changes in bacterial community
414 structure within either the organic or mineral horizons ($P = 0.36$ and $P = 0.67$,
415 respectively; Online Resource Fig. S6; Table 2), even when the analyses were performed
416 for each individual date of sampling (data not shown). Similarly, there were no
417 significant changes in archaeal community structure in the mineral horizon due to N
418 treatment ($P = 0.76$), or in fungal community structure (Online Resource Fig. S6; Table
419 2) within the organic or mineral horizons ($P = 0.88$, $P = 0.46$, respectively).
420
421 **Variation in bacterial, archaeal genes and fungal amplicons abundance**
422 Variation in bacterial, archaeal and fungal abundance was investigated by Q-PCR
423 analysis of 16S rRNA genes (bacteria and archaea) and ITS regions (fungi). Abundance
424 data was generated from all plots and at every time point for all three target amplicons,
425 with the exception of a single plot for bacteria (mineral horizon within a N treatment plot
426 at Day+7) and a single plot for archaea (mineral horizon within a N treatment plot at
427 Day+7). Bacterial and archaeal 16S rRNA genes and fungal ITS regions were all more
428 abundant 2x, 2.8x and 1.1x, respectively) in the mineral horizon than in the organic
429 horizon (Fig. 3). Bacterial gene abundance was significantly (ANOVA, $F = 8.63$, $P =$
430 0.0051) higher in the mineral horizon regardless of the date of sampling and treatment,
431 but the Tukey HSD test did not reveal any significant ($P > 0.05$) differences between soil
432 horizons for each specific date of sampling and treatment. Archaeal gene abundances
433 were significantly ($F = 54.54$, $P = 2.14\ 10^{-9}$) higher in the mineral horizons than the

organic, such as for the control plots at Day-2 and Day+1 (Fig. 3b). Despite the fungal amplicon abundance being slightly higher in the mineral than in the organic horizon, the difference was not significant ($P = 0.43$; Fig. 3c).

Bacterial 16S rRNA gene numbers did not change significantly ($P > 0.05$) between dates of sampling in the organic horizon (Fig. 3a). However, bacterial 16S rRNA gene abundance increased significantly (ANOVA, $F = 4.59$, $P = 0.021$) regardless of the treatment within the mineral horizon by 2.9 times between Day-2 and Day+7, but a Tukey HSD test did not show specific differences between dates of sampling for a specific treatment. Archaeal 16S rRNA gene abundances did not show any significant ($P > 0.05$) changes between dates of sampling in either soil horizon (Fig. 3b). In contrast, abundance of fungal ITS regions increased between Day-2 and Day+7 by 3.3 times in the organic and by 4 times in the mineral horizons (Fig. 3c). The differences between date of sampling were significant for the organic (ANOVA, $F = 4.50$, $P = 0.022$) and mineral (ANOVA, $F = 7.30$, $P = 0.003$) horizons regardless of treatments, but the Tukey HSD test did not reveal significant differences between dates of sampling for a specific treatment.

Abundances of bacterial, archaeal rRNA genes and fungal ITS amplicons were not significantly influenced ($P > 0.05$) by N treatment for either soil horizon or at any dates of sampling (Fig. 3).

Relationship between microbial community structure, microbial gene abundance and soil chemistry

Relationships between microbial community structure, soil chemistry, microbial structure or microbial genes/amplicons abundance were investigated in both soil horizons

simultaneously, to reveal drivers of the difference in microbial communities between soil horizons, and from the organic or mineral horizons separately to reveal the drivers of the microbial communities within a specific soil horizon over time. Bacterial community structure from both soil horizons was significantly and strongly correlated (P = 0.001) with soil water content (ρ = 0.53), N content (ρ = 0.43), ^{13}C content (ρ = 0.37), C content (ρ = 0.33), and weakly correlated with ^{15}N content (ρ = 0.23) and soil pH (0.22) (Fig. 4). Bacterial community structure from the organic horizon was significantly but weakly correlated with C/N (ρ = 0.14, P = 0.039). In contrast, bacterial community structure from the mineral horizon was significantly and strongly correlated with ^{13}C content (ρ = 0.32, P = 0.001), and soil water content (ρ = 0.31, P = 0.001), and more weakly with N content (ρ = 0.27, P = 0.003), ^{15}N content (ρ = 0.24, P = 0.006) and soil pH (ρ = 0.16, P = 0.034). Bacterial gene abundance from both soil horizons was significantly correlated with C/N (ρ = -0.48, P = 0.00002) and were close to significantly correlated with C content (ρ = -0.26, P = 0.052; Fig. 5). Similarly, bacterial gene abundance from the organic horizon was significantly correlated with C content (ρ = -0.38, P = 0.045), and C/N (ρ = -0.37, P = 0.047). In contrast, bacterial gene abundance in mineral horizons was significantly correlated with soil water content (ρ = 0.59, P = 0.0007), ^{13}C content (ρ = -0.57, P = 0.001), ^{15}N content (ρ = -0.54, P = 0.002), N content (ρ = 0.54, P = 0.003) and C content (ρ = 0.40, P = 0.032) (Fig. 5).

Correlations between archaeal community structure from both soil horizons and soil chemistry could only be investigated at Day-2 and showed a significant correlation only with C/N ratio (ρ = 0.23, P = 0.01; Fig. 4). Archaeal community structure from the organic horizon was not significantly correlated with any of the soil chemistry variables,

but was close to being significantly correlated to soil pH ($\rho = 0.41$, $P = 0.051$). In contrast, archaeal community structure from the mineral horizon was significantly correlated to several variables: ^{13}C content ($\rho = 0.3$, $P = 0.003$), C/N ($\rho = 0.25$, $P = 0.007$), C content ($\rho = 0.24$, $P = 0.009$), soil pH ($\rho = 0.20$, $P = 0.005$), and ^{15}N content ($\rho = 0.19$, $P = 0.002$). Archaeal gene abundance from both soil horizons showed significant and negative correlations with C content ($\rho = -0.62$, $P = 1.88\ 10^{-7}$), C/N ($\rho = -0.58$, $P = 1.87\ 10^{-6}$), soil water content ($\rho = -0.55$, $P = 8.19\ 10^{-6}$), N content ($\rho = -0.53$, $P = 1.94\ 10^{-5}$), and was positively correlated with ^{13}C content ($\rho = 0.37$, $P = 0.005$) and soil pH ($\rho = 0.32$, $P = 0.013$; Fig. 5). Archaeal gene abundance from the organic horizon was not significantly correlated with any soil chemistry variables, while archaeal gene abundance from the mineral horizon was significantly correlated only with C/N ($\rho = -0.39$, $P = 0.039$; Fig. 5).

Fungal community structure from both soil horizons was significantly, but weakly, correlated with ^{13}C content ($\rho = 0.16$, $P = 0.001$), ^{15}N content ($\rho = 0.10$, $P = 0.015$), N ($\rho = 0.1$, $P = 0.01$), C ($\rho = 0.09$, $P = 0.027$) and soil water content ($\rho = 0.09$, $P = 0.038$) (Fig.4). Fungal community structure from organic or mineral horizons were not significantly correlated with any soil chemistry variables. Fungal amplicon abundance from both soil horizons was significantly and negatively correlated with C/N ($\rho = -0.45$, $P = 3.2\ 10^{-4}$), and ^{15}N content ($\rho = -0.26$, $P = 0.044$; Fig. 5). Fungal amplicon abundance from the organic horizon was significantly correlated with C/N ($\rho = -0.53$, $P = 0.003$) and C content ($\rho = -0.50$, $P = 0.006$), while fungal amplicon abundance from the mineral horizon was only significantly correlated with ^{15}N content ($\rho = -0.51$, $P = 0.004$).

Microbial community structures or gene abundances showed also intercorrelations between communities. Hence, bacterial community structure from both soil horizons was significantly correlated with archaeal ($\rho = 0.21$, $P = 0.008$) and fungal ($\rho = 0.25$, $P = 0.025$) community structure, and also with bacterial gene abundance ($\rho = 0.17$, $P = 0.008$), and archaeal gene abundance ($\rho = 0.22$, $P = 0.001$; Fig. 4). Archaeal community structure from both soil horizons was significantly correlated to archaeal gene abundance at Day-2 ($\rho = 0.26$, $P = 0.001$). Microbial community structure from a specific soil horizon showed less significant correlations between communities than for both soil horizons. Hence, bacterial community structure from the mineral horizon was significantly correlated with archaeal community structure ($\rho = 0.19$, $P = 0.044$) and bacterial gene abundance ($\rho = 0.25$, $P = 0.023$) from the mineral horizon. Archaeal community structure from the organic horizon was close to being significantly correlated with fungal community structure from the organic horizon ($\rho = 0.23$, $P = 0.071$), while archaeal community structure from the mineral horizon was close to being significantly correlated with archaeal gene abundance from the mineral horizon ($\rho = 0.13$, $P = 0.071$). Bacterial gene abundance from both soil horizons was significantly correlated with fungal amplicon abundance ($\rho = 0.70$, $P = 1.12\ 10^{-9}$) and archaeal gene abundance ($\rho = 0.65$, $P = 3.54\ 10^{-8}$), and these two communities were also significantly correlated to each other ($\rho = 0.49$, $P = 9.08\ 10^{-5}$; Fig. 5). Similarly, bacterial gene abundance from the organic or mineral horizon was significantly correlated with fungal amplicon ($\rho = 0.72$, $P = 09.41\ 10^{-6}$, $\rho = 0.70$, $P = 2.57\ 10^{-5}$; respectively) archaeal gene ($\rho = 0.69$, $P = 3.15\ 10^{-5}$, $\rho = 0.50$, $P = 0.007$; respectively) abundance and these two communities were significantly correlated with each other ($\rho = 0.77$, $P = 1.07\ 10^{-6}$, $\rho = 0.46$, $P = 0.013$; respectively).

Discussion

Variation in soil bacterial, archaeal and fungal community structure and abundance between soil horizons

The structure of both bacterial and archaeal communities differed significantly between organic and mineral soil horizons (Fig. 2; Table 2). Variation in bacterial community structure in relation to soil horizons or soil depths has been reported in polar regions, including subarctic heathland (Rinnan et al. 2007) and High Arctic tundra (Wallenstein et al. 2007; Lee et al. 2013). In contrast, little is known about changes in archaeal community structure with soil depth or between horizons. Lee et al. (2013) also found that archaeal community structure differed between organic and mineral horizons in High Arctic tundra located ~2 km from the current study site, using T-RFLP analysis, but targeting different regions of the 16S rRNA genes, and utilising different restriction enzymes from our study, highlighting the consistency of these results. In the current study, differences in bacterial and archaeal community structure between soil horizons were not related to changes in the richness or evenness (Shannon index) of terminal restriction fragments (T-RF) as no significant differences were found between soil horizons and/or between dates of sampling (data not shown). In contrast, Lee et al. (2013) found a lower Shannon index for archaea within the mineral horizon. Correlation analysis using RELATE tests showed that soil water content was the dominant driver of bacterial community structure, with total N, C and ^{13}C contents also important and to a lesser extent ^{15}N and soil pH. Variation in water content and C content (Zhou et al. 2002; Fierer et al. 2003) has previously been proposed as important determinants of soil microbial

548 community structure, in addition to soil type (Girvan et al. 2003) and soil pH (Fierer and
549 Jackson 2006). Soil pH was also shown to be an important driver of bacterial community
550 structure in subarctic soil (Ma¨nnisto¨ et al. 2007) and to bacterial diversity in dry heath
551 tundra across the Arctic (Chu et al. 2010), although it does not seem to be a dominant
552 driver explaining the different microbial communities between soil horizons. In this
553 study, differences in the structure of the archaeal community between soil horizons, prior
554 to N treatment, were only correlated with variation in the C/N ratio, although it is
555 recognised that correlation analysis post-N treatment was not possible. Hence, organic
556 matter quality and quantity seem to be the dominant driver of the bacterial and archaeal
557 community structure in the different soil horizons.

558 Bacterial and archaeal gene abundance was higher in the mineral than in the
559 organic horizon, especially archaea which were ~2.8 times more abundant in the mineral
560 horizon (Fig. 3a, b). These results are in contrast to Lee et al. (2013) who showed no
561 difference in bacterial and archaeal gene abundance between horizons (organic versus
562 mineral) in High Arctic tundra. However, the knowledge of microbial abundance with
563 soil horizons in Arctic soil is limited. The effects of PCR inhibitors such as humic acids
564 co-extracted with DNA could potentially explain the lower gene abundance within the
565 organic horizon. However, when the amount of DNA template was increased (from 1 to 2
566 ll) in PCRs, there was no evidence of PCR inhibition due to potential increase in
567 concentration of PCR inhibitors. Moreover, the DNA extraction kit used herein included
568 a DNA purification step, reducing the co-extraction of humic acids with DNA and has
569 been shown to be an efficient DNA extraction protocol (Ning et al. 2009; Engel et al.
570 2012). This difference may also be partly explained by a possible lower DNA extraction

efficiency from the organic horizon samples. The increases in microbial abundance observed within the mineral horizon within our study are not readily explained by soil water, carbon and nitrogen content, which were higher in the organic horizon (Table 1). Soil water content was 1.59 higher in the organic than in the mineral horizon, although the volumetric water content was ~3 times higher in the mineral than in the organic horizon, leading to higher water availability in the mineral horizon than in the organic horizon. A microcosm experiment using samples from the experimental site showed an increase in bacterial abundance after water addition within the organic horizon only, indicating that water availability might be limiting bacterial (but not archaeal) abundance within the organic horizon (Blaud et al. in preparation). In contrast, archaeal gene abundance showed strong and negative correlations with C concentration, C/N ratio and water, but positive correlations with ^{13}C content and soil pH. The negative correlations with C and C/N ratio may indicate that nutrients sources are not readily available for archaea or they are outcompeted by other microorganisms to access/use nutrients, which are supported by the strong correlations with bacterial and fungal abundance (Fig. 4). Overall, the mineral horizon in High Arctic tundra could represent a hotspot for bacterial and archaeal abundance and also to their activity which ould explain the higher ^{13}C and ^{15}N contents found in the mineral horizons (Online Resource Fig. S3).

No significant differences were found in fungal community structure and abundance between organic and mineral soil horizons (Fig. 2c; Fig. 3c; Table 2). Lee et al. (2013) also found no difference in fungal community structure investigated by T-RFLP (ARISA was used in the current study) and abundance (also investigated by Q-PCR) between soil horizons in High Arctic tundra near our research site. In contrast,

Robinson et al. (2004) showed that the diversity of fungal communities (assessed by culture dependent approaches) was higher in the organic soil than in mineral soil in tundra near Ny-Ålesund, and Deslippe et al. (2012) also found that the fungal community structure differed between organic and mineral soil horizons. In the tundra system studied here, the absence of significant differences in fungal community structure and abundance between soil horizons is unlikely due to the sampling at the end of the summer, where the environmental conditions change (e.g. decreases in daily temperature, and the onset of plant senescence) stimulating fungal growth via the input of organic matter from senescing plants (Nemergut et al. 2005), as Lee et al. (2013) sampled in June and July and did not find difference in structure and abundance, whilst Deslippe et al. (2012) found difference in fungal structure between horizons by sampling in July and August. Perhaps the absence of a difference could be explained by the distribution of fungal mycelia, probably present across both soil horizons for each fungal species because of the sizes of fungal mycelia that spread in soil and the relatively small size of soil samples taken (10 cm depth including organic and mineral horizons) (Landeweert et al. 2003). Moreover, fungal community structure was weakly correlated with a few soil chemistry variables (Fig. 4) with these weak correlations perhaps explained by considerable heterogeneity in fungal community structure. Only the variability in fungal abundance within soil horizons was negatively correlated with the C/N ratio which could indicate the role of fungi in decomposition of recalcitrant organic matter.

Short-term variation in bacterial, archaeal and fungal community structure and abundance

617 Bacterial community structure did not change over time, except in the organic horizon
618 (Fig. 2, Online Resource S5; Table 2), but the low ANOSIM R value (0.17) indicated
619 weak separation between Day - 2 and Day + 1. In contrast, significant changes in
620 bacterial community structure were observed (via T-RFLP analysis) within tundra soil in
621 Alaska (McMahon et al. 2011), but over longer timescales of between June and late
622 summer (August). Bacterial abundance increased during the 9-day plot experiment within
623 the mineral horizon and significantly correlated with the decrease over time in ^{15}N
624 enrichment, which could indicate an increase in bacterial activity coupled with an
625 increase in abundance. Hence, the transition between summer to autumn, during which
626 the experiment took place, when the plants start senescing, may result in an increase in
627 organic matter which stimulates bacterial mineralisation and growth. Previously,
628 increases in bacterial biomass (measured by cell counting) in the organic horizon were
629 seen over a longer period (between mid-July and mid-August 2002) at a location ~2 km
630 north from our experimental plots (Stapleton et al. 2005). However, such increases were
631 not observed by these authors in plots sampled during 2001 over a similar duration and
632 time period, indicating that changes in bacterial abundance within the organic horizon
633 over the summer period may differ between years, which might explain the absence of
634 bacterial abundance change in the current study.

635 Archaeal community structure within the mineral horizon showed greater
636 variability during the plot experiment than was seen in the structure of either bacterial or
637 fungal communities (Fig. 2; Table 2). Changes in archaeal community structure in the
638 mineral horizon were correlated with the decrease in ^{13}C and ^{15}N enrichment and C/N
639 ratio, suggesting that changing in soil organic matter might affect archaeal community

640 composition, via either a direct effect on archaeal activity, or indirectly due to an increase
641 in competition with bacteria and fungi which increased in abundance. Longer-term
642 variation in archaeal community structure has previously been demonstrated, via DGGE
643 analysis, in a peat soil located ~2 km north-east to our experimental plots, but over longer
644 period of time between July and August (Høj et al. 2005). Archaeal 16S rRNA genes
645 could not be amplified from organic horizon samples from all of the experimental plots to
646 enable T-RFLP analysis at Day + 1 and Day + 7, which initially suggested a decline in
647 archaeal abundance within the organic horizon over time. However, when archaeal
648 community abundance was investigated by Q-PCR, using a different primer combination,
649 archaeal 16S rRNA genes were amplified from the organic horizon of all plots at all time
650 points and, furthermore, revealed no short-term temporal variation in archaeal abundance
651 within the organic horizon. Differential detection of archaea by different primers is
652 unsurprising and may be a consequence of the primer sets used for T-RFLP and Q-PCR
653 targeting different archaeal taxa and/or populations. This emphasises the importance of
654 using multiple primer combinations and/or methodologies to avoid methodological bias
655 when studying archaeal communities (Lueders and Friedrich 2003).

656 Fungal community structure changed over time, although only in the mineral
657 horizon but was not correlated with any environmental variables. Fungal amplicon
658 abundance increased in both soil horizons over time, but different drivers explained this
659 increase, with fungal abundance correlated with the decrease in C and C/N ratio in the
660 organic horizon and to the 15N content in the mineral horizon. In contrast, using culture-
661 dependent approaches, Robinson et al. (2004) did not find significant changes in fungal
662 assemblages over a longer period (end of June to start of August) within tundra soil

663 located ~5 km to the west of our site. The current experiment was sampled at the
664 transition between summer and autumn, during which time environmental conditions
665 change, with decreases in daily temperature, increases in the diurnal oscillation in air
666 temperature and the onset of plant senescence. Nemergut et al. (2005) described this
667 period within Alpine tundra as being characterised by important changes in microbial
668 communities, dominated by changes in the fungal community that are able to decompose
669 recalcitrant organic matter. Thus, the correlations between fungal abundance C, ^{15}N and
670 C/N may indicate an increase in plant material decomposition by fungi. In parallel, the
671 change in fungal structure in the mineral horizon could be related to a reduction in
672 mycorrhizal fungi due to plant senescence reducing C allocation to mycorrhizas. Activity
673 of mycorrhizal fungi was shown to contribute to soil ^{15}N enrichment (Etcheverrı´a et al.
674 2009) which decreased over time, supporting a decrease in mycorrhizal fungi and an
675 increase in saprophytic fungi decomposition organic matter.
676
677 **Acute nitrogen deposition events do not have a short-term impact on bacterial,**
678 **archaeal and fungal community structure or abundance**
679 Bacterial, archaeal and fungal community structure and gene abundance were not
680 affected by the simulated acute nitrogen deposition event, within either soil horizon or
681 over time (7 days post-N treatment). In the two prior experiments that have utilised long-
682 term chronic (rather than acute) N treatments using a comparable N addition rate, effects
683 on microbial communities have been variable, with no changes observed in microfungal
684 diversity and richness after 2 years of N addition at 5 (but also at 50) kg N ha^{-1} yr^{-1}
685 (Robinson et al. 2004), whilst Stapleton et al. (2005) found an increase in bacterial

686 biomass 44 days after an N application of 5 kg N-NH_4 ha^{-1} yr^{-1}. Other prior research has
687 utilised N addition rates that are more representative of N fertilisation (i.e. very large N
688 inputs and/or combined with other nutrients), as opposed to N deposition events and have
689 also shown variable microbial responses. For example, bacterial community structure was
690 found not to vary, via DGGE analysis, in Canadian Arctic tundra over a 15-year period,
691 despite N additions at rate of 100 and 500 kg ha^{-1} yr^{-1} of NPK fertilizer (Lamb et al.
692 2011). In contrast, PLFA profiling revealed compositional changes in bacterial
693 communities in Swedish Arctic heathland following a total N treatment of 400 kg N ha^{-1}
694 over a 4-year period, although microbial biomass was not affected (Schmidt et al. 2000).
695 Rinnan et al. (2007) also found the structure of the bacterial communities, investigated by
696 PLFA, within the organic and mineral horizons of subarctic heath (near to the site studied
697 by Schmidt and colleagues) was affected after 15 years of N addition with a total N load
698 of 1250 kg N ha^{-1}, and the total PLFA content increased with fertilisation by 16 % in the
699 organic horizon but by 27 % in the mineral horizon. It is probable that the modest
700 nitrogen inputs used in this current plot experiment to simulate an acute N deposition
701 event do not reach the critical load of the tundra ecosystem under which ecosystem
702 changes occur (Jefferies and Maron 1997), although it should be recognised that our
703 current study has investigated potential impacts over a short duration and towards the end
704 of the Arctic summer and that the plant cover (especially the bryophytes) may have
705 retained part of the N applied (Tye et al. 2005). Furthermore, changes in the
706 concentration of N, ^{15}N, NH_4^+, and NO_3^- between the control and N treatment for both
707 soil horizons and over time could not be detected, as N addition rate was within the
708 natural variation of N, NH_4^+ and NO_3^- within the organic and mineral horizon. Other

studies applying similar or higher amounts of N in Arctic tundra did not find any significant changes in total soil N content (Robinson et al. 2004; Tye et al. 2005), or even soil inorganic N, and only the use of ^{15}N-labels revealed the increase in N within the soil due to N addition (Tye et al. 2005). A subsequent study will report on microbial community responses to acute N deposition within Arctic tundra soil over a full summer period and in response to higher rates of simulated N deposition, using ^{15}N-labelled NH_4NO_3 to follow the fate of N within soil horizons.

Conclusions

In this study, variation in the structure and abundance of bacterial, archaeal and fungal communities was investigated within tundra soils in the High Arctic. Bacterial and archaeal community structure was found to vary significantly between organic and mineral horizons, reflecting environmental variability therein. Fungal community structure also varied between the two soil horizons but not significantly. Interestingly, and in contrast to prior research, the abundance of bacteria and archaea was higher in the mineral horizon than in the organic horizon and, in particular, for archaea. The quality and quantity of organic matter seem to be important drivers of bacterial and archaeal communities between soil horizons. Hence, the mineral horizon may represent an important spot for microbial diversity and activity, which have been scarcely studied in Arctic tundra.

Soil horizon- and/or microbial domain- and/or kingdomspecific variability was observed during this short-term field plot experiment. In particular, archaeal community

731 structure varied with time in the mineral horizon, in relation to decreases in total C
732 content and in the C/N ratio, whilst short-term changes in the structure and abundance of
733 fungal communities were also observed within the mineral horizon. Bacterial abundance
734 also increased within the mineral horizon over time. Microbial communities have been
735 rarely studied over shorter periods of time (i.e. over few days), arguing that long periods
736 of time are needed for microbial communities to respond to environmental changes due to
737 the harsh environmental conditions. However, this study showed that microbial
738 communities can change in structure and abundance over a few days, and this should be
739 considered when assessing the effect of global changes on microbial communities in
740 Arctic tundra soil.

741 Following the experimental simulation of an acute atmospheric nitrogen
742 deposition event, no short-term response was seen by any of the three microbial groups
743 studied. Prior to this study, comparable N treatments had focused either on a single
744 microbial group, single sample point and/or considered the soil as a single compartment,
745 albeit investigating changes over several time periods and soil depths, than studied
746 herein. The absence of a short-term N deposition event effect on these tundra microbial
747 communities may be explained by the fact that N is not limiting for microbial growth
748 within either soil horizon, although it is recognised that potential impacts on different
749 functional guilds within the nitrogen cycle have not been investigated.

750 Overall, this study reveals the importance of studying both structural and
751 quantitative variability both between and within each microbial domain (and/or kingdom)
752 n tundra microbial communities in the High Arctic and, further, that different soil
753 horizons should be studied as independent environmental compartments to fully

754 understand how these microbial communities will respond to future environmental
755 change over short (days) and long period of time (months and years).
756

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765

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Table 1 chemical characteristic of organic and mineral soil horizons in experimental plots prior to N treatment. Means values ± standard deviation (n = 10) are shown from soils sampled at Day-2.

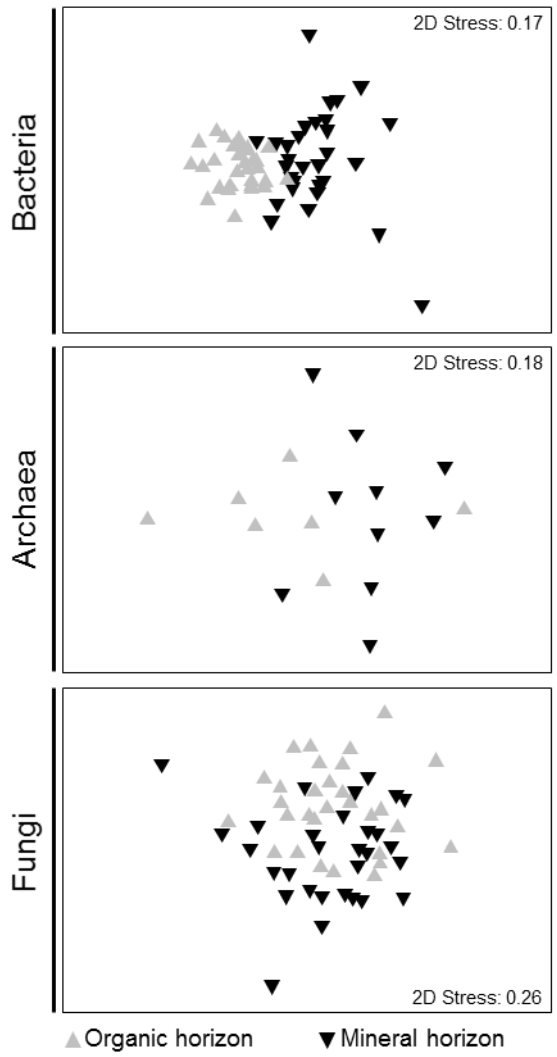
	Organic horizon	Mineral horizon
Water content (%)	59.6 ± 6.3	35.2 ± 12.7
pH aqueous	6.4 ± 0.3	6.6 ± 0.2
Total C (g kg^{-1} soil)	18.5 ± 4.3	6.4 ± 3.8
Total N (g kg^{-1} soil)	0.90 ± 0.20	0.41± 0.22
C/N	20.6 ± 1.5	14.8 ± 1.6
^{13}C (atom (%)	1.081 ± 0.0019	1.083 ± 0.0019
^{15}N (atom %)	0.3669 ± 0.00065	0.3680 ± 0.00065
$N\text{-}NO_3^-$	41.35 ± 46.16	11.07 ± 37.89
NNH_4^+	34.26 ± 38.96	14.06 ± 15.89

964 **Table 2** Two-way ANOSIM comparison showing variation in bacterial, archaeal and
965 fungal community structure between soil organic and mineral horizons; day of sampling
966 and in response to simulated episodic nitrogen deposition (N treatment; 4 kg N ha^{-1} yr^{-1}
967 ~pH 4 *vs.* Control; water ~pH 6). ANOSIM R and *P* values were generated from a Bray-
968 Curtis similarity matrices derived from T-RFLP relative abundance data (bacteria and
969 archaeal 16S rRNA genes) and ARISA relative abundance data (fungi). Day-2: before N
970 treatment; Day+1: one day after N treatment; Day+7: seven days after N treatment.
971 ANOSIM R values (and *P* values in brackets) are given. Significant values at $P < 0.05$
972 are shown in bold text.

Soil horizons	Factors compared	Bacteria	Archaea	Fungi
	Organic *vs.* Mineral	**0.55 (0.00005)**	**0.38 (0.008)**[a]	0.09 (0.08)
Organic	Day-2 *vs.* Day+1	0.13 (0.084)	nd	-0.02 (0.53)
	Day-2 *vs.* Day+7	**0.17 (0.046)**	nd	0.04 (0.35)
	Day+1 *vs.* Day+7	0.13 (0.092)	nd	0.02(0.39)
Mineral	Day-2 *vs.* Day+1	0.01 (0.40)	**0.16 (0.03)**	0.002 (0.48)
	Day-2 *vs.* Day+7	0.01 (0.38)	**0.39 (0.007)**	**0.29 (0.009)**
	Day+1 *vs.* Day+7	-0.09 (0.86)	0 (0.41)	0.18 (0.053)
Organic	Control *vs.* N treatment[b]	0.03 (0.36)	nd	-0.10 (0.88)
Mineral	Control *vs.* N treatment[b]	-0.04 (0.67)	-0.07 (0.76)	0.004(0.46)

973 [a] Comparison was based on data from Day-2 only; nd = not determined (see text for
974 details)

975 [b] The control *vs.* N treatment show results tested for Day+1 and Day+7 simultaneously.
976 No significant differences were found when the analysis where performed for each
977 individual date of sampling (data not shown).



978

979 **Fig. 1** non-metric multidimensional scaling (nMDS) plots showing variation in bacterial,
980 archaeal and fungal community structure between organic and mineral horizons during
981 late summer 2009 (for all dates of sampling) for the N treatment (4 kg N ha^{-1} yr^{-1} ~pH 4)
982 and control (water ~pH 6) plots. nMDS plots were generated from Bray-Curtis similarity
983 matrices derived from T-RFLP relative abundance data (bacterial and archaeal 16S rRNA
984 genes) and ARISA relative abundance data (fungi ITS). nMDS of Archaea shows data
985 from Day-2 only (see text for details). Symbols and 2D stresses are as indicated.

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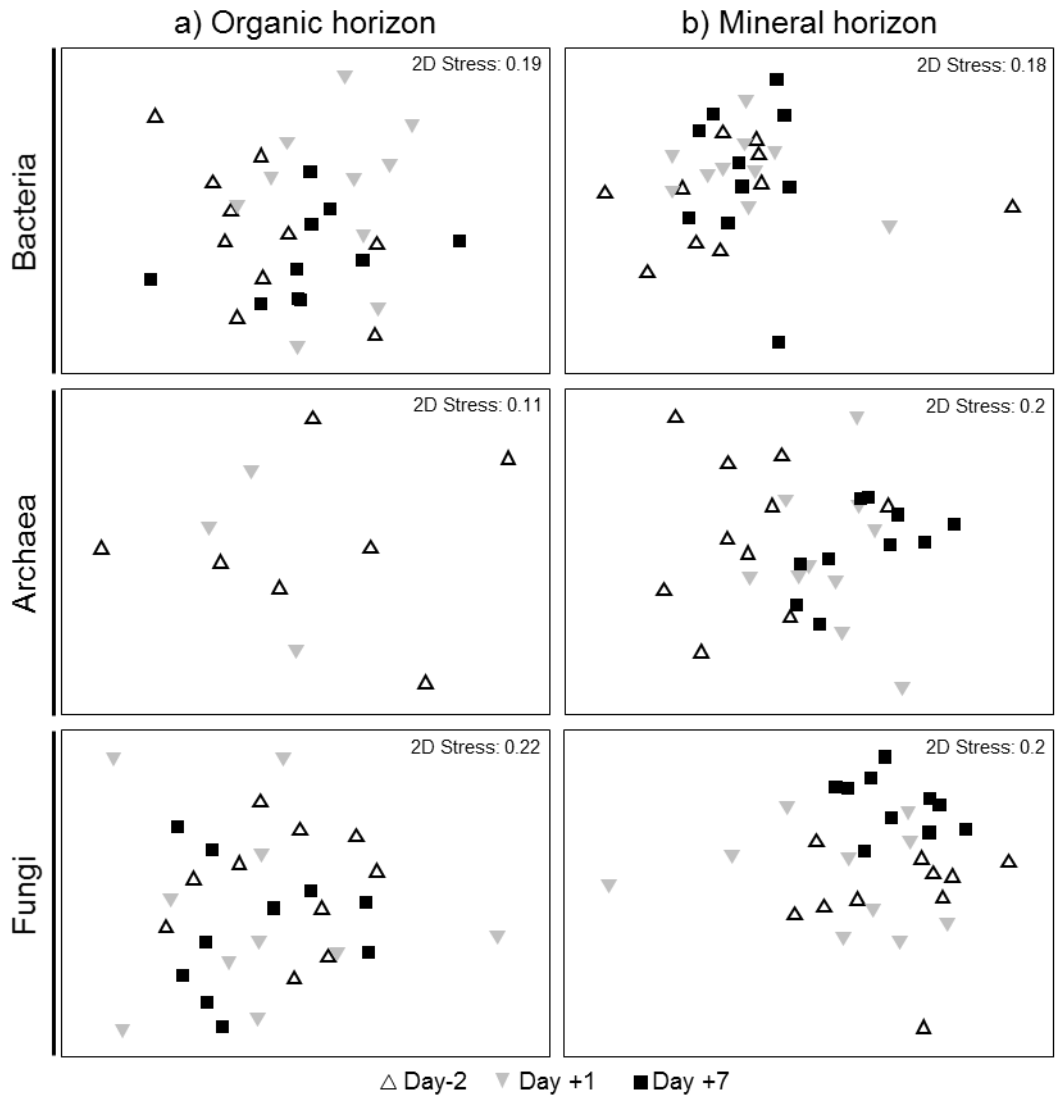

987

988 **Fig. 2** non-metric multidimensional scaling (nMDS) plots showing variation in bacterial,
989 archaeal and fungal community structure between dates of sampling (Day-2, Day+1 and
990 Day+7) within the **a)** organic horizon and **b)** mineral horizon for the N treatment (4 kg N
991 ha^{-1} yr^{-1} ~pH 4) and control (water ~pH 6) plots. nMDS plots were generated from Bray-
992 Curtis similarity matrices derived from T-RFLP relative abundance data (bacterial and
993 archaeal 16S rRNA genes) and ARISA relative abundance data (fungi ITS). Symbols and
994 2D stresses are as indicated.

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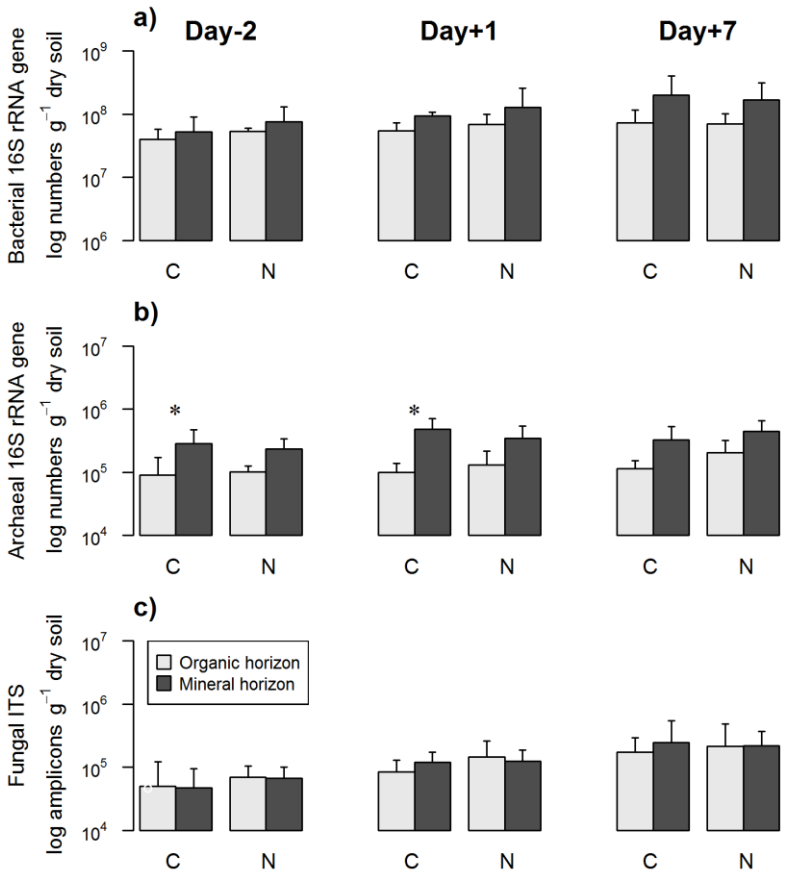

996

997 **Fig. 3** Variation in abundance of **a)** bacteria and **b)** archaea (log 16S rRNA gene numbers
998 g^{-1} dry soil) and **c)** fungi (log ITS amplicons g^{-1} dry soil) between organic and mineral
999 soil horizons (as indicated), over time (Day-2, Day+1 and Day+7) and between N
1000 treatment (**N**: 4 kg N ha^{-1} yr^{-1} ~pH 4) and control (**C**: water ~pH 6) plots. Day-2: before N
1001 application; Day+1: one day after N application; Day+7: seven days after N application.
1002 Means values ± standard deviation (n = 5, except where mentioned in the text) are shown.
1003 Gene numbers were calculated from the standard curves: bacteria: r^2 = 0.991, y (intercept)
1004 = 36.48, E (amplification efficiency) = 94.2%, NTC C_t = 29.4; archaea: r^2 = 0.995, y =
1005 36.95, E = 85.9%, NTC C_t = 32.4; fungi: r^2 = 0.899, y = 32.76, E = 138.5%, NTC C_t =
1006 37.3. * indicates a significant (P < 0.05) difference between the organic and mineral
1007 horizons for a specific date of sampling and treatment.

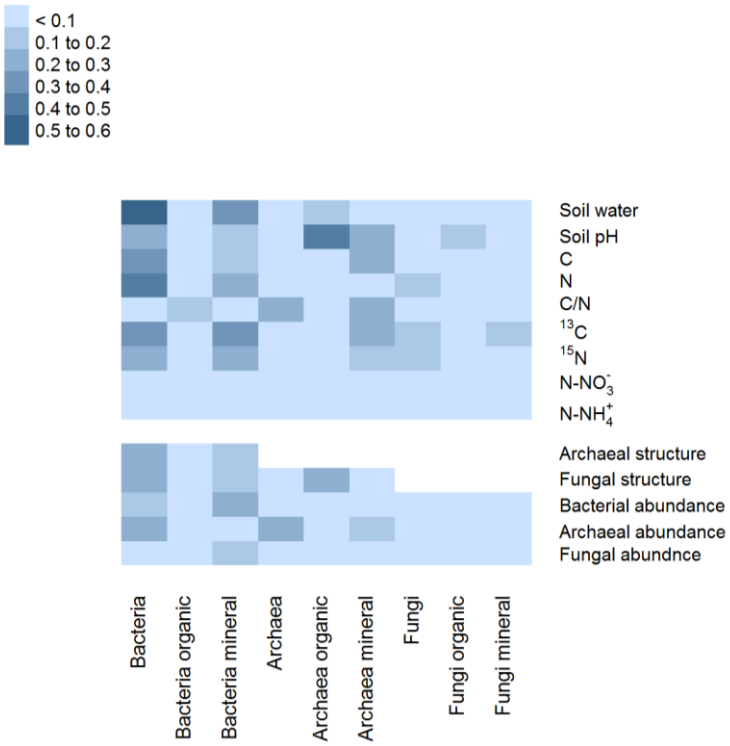

Fig. 4 Heat map of Spearman's rank correlation coefficients between bacterial, archaeal and fungal community structure, and soil chemistry or bacterial, archaeal and fungal gene abundance. The correlations were performed with communities structure from both organic and mineral horizons simultaneously or for the organic and mineral horizons separately (named organic and mineral in the heat map, respectively). Correlations were calculated using the RELATE test (PRIMER-E software) from Bray-Curtis similarity matrices derived from T-RFLP relative abundance data (bacteria and archaeal 16S rRNA genes) and ARISA relative abundance data (fungi), and Euclidean matrices derived from soil chemistry and 16S rRNA gene (bacteria and archaea) and fungal ITS region abundances. Correlations for Archaeal community structure from both soil horizons were calculated using data from Day-2 samples only (see text for details), while correlations for Archaea in organic horizon were calculated using data from 10 samples (7 samples Day-2 and 3 Day+1; see text for details).

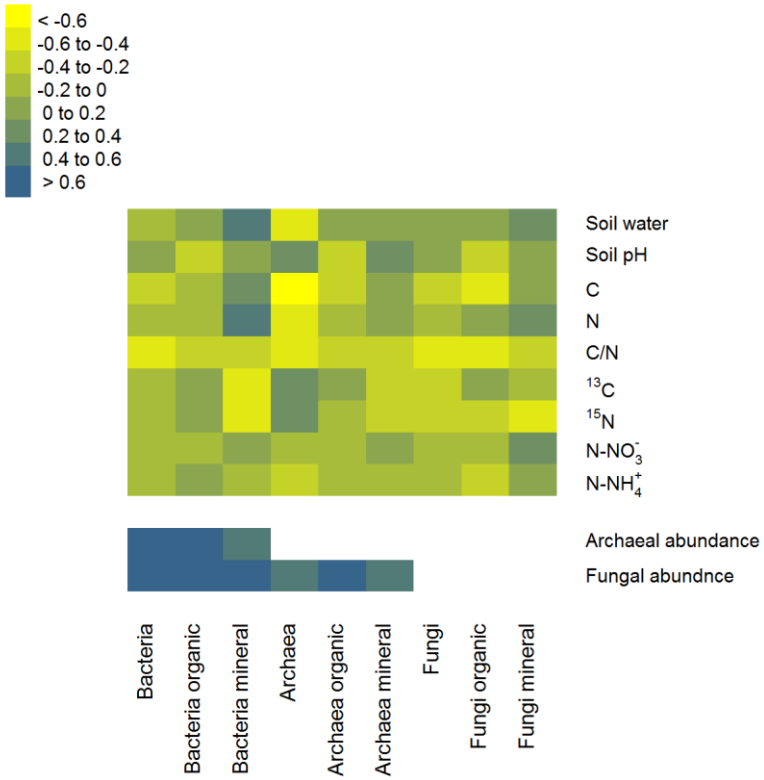

Fig. 5 Heat map of Spearman's rank correlations coefficient between bacterial, archaeal and fungal community gene/amplicon abundance, and soil chemistry or bacterial, archaeal and fungal gene/amplicon abundance. The correlations were performed with communities gene/amplicon abundance from both organic and mineral horizons simultaneously or for the organic and mineral horizons separately (named organic and mineral in the heat map, respectively).

Supplementary material

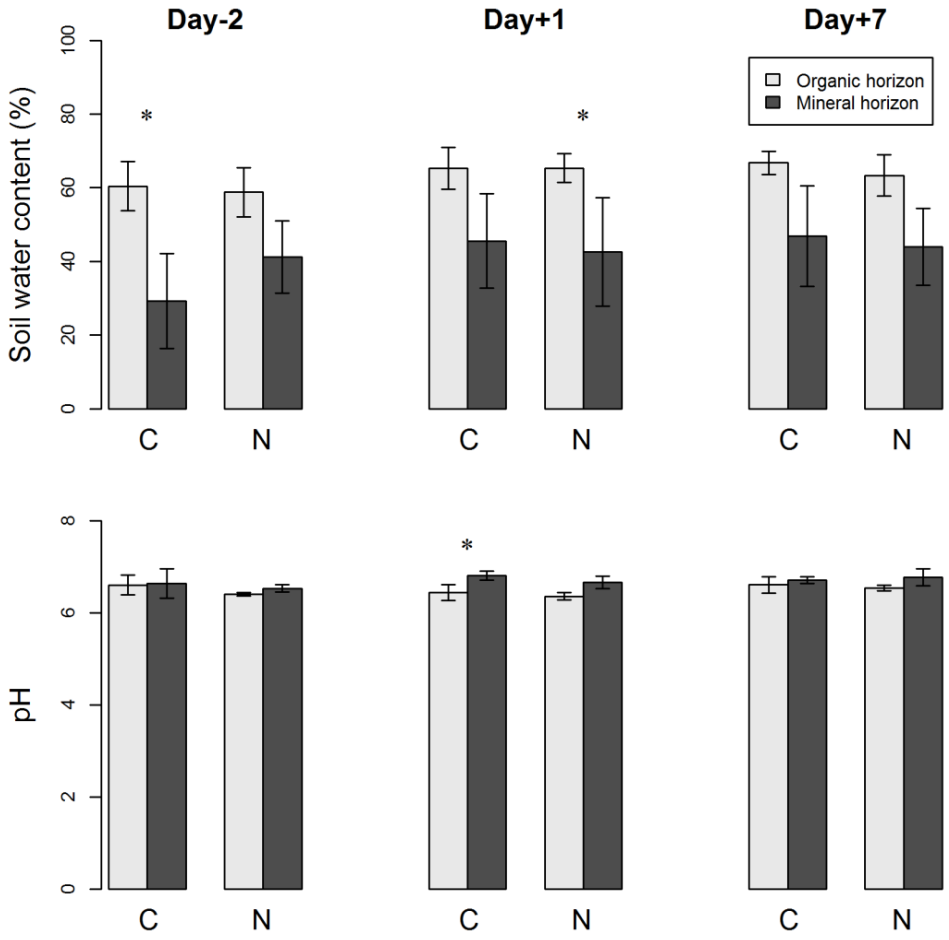

Fig. S1 Variation in soil water content (%) and soil pH between the organic and mineral horizons (as indicated), over time (Day-2, Day+1 and Day+7) and between N treatment (**N**: 4 kg N ha^{-1} yr^{-1} ~pH 4) and control (**C**: water ~pH 6) plots. Day-2: before N application; Day+1: one day after N application; Day+7: seven days after N application. Means values ± standard deviation (n = 5, except at Day+1 for the organic horizon of the N treatment for which n = 4). * indicates significant (P < 0.05) difference between the organic and mineral horizons, for a specific date of sampling and treatments.

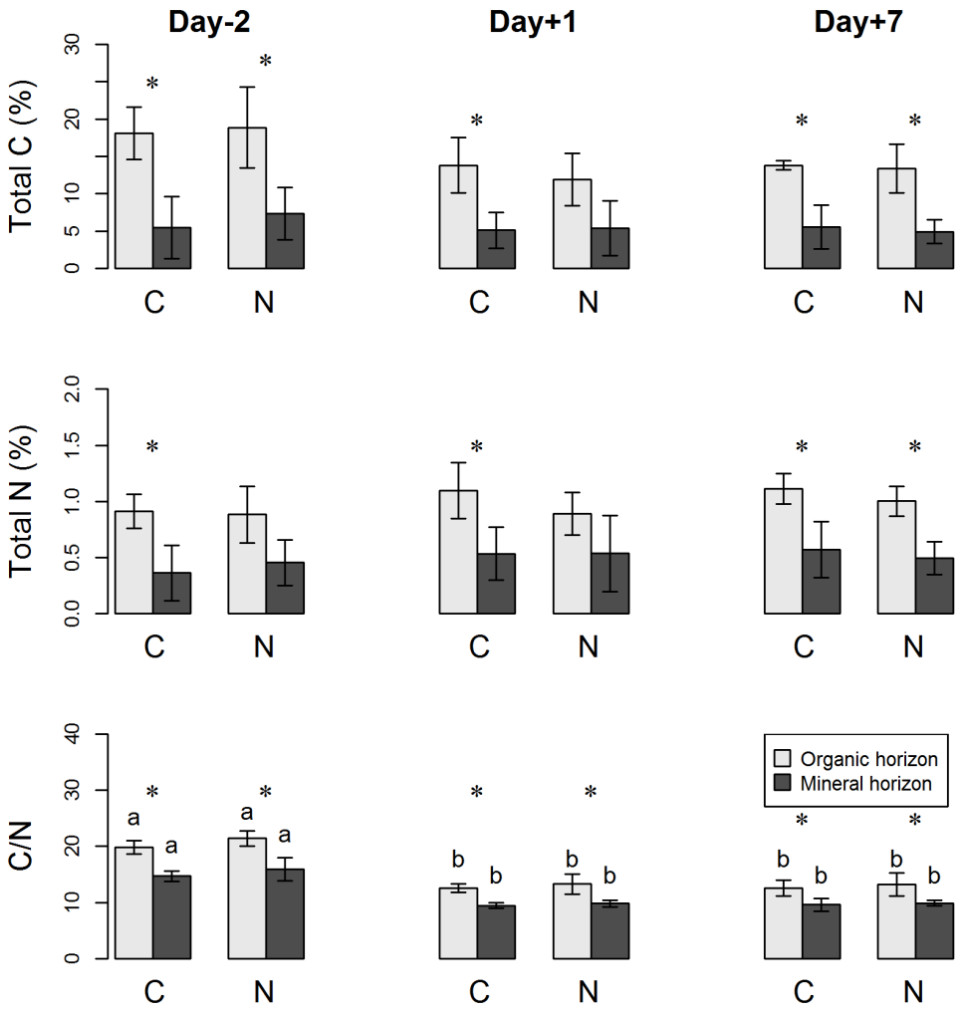

Fig. S2 Variation in C and N content (%) and C/N ratio between the organic and mineral horizons (as indicated), over time (Day-2, Day+1 and Day+7) and between N treatment (**N**: 4 kg N ha^{-1} yr^{-1} ~pH 4) and control (**C**: water ~pH 6) plots. Day-2: before N application; Day+1: one day after N application; Day+7: seven days after N application. Means values ± standard deviation (n = 5, except at Day+1 for the organic horizon of the N treatment for which n = 4). * indicates significant (P < 0.05) difference between the organic and mineral horizons, for a specific date of sampling and treatments. Lower case letters (a, b) indicate significant (P < 0.05) difference between dates of sampling for a specific soil horizon and treatment.

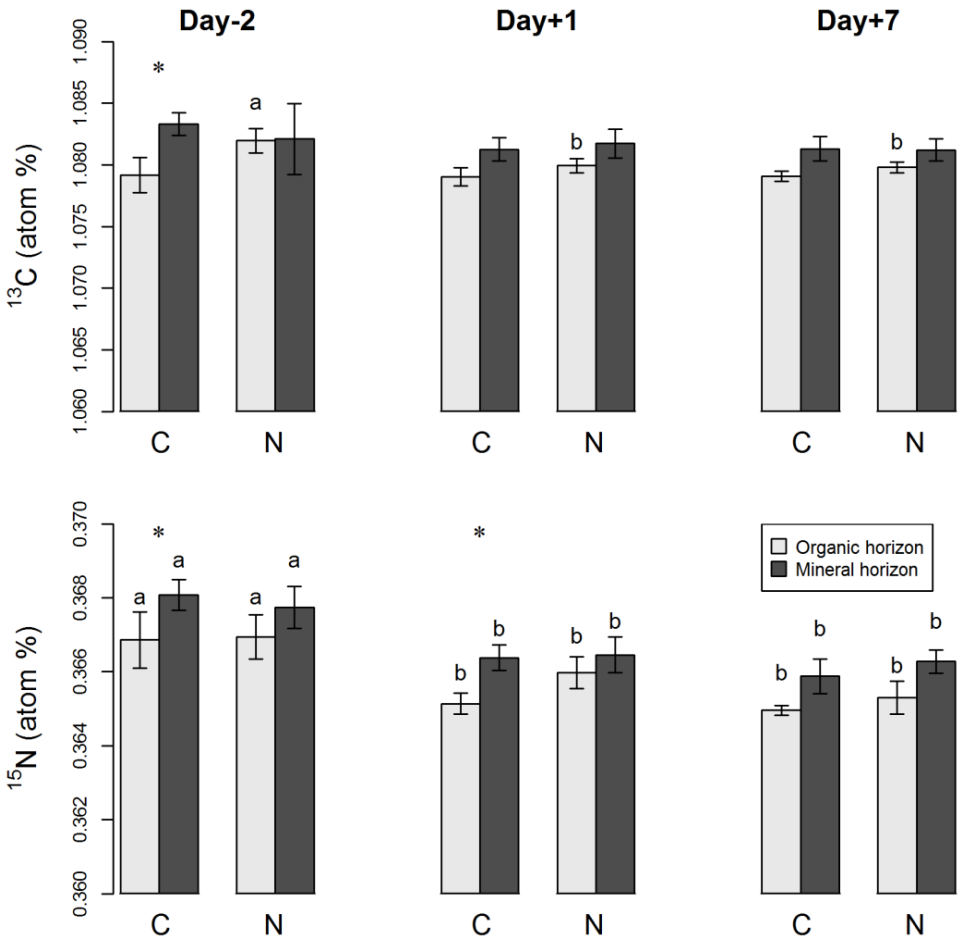

Fig. S3 Variation in ^{13}C and ^{15}N content (atom %) between the organic and mineral horizons (as indicated), over time (Day-2, Day+1 and Day+7) and between N treatment (**N**: 4 kg N ha^{-1} yr^{-1} ~pH 4) and control (**C**: water ~pH 6) plots. Day-2: before N application; Day+1: one day after N application; Day+7: seven days after N application. Means values ± standard deviation (n = 5, except at Day+1 for the organic horizon of the N treatment for which n = 4). * indicates significant (P < 0.05) difference between the organic and mineral horizons, for a specific date of sampling and treatments. Lower case letters (a, b) indicate significant (P < 0.05) difference between dates of sampling for a specific soil horizon and treatment.

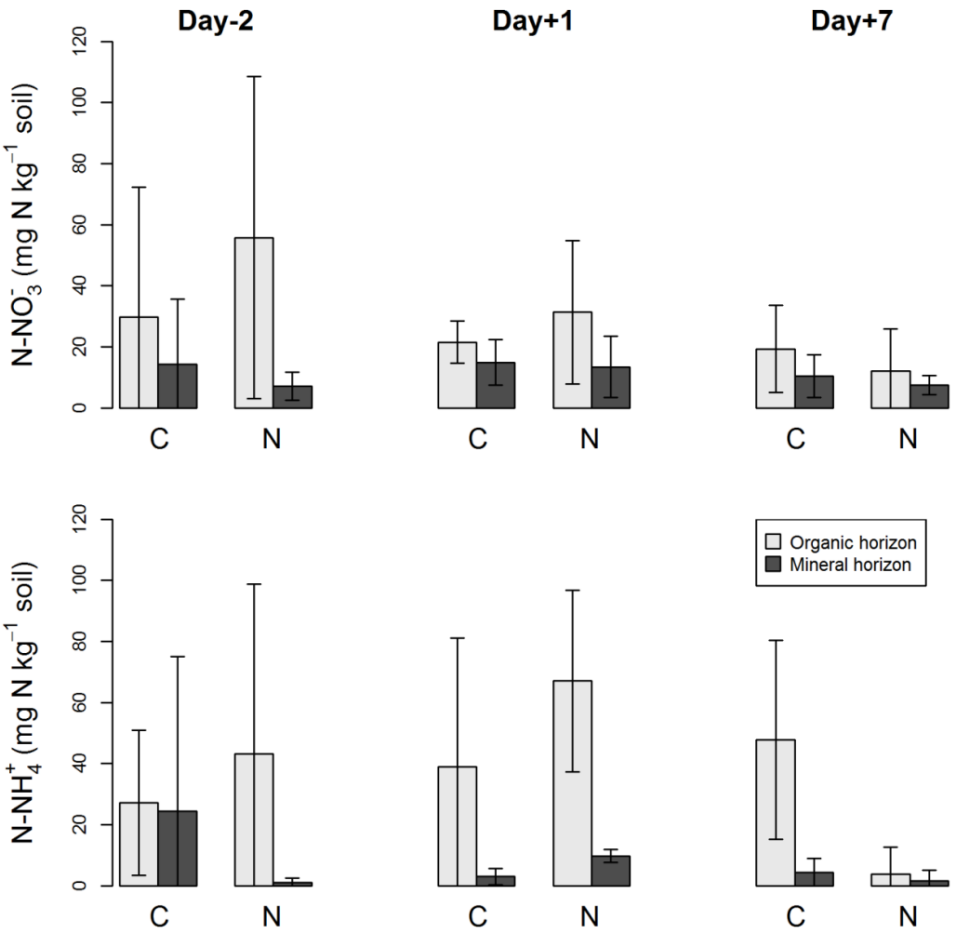

Fig. S4 Variation in soil N-NO_3^- and N-NH_4^+ content (mg N kg^{-1} soil) between the organic and mineral horizons (as indicated), over time (Day-2, Day+1 and Day+7) and between N treatment (**N**: 4 kg N ha^{-1} yr^{-1} ~pH 4) and control (**C**: water ~pH 6) plots. Day-2: before N application; Day+1: one day after N application; Day+7: seven days after N application. Means values ± standard deviation (n = 5, except at Day-2 in the organic and mineral horizon of the N treatment and at Day+1 for the organic horizon of the N treatment for which n = 4).

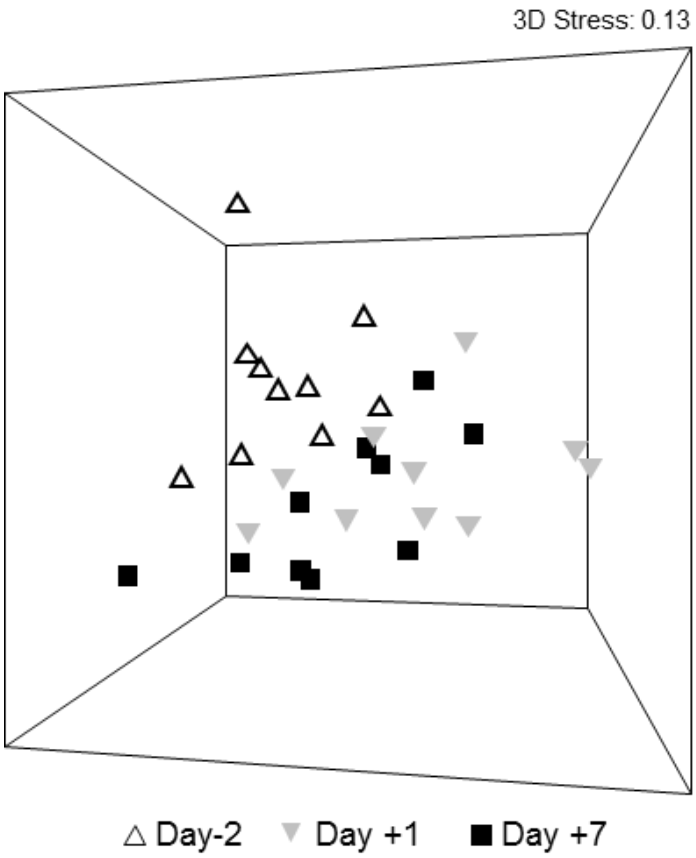

Fig. S5 3D non-metric multidimensional scaling (nMDS) plot showing variation in bacterial community structure during late summer 2009 between dates of sampling (Day-2, Day+1 and Day+7) within the organic horizon of the N treatment (4 kg N ha^{-1} yr^{-1} ~pH 4) and control (water ~pH 6) plots. nMDS plots was generated from Bray-Curtis similarity matrix derived from T-RFLP relative abundance data of bacterial 16S rRNA genes. Symbols and 3D stresses are as indicated.

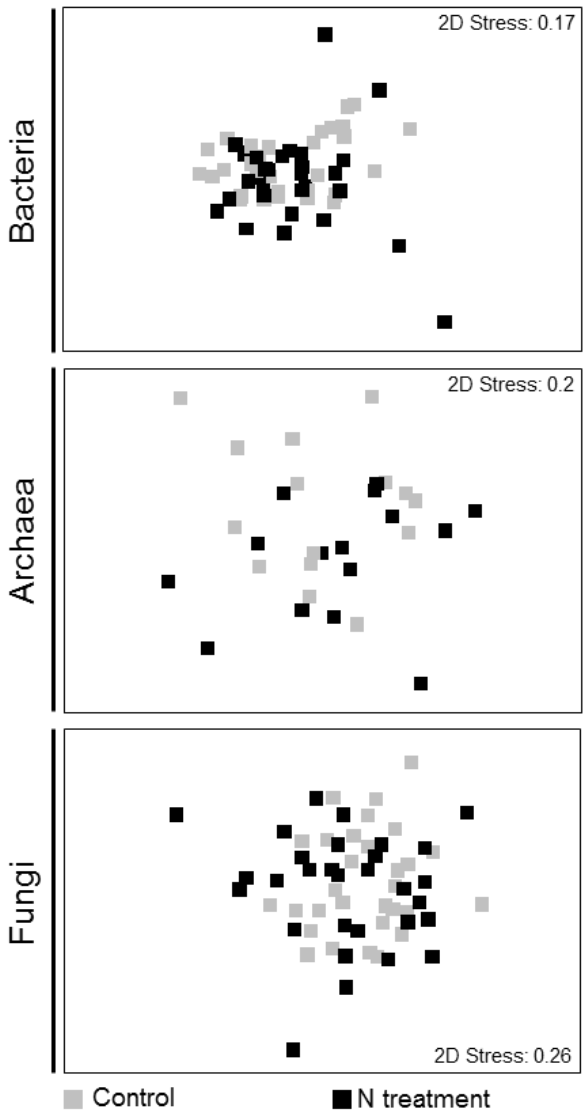

Fig. S6 non-metric multidimensional scaling (nMDS) plots showing variation in bacterial, archaeal and fungal community structure between the N treatment (4 kg N ha^{-1} yr^{-1} ~pH 4) and control (water ~pH 6) plots of the organic and mineral horizons during late summer 2009 (for all dates of sampling). nMDS plots were generated from Bray-Curtis similarity matrices derived from T-RFLP relative abundance data (bacterial and archaeal 16S rRNA genes) and ARISA relative abundance data (fungi ITS). nMDS of Archaea shows data from mineral horizon only (see text for details). Symbols and 2D stresses are as indicated.