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Role of microbial communities in conferring resistance and resilience of soil carbon and nitrogen cycling following contrasting stresses

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Abstract

Soils frequently experience environmental stresses that may have transient or persistent impact on important ecosystem services, such as carbon (C) and nitrogen (N) cycling. Microbial communities underpin resistance (the ability to withstand a stress) and resilience (the ability to recover from a stress) of these functions. Whilst functional stability and resilience have been studied extensively, the link to genetic stability is missing. In this study, the resistance and resilience of C mineralization, ammonia oxidation and denitrification, their associated gene abundances (16S rRNA, bacterial *amoA*, *nirK*, *nirS*, *nosZ-I* and *nosZ-II*) and bacterial community structures (T-RFLP 16S rRNA) were compared in two managed soils for 28 days after stressing the soils with either a persistent (1 mg Cu soil g^{-1}) or a transient (heat at 40 °C for 16 h) stress. The average resistance of C mineralization to Cu was 60%, which was significantly greater than the resistance of ammonia oxidation (25%) and denitrification (31%) to Cu. Similarly, the average resilience of C mineralization to Cu was 52%, which was significantly greater than the resilience of ammonia oxidation (12%) and denitrification (18%) to Cu. However, this pattern was not significant after heat stress, indicating the critical role of different stressors. Changes in total bacterial community structure rather than abundance of 16S rRNA reflected the responses of C mineralization to Cu and heat. Both Cu and heat significantly decreased functional gene abundance (*amoA*, *nirK*, *nirS*, *nosZ-I* and *nosZ-II*), however, significant recovery of denitrifying gene abundance was observed after 28 days following heat. There was lack of constant relationships between functional and genetic stability, highlighting that soil physiochemical properties, the nature of the stressor, and microbial life history traits combine to confer functional resistance and resilience. Genetic responses on their own are therefore inadequate in predicating changes to soil functions following stresses.

Keywords: microbial community, mineralization, denitrification, ammonia oxidation, stresses, sustainability

1. Introduction

Soil ecosystems are undergoing unprecedented disturbances from anthropogenic activities and climate extremes. A key challenge is how to maintain soil sustainability under a situation where severity and frequency of anthropogenic stresses are increasing [1]. It is not just the ability of an ecosystem to withstand these stresses (resistance), but their ability to recovery (resilience) over time that is important to understanding soil ecosystem sustainability, as appreciated in a wide body of research [2,3]. Stresses are often classified as transient or persistent depending on their duration [4].

Transient stresses are usually abrupt and intense and are associated with climate extremes, such as droughts and heat waves [1]. Although transient stresses are short-term, their effects to soil ecosystem could be detrimental. Heat stress is a typical transient stress because it gives soils a short shock in temperature with minimal impact to other soil physicochemical properties [5]. For example, a heat stress (50 °C) could diminish soil microbial biomass by 60% and beta-glucosidase activity by 50% [6]. By contrast, the effects of persistent stresses (e.g. heavy metal contamination) are long-lasting and continuous [4]. With the excessive uses of Cu-based pesticides and Cu-contaminated manure, agricultural soils are continuously exposed to the accumulation of Cu toxicity [7]. The long-term use of Cu-based pesticides has been reported to cause adverse effects on soil enzyme activities and organisms [7]. Microorganisms in forest soils which are adjacent to a metal smelter are also facing deleterious impact from increased metal pollution [8].

Stresses on soils affect the extraordinary diversity of microbial communities and their capacity to drive carbon (C) and nitrogen (N) fluxes between the atmosphere and terrestrial ecosystems, and in regulating soil nutrient availability [1,9]. Many aerobic heterotrophic microbial communities with large species diversity are responsible for the largest flows of C in soil, including C mineralization and storage [10]. In contrast, denitrification and nitrification which are the important parts of N cycling that usually involve specific N-transforming microorganisms. Ammonia oxidation where ammonia is oxidized to hydroxylamine using ammonia monooxygenase (the rate limiting step of nitrification) is carried out by aerobic ammonia-oxidizing bacteria (AOB) and archaea (AOA) [11]. AOA belong to the class *Nitrososphaeria* within the phylum *Thaumarchaeota* [11]. AOB are limited to a small number of genera *Nitrosomonas*, *Nitrosospira* (*Betaproteobacteria*) and *Nitrosococcus* (*Gammaproteobacterial*) [12]. Although AOA and AOB could coexist and contribute to ammonia oxidation in soil, AOB have been found to play a crucial role driving ammonia oxidation in managed grassland and arable soils [13,14]. In contrast, complete denitrification where nitrate is reduced to dinitrogen (N_2) involves a series of enzymes reactions and diverse microbial communities which are phylogenetically more heterogeneous than ammonia oxidizers [15]. Denitrifying bacteria have been identified in over 50 genera with over 125 different species and are estimated to represent 10-15% of bacteria populations in water, soil and sediment [16]. Among denitrifying bacteria, *nirK*- and *nirS*- harbouring bacteria produce nitrite reductase to reduce nitrite (NO_2^-) to nitric oxide (NO), and *nosZ*- containing bacteria generate nitrous oxide reductase to catalyse nitrous oxide (N_2O) reduction to dinitrogen [12]. Therefore, *nirK*, *nirS* and *nosZ* are important genes to determine the production of N_2O and N_2 during denitrification.

According to the insurance hypothesis, high diversity insures against declines in ecosystem function because many species provide a greater guarantee that some will maintain their function if others are killed or inhibited by stresses [17]. Thus species richness or diversity is typically considered a key attribute associated with resistance and resilience [17]. Several studies have aimed to capture the relationship between functional resilience and biodiversity. Griffiths et al. (2000) [18] found that loss of biodiversity impaired the resistance of decomposition to a spike in Cu. In contrast, a more recent study focused on the stability of both general (e.g. substrate-induced respiration) and specific (e.g. nitrification and denitrification) functions to heavy metal contamination and herbicides, and found bacterial diversity had no significant effect on the stability of these functions [19]. A weak association between microbial functions and microbial diversity was also reported in soils following transient stresses. Selmants et al. (2016) [20] did not found a significant relationship between bacterial richness and the response of microbial respiration to warming in a forest soil. Likewise, the resilience of multifunctionality in a forest soil to drought was found to be not related to the bacterial diversity [21]. These contrasting findings suggested that soil functional resistance/resilience may not only be related to total microbial diversity, but also to specific microbial communities pertaining to the function.

Griffiths and Philippot (2013) [3] reviewed 51 studies on microbial resistance and resilience in soil systems, of which most focused on C processes (e.g. C mineralization/ respiration), and only 3 studied both C and N processes (e.g. denitrification and nitrification). Fewer of these studies investigated the soil microbial communities, and even fewer explored the specific communities underlying measured functions which could be sensitive indicators of resilience [21]. Although the research interest in soil sustainability has boomed, there is a lack of evidence of the linkage of functions and underpinning microbial communities involving both C and N processes to stresses, particularly in relation to transient stresses typical of climatic variability and persistent stresses typical of pollution. Our objectives were (1) to compare the responses of selected C and N processes to experimental heat or Cu stresses, (2) to determine the response of bacterial communities to these experimental stresses, and (3) to identify any relationships between microbial communities and the corresponding functional resilience.

To do this we examined two soils of similar texture and climate, but under different management practices that altered C composition. Changes in C mineralization, ammonia oxidation, denitrification, functional gene abundance relating to these processes and bacterial community structure were measured immediately after heat- and Cu- induced stresses and during subsequent recovery over 28 days. Sampling encompassed fast alterations to microbial community structure that can occur in 1 day [22], up to 28 days of incubation where a range of studies have demonstrated temporal dynamics to heat stress decrease [5,6,23]. Microbial communities and functions were speculated to recover from heat due to the short-term exposing time, but not recover from Cu because of its long-lasting effects. C mineralization was hypothesized to be more resistant and resilient than denitrification and ammonia oxidation to the same stress, reflecting different degrees of heterogeneous phylogeny and functional redundancy. We hypothesized that changes in C and N processes were positively correlated to the changes in underlying bacterial abundances. In particular, the positive links between ammonia oxidation and bacterial *amoA* were expected to be the strongest in these managed soils.

2. Materials and Methods

2.1 Soil samples

Two soils were collected in Aberdeenshire in north-east Scotland. The climate was temperate oceanic with the average temperature ranging from 9 to 16 °C during June to August, and the average temperature ranging from 0 to 6 °C during December to February. The annual average rainfall was 753 mm with 64 mm during June to August and 62 mm during December to February. The soils were sampled according to two land uses: a managed grassland soil at Craibstone (57° 11' 19'' N, 2° 12' 43'' W) and an arable soil at Insch (57° 20' 36'' N, 2° 36' 20'' W) in January 2015 when the soils had received no chemical treatment in the month preceding soil sampling. At each sample site, three surface soil samples (0–20cm in depth) approximately 10 kg in weight were randomly collected from different locations at the same site and were mixed thoroughly to form a representative sample of that soil. The litter layer, visible fauna, and vegetation were removed before the soils were passed through a 4 mm metal sieve at field water content and stored at 4 °C.

For each soil, four replicates were measured for soil characteristics before subjecting to stresses. Soil physicochemical properties were measured following standard methods [24]. Soil microbial biomass carbon (MBC) was analysed by chloroform fumigation. Soil mineral N (NO_3^--N and NH_4^+-N) was extracted by shaking with 2 M KCl for 1 hour and analysed using a continuous flow analyser (Skalar San++ 4800, Netherlands). Dissolved organic carbon (DOC) was analysed using a TOC analyser (Dohrmann DC-80, UK). Total N and organic C were analysed using a Thermo Scientific Flash 2000 elemental analyser. Soil pH was determined in 0.01M $CaCl_2$ at a ratio of 1:2 (soil: solution). Soil characteristics and their WRB (World Reference Base) classification are presented in Table 1.

2.2 Resistance and resilience assay

Soil moisture of sieved soils at field water content was adjusted to 60% water-filled pore space (WFPS) and incubated for 7 days at 20 °C prior to analysis. This allowed for mineralization of nutrients released during sieving and the equilibration of soil moisture. Our selection of stresses follows standard approaches used in a large body of previous research exploring soil resilience [5,23,25]. For each soil, aliquots were exposed to either a heat or Cu stress or were unstressed as a control, with four replicates for each soil and stress. Each aliquot contained 250 g dry-weight equivalent of soil loosely packed to a bulk density of 1.1 g cm^{-3} in a 500 ml pot (89 mm diameter, 80.4 mm height). The Cu- perturbed soils were amended with 2.5 ml of 1.57 M $CuSO_4.5H_2O$ to obtain a concentration of 1 mg Cu soil g^{-1}. Cu concentration above 0.17 mg g^{-1} could result a significant loss of bacterial biodiversity [26], thus we expected 1 mg Cu g^{-1} could be a strong stress for these soils. Both heat- and un-stressed soil had 2.5 ml of sterile distilled water added. Because at the sampling period the average soil temperature ranges from 0 to 6 °C, a temperature of 40 °C could induce a large transient stress. This temperature was documented to significantly impair key functions in temperature soils [5]. The heat- stressed soils were incubated at 40 °C for 16 h, in a sealed pot to prevent any water loss, while both Cu- and un- stressed soil were incubated at 20 °C for 16 h. After 16 h, all pots were then incubated at 20 °C for the remainder of the resilience assay.

Soil C mineralization, potential ammonia oxidation, and potential denitrification were measured at intervals of 1, 3, 7, 14, and 28 days after the stress was imposed. At each sampling time, each aliquot of soil was gently mixed, and subsamples were taken for analysis (described below). Subsamples were also collected and stored at -80 °C for later analysis of functional gene abundance and microbial community structure (described below).

2.3 Soil C mineralization

Soil C mineralization was measured as the short-term decomposition of organic C compounds. A stock solution to mimic root exudate containing 86.8 mM fructose, 86.8 mM glucose, 43.4 mM sucrose, 130.2 mM succinic acid, 130.2 mM malic acid, 86.8 mM arginine, 173.6 mM serine and 173.6 mM cysteine was prepared and filter- sterilised [27]. Two g soil was mixed with 120 µl of this solution to give a concentration of 3 mg C soil g^{-1} and stored in an air-tight gas vial (22 ml) over 24 hours at 20 °C, prior to measurement of CO_2-C concentration on a gas chromatograph (Agilent 7890A, UK) with a thermal conductivity detector (Agilent Technologies, UK).

2.4 Potential ammonia oxidation

Potential ammonia oxidation (referred to here as ammonia oxidation) was determined by the chlorate inhibition method [28]. Briefly, 50 ml solution (0.5 mM $(NH_4)_2SO_4$ + 10 mM $NaClO_3$, pH 7.2) was added to 10 g soil. The samples were then shaken on a rotary shaker (180 rpm) at 20 °C for 24 h, with the resulting slurry being centrifuged at 10,000 g min^{-1} for 10 min. Nitrite-N concentration was determined in 0.4 ml supernatant amended with 0.1 ml sulphanilamide, 0.1 ml naphthylethylene diamide and 2.4 ml H_2O at λ=543 nm on a spectrophotometer (Agilent 8453 UV-visible Spectroscopy System, UK).

2.5 Potential denitrification

Potential denitrification (referred to here as denitrification) was determined following anaerobic incubation of soil samples in the presence of 10% (v/v) acetylene (C_2H_2) [29]. A 20 ml solution of 25 mM glucose + 3.57 mM KNO_3 was mixed with 20 g soil in a 200 ml flask, followed by flushing with helium at 600 ml min^{-1} for 2 min. A 20 ml headspace sample was then removed from each flask by syringe and replaced with 20 ml C_2H_2 to give a headspace concentration of 10% (v/v) C_2H_2. The flasks were shaken on a rotary shaker (150 rpm) at 20 °C for 5 h. Gas samples (22 ml) for analysis of N_2O-N concentration were collected at 5 h, stored in pre-evacuated 22 ml vials and analysed on a gas chromatograph (Agilent 7890A, UK) equipped with an electron capture detector (Agilent Technologies, UK).

2.6 Soil DNA extraction

DNA of the soil stored at -80 °C was extracted by a phenol-chloroform method [30]. Briefly, 1 g of soil was suspended in 2 ml of solution (0.12 M Na_2HPO_4 + 1% SDS) which contained 1×10^6 copies of a mutated reference gene to determine extraction efficiency [31]. One ml of the resulting slurry was further bead-beaten for 4.5 mins at 30 Hz (TissueLyser, Qiagen, Hilden, Germany). The slurry was then centrifuged at 5,000 rpm for 10 mins, and the aqueous phase was transferred to new 96-well microplates (Qiagen, Hilden, Germany), where the soil DNA was purified with phenol/ chloroform/ isoamyl alcohol, and precipitated with isopropanol/ sodium acetate, and treated with polyvinylpolypyrrolidone. The extracted DNA was stored at -80 °C for later analysis.

2.7 PCR and T-RFLP of 16S rRNA

Bacterial DNA was amplified from 1:10 diluted DNA extracts. The primers used were 16F27 with labelled 8'-FAM (AGA GTT

TGA TCC TGG CTC AG) and 1392R (ACG GGC GRT GTG TAC A) [32]. Fourteen µl 'master mix' which contained 0.3 U DNA polymerase (Platinum Taq DNA Polymerase High Fidelity, Invitrogen, UK), 6 pM each primer, 1.5 µl of 10X High Fidelity Buffer (Invitrogen), 1 U *Hha* I (Promega, UK), 3.75 mM dNTPs, 3 mM $MgSO_4$ and 10 µg bovine serum albumin (BSA) was digested at 37 °C for 40 min. One µl template was combined with 'master mix' to form 15 µl final reaction mix. PCR was performed with a hot start step of 94 °C for 4.5 mins, followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 68 °C for 90 s; cycling was completed by a final extension period of 68 °C for 10 mins. PCR products showed equal intensity after visualization by agarose gel electrophoresis (1.5%) with 1X Tris/Borate/EDTA as the buffer.

Three µl fluorescent PCR products were digested with 0.5 µl *Alu* I restriction enzyme (Promega, UK) at 37 °C. One µl one to ten diluted digests were mixed with 0.25 µl 1200 LIZ dye Size Standard (Life Technologies, UK) and 8.75 µl formamide (Life Technologies, UK). Samples were analysed by an ABI 3730 automated sequencer (Thermo Fisher Scientific, UK). Peaks of resulting electropherograms were analysed by GeneMapper

(Applied Biosystems, CA, USA) and processed following Deng et al. (2010) [30].

2.8 qPCR for quantification of functional genes and total bacteria

For relative qPCR, 16S rRNA, *amoA*, *nirK, nirS*, *nosZ-I* and *nosZ-II* gene copy numbers were quantified including amplification of the added reference target as described in Daniell et al. (2012) [31]. Amplification was performed on a LightCycler 480 Sybr Green qPCR (Roche, UK). qPCR for functional gene (bacterial *amoA*, *nirK*, *nirS*, *nosZ-I*), total bacterial (16S rRNA) and the reference fragment abundance were performed in 20 µl reaction mixtures containing 10 µl SYBR Green 1 Master Mix (Applied Biosystems, UK); 0.5 µl of BSA, 1 µl of 10 pM of each primer, 2 µl template DNA (10-fold dilutions of soil nucleic acid extracts) and DNase-free water. In the reaction mixtures of the *nosZ-II*, each primer was increased to 2 µl with the proportional decrease of water. Standard curves were obtained using serial dilutions of linearized plasmids containing cloned *amoA*, *nirK*, *nirS*, *nosZ-I*, *nosZ-II*, 16S rRNA genes and the reference fragment. No-template controls gave null or negligible values. The primers and thermal cycling conditions for amplification of target genes are described in Table S1.

2.9 Data analysis

All statistical analysis were performed using R 3.5.2 [33] except for analysis of similarity (ANOSIM) which was conducted using Primer 7 (PRIMER-E limited, Auckland, New Zealand).

Stability (%) was calculated as the reduction in biological functions of the stressed soil compared with the unstressed control at day t [23]:

$$f(t) = \frac{Stressed\ indicator\ (t)}{Unstressed\ indicator\ (t)} \times 100$$

Resistance was defined as stability measured at day 1 after perturbation, while resilience was estimated as the integrative stability after day 1 up to 28 days following stress [27].

$$Resilience = \int_{1}^{28} f(t)dt/(28 - 1)$$

The mean and standard deviation of resistance and resilience was estimated using all possible combinations of stressed and unstressed soils.

One-way analysis of variance (ANOVA) followed by a post hoc Tukey’s test was used to detect the differences of physicochemical properties between soils. Two-way ANOVA was performed to determine the significance of stress, time and their interaction on functions, the copy number of genes as well as the functional and genetic stability in each soil. Pearson correlations of functional and genetic stability were performed to detect relationships between functions and the underlying bacteria communities.

For each soil, 16S rRNA T-RFLP data was normalized by Hellinger transformation and Bray-Curtis dissimilarity matrices were constructed under the “vegan” package [34]. Ordinations were produced using non-metric multidimensional scaling (NMDS) and significant effects of stress, time and their interactions on the bacterial community structure were tested using a two-way PERMANOVA. A one-way ANOSIM on Bray-Curtis resemblance matrix in each soil was performed to determine pair-wise comparison between different treatments. A separate NMDS on Bray-Curtis distance of bacterial communities in the unstressed soils was performed to distinguish bacterial community structure influenced by soil properties.

3. Results

3.1 Resistance and resilience of soil functions to Cu and heat stress

Over the 28 days incubation the unstressed Craibstone soil exhibited 7.0 µg C g^{-1} h^{-1} C mineralization, 14.8 µg N g^{-1} d^{-1} ammonia oxidation and 356.9 ng N g^{-1} h^{-1} denitrification on average (Table S3). These were significantly ($P < 0.001$) decreased by Cu as well as heat (Table S2). The unstressed Insch soil exhibited 13.0 µg C g^{-1} h^{-1} C mineralization, 13.0 µg N g^{-1} d^{-1} ammonia oxidation and 280.8 ng N g^{-1} h^{-1} denitrification on average, which were also significantly ($P < 0.001$) decreased by Cu and heat (Table S2).

For both soils, functional stabilities in response to Cu significantly ($P < 0.001$) varied by the measured function (Table 2). C mineralization in the Insch soil was significantly ($P < 0.05$) more resistant and resilient to Cu compared to denitrification and ammonia oxidation (Figure 1). C mineralization in the Craibstone soil was also significantly ($P < 0.05$) more resilient to Cu in comparison to denitrification and ammonia oxidation in this soil (Figure 1).

The stability to heat varied significantly ($P < 0.001$) between functions in the Craibstone soil (Table 2), where C mineralization was significantly ($P < 0.05$) more resistant and resilient compared to ammonia oxidation, and was significantly ($P < 0.05$) more resilient than denitrification (Figure 1). In the Insch soil, the stability to heat did not vary significantly between functions (Table 2), and there were also no differences in resilience between functions (Figure 1).

3.2 Resistance and resilience of bacterial abundances to Cu and heat stress

In both soils, applying stress had a significant ($P < 0.05$) impact on the abundance of genes (Table S2). One day after Cu stress, the gene copy number of 16S rRNA, *amoA*, *nirS*, *nirK*, *nosZ-I* and *nosZ-II* in both soils significantly ($P < 0.05$) decreased (Table S4 and S5). One day following heat stress, significant ($P < 0.05$) decreases in genetic abundance were observed in *amoA*, *nirS*, *nirK*, *nosZ-I* and *nosZ-II* (Table S4 and S6).

The genetic stability to Cu significantly ($P < 0.01$) varied by the measured genes and time (Table 2). 16S rRNA was significantly ($P < 0.05$) more resilient to Cu compared to bacterial *amoA* in both soils (Figure 2). There was no significant difference of the resilience to Cu between denitrifying genes in both soils.

Similarly, the stability of gene abundance to heat significantly ($P < 0.001$) varied with gene identity and time (Table 2). The abundance of 16S rRNA was significantly ($P < 0.05$) more resistant to heat than bacterial *amoA* and all measured denitrifying genes in both soils (Figure 2). 16S rRNA was also significantly ($P < 0.05$) more resilient to heat than *amoA* in both soils. Among denitrifying genes, *nirS* and *nosZ-I* were significantly ($P < 0.05$) more resilient than *nirK* and *nosZ-II* in the Craibstone soil; *nirK* was significantly ($P < 0.05$) more resilient than *nirS* in the Insch soil (Figure 2).

3.3 Changes in bacterial community structure

Non-metric multidimensional scaling (NMDS) demonstrated a clear separation of the bacterial community structure by soil (Figure S1). Applying heat and Cu stress markedly shifted the community structure away from the unstressed control (Figure 3). PERMANOVA results showed significant ($P < 0.001$) effects of stress, time and their interactions on the bacterial community structure in both soils (Table 3). Compared to the unstressed control, Cu did not significantly ($P < 0.05$) shift the bacterial community structure until 14 days after stress in both soils (Table S7 and S8 and Figure 3). The bacterial community structure did not recover from Cu stress in either soil. In both soils, heat significantly ($P < 0.05$) altered the bacterial community structure away from that of the control just one day after the stress. Over time, the difference of the bacterial community structure between heat stressed and unstressed was smaller though still significantly ($P < 0.05$) different (Figure 3 and Table S7 and S8).

3.4 Relationships between functional and genetic stability to Cu and heat

The relationships between functional and genetic stability varied between soils, stresses and time (Table 4). There was no significant correlation between the stability of C mineralization and 16S rRNA across 5 time points in the Craibstone soil under either Cu or heat stress. However, a significant relationship was detected in the Insch soils after 1, 7 and 28 days following Cu, and after 1 and 14 days following heat stress.

When soils were under Cu stress, the stability of ammonia oxidation was significantly positively correlated with *amoA* at day 7 in the Craibstone soil (r =0.615, $P < 0.05$), whereas no significant relationship was found in the Insch soil over 28 days. There was a significant positive correlation between the stability of ammonia oxidation and *amoA* (r =0.729 $P < 0.01$) in the Craibstone soil after 3 days following heat stress. However, a significant ($P < 0.05$) negative correlation between the stability of ammonia oxidation and *amoA* was found in the Insch soil after 14- and 28-days following heat.

The stability of denitrification was not significantly correlated with *nirS* in the Craibstone soil regardless of stresses, but significantly positively correlated with *nirS* in the Insch soil after 14 days following Cu (r =0.631, $P < 0.01$). Significant ($P < 0.05$) positive correlations between the stability of denitrification and the stability of *nirK*, *nosZ-I* and *nosZ-II* were also found in the Craibstone soil after 7 days following Cu. In the Insch soil, denitrification was significantly ($P < 0.05$) positively correlated with *nirK* and *nosZ-I* after 1 day following Cu. After one day following heat stress, the stability of denitrification was significantly positively correlated with *nosZ-II* in the Craibstone soil (r =0.710, $P < 0.001$), and *nirK* in the Insch soil (r =0.578, $P < 0.05$).

4. Discussion

4.1 The resistance and resilience of soil functions to Cu and heat

We verified our hypothesis that C mineralization will be more stable than denitrification and ammonia oxidation under Cu stress (Figure 1). However, this pattern became less significant after heat stress was imposed on soil. The results challenge previous conclusions that processes carried out by the general microbial community (e.g. C mineralization) are more stable than those carried out by narrower and specialised microbial populations (e.g. ammonia oxidation) [18,25]. Differences of functional resistance and resilience varied with the nature of the imposed stress. Heat, a transient stress is relatively discrete and intense having the potential to shift resource supplies and alter biomass, whereas Cu as a persistent stress is continuous and likely to increase in intensity through time [2]. It is likely that the soil microbial communities respond differently to these contrasting stresses (discussed below).

4.2 Resistance and resilience of microbial communities to Cu and heat stress

Compared to the unstressed control, the bacterial community structure shifted significantly ($P < 0.05$) just one day after heat, although the copy number of 16S rRNA remained relatively stable (Figure 2 and 3). Similarly, distinct responses of genetic structure and abundance of microbial communities to warming (5 °C above ambient) have been observed previously [35]. Microorganisms have evolved appropriate life-strategies to survive in stressful situations. For example, *Actinobacteria* increase their synthesis of ribosomes when stressed by desiccation, and then grow quickly as soon as conditions become more favourable for nutrient acquisition [36]. In our two soils, heat tolerant bacteria, such as *Actinobacteria* may have employed this “preparedness strategy” to accelerate their cell division and increase their biomass [36]. The competition between tolerant and sensitive species following a stress would allow the remaining tolerant species to be detected in higher densities in the absence of their competitors [37], resulting in an altered community structure. After 28 days, the difference between heat- and un- stressed soil was smaller (Table S7 and S8) which is in accordance with heat being an abrupt, intense but short-term stress. Removing heat would have given time for the microbial communities to resuscitate and reconstruct to another stable state.

In contrast, the bacteria community structure remained unchanged until 14 days after Cu addition in both soils (Figure 3), complying with the nature of Cu as a gradual but persistent stress [2]. Bacterial communities can adapt to Cu stress by gathering the required resistance genes through horizontal gene transfer, mainly mediated by plasmids or transposons [38]. However, the transfer of genetic information and expression is energy-consuming [38]. Lack of C and energy supplies possibly caused restricted recovery of bacterial from Cu in these two soils (Figure 3 and Table S7 and S8). The response of the bacterial community structure showed a strong time-dependence (Figure 3 and Table S7 and S8) that could have resulted from Cu diffusion through soil pores and aggregates and microbial evolutionary adaptation allowing Cu tolerant bacteria to prevail in the continuous stressful condition [39]. Considering this incubation experiment lasted only 28 days it may not have been possible to detect the recovery of bacterial communities following the decrease of Cu bioavailability which could be resulted from the formation of complexes with inorganic or organic ligands or association with mineral colloids [40].

Denitrifying genes were not completely resistant to heat but a significant recovery in gene copy number was observed after 28 days following heat (Figure 2), in agreement with a previous study demonstrating a low resistance but high resilience of denitrifying genes to drought [41]. A significant recovery following heat may be a result of a resuscitation of dormant denitrifiers supported by the flush of available C and N after the heat-induced breakdown of links between soil aggregates and organic-mineral components [42]. The recovery of *nosZ*-harbouring denitrifiers (Figure 2) in particular could help the completion of denitrification where N_2 rather than N_2O would be the end product, which could alleviate the excessive emission of N_2O from denitrification after recurring heat waves [15]. The stability to heat varies with genes (Table 2 and S6). Such a different response of denitrifiers to the changes of environment could be due to their phylogenetic and physiological variation [43]. Cu has an extensive involvement in the denitrification pathway, such as a co-factor in Cu-containing nitrite reductase (*nirK*) and nitrous oxide reductase (*nosZ*) [12]. Therefore, soils that are Cu deficient could result in incomplete denitrification and a rise in N_2O emission [44]. However, in this study the gene copy number of all measured denitrifying genes were significantly decreased by 1 mg Cu g^{-1}, (Table S4), suggesting this concentration is lethal to denitrifying bacteria. Indeed, the Cu concentration in our study largely exceeds previously reported ideal concentration (150–200 μg g^{-1}) to maximize the activity of *nirS*- and *nosZ*- harbouring bacteria [45].

In this study, we mainly focused on AOB because both soils are under management, thus AOB are likely to play a dominant role in ammonia oxidation [13,14]. Moreover, these soils remained slightly acid (pH 4.95 and 5.30 for Craibstone and Insch, respectively) which are within the preferred soil pH range of AOB *Nitrosospira* clusters 2 [46]. We found that AOB were vulnerable to these stresses (Figure 2). High sensitivity of AOB to increasing temperature [41] and Cu contamination [47] were also reported. The bacterial membrane is permeable to ions which increases the contact of AOB to Cu^{2+} through the pore water [47]. Additionally, constructing a Cu-tolerant ammonia oxidizing community requires long-term exposure and highly depends on the native composition [48], thus very little recovery was seen in AOB abundance over the time frame of this study. The detrimental impact on bacterial *amoA* copy number following heat could be a result of the narrow thermodynamic trait in AOB [49].

4.3 Relationships between microbial communities and functions under stress

In contrast to the hypothesis that functional stability was positively correlated to the underlying genetic stability, the relationship between functional and genetic stability was not constant and varied between soils, stresses and time (Table 4). In the Craibstone soil, the stability of C mineralization was not significantly correlated with 16S rRNA under either stress. This is consistent with previous studies that have showed no significant relationship between bacterial diversity and microbial respiration under the stress of a mixture of heavy metals and the herbicide Siduron [19] and no significant relationship was found between CO_2 fluxes and bacterial abundance under a 5.2 °C warmer condition [20]. However, a significant correlation between C mineralization and 16S rRNA under either stress in the Insch soil was observed (Table 4), suggesting that soil native bacterial composition (Figure S1) and physicochemical properties (Table 1) could play an important role in determining this relationship. AOB have been reported to play a dominant role in controlling ammonia oxidation in a N-rich grassland soil and an arable soil [13,14]. However, in our two managed soils ammonia oxidation was not consistently correlated with the stability of bacterial *amoA* (Table 4). Our result aligns with a recent study which found no significant correlation between nitrification and the abundance of any nitrifier group (AOB, AOA and nitrite oxidizers) [49]. The decoupling between the response of ammonia oxidation and bacterial *amoA* to the stresses could also reflect that not all the qPCR-quantified *amoA* are active [50]. We acknowledge that AOA could also contribute to ammonia oxidation in a stressed environment. Therefore, we suggest future studies on the responses of AOA, AOB and their transcriptional activities will help to explain the response of ammonia oxidation to contrasting stress. Denitrification was not correlated with any of these measured denitrifying genes constantly over 28 days, which could be a result of competition within functionally similar taxa for the same niche, such as the competition between *nirS* and *nirK*, and between *nosZ-I* and *nosZ-II* [51].

We offer the following explanations for the lack of constant relationship between functional and genetic stability over time. Firstly, soil native microbial composition (Figure S1) and physicochemical properties (Table 1) could determine the direction of the relationship between functions and genetic abundance as two soils exhibited contrasting relationship between functional and genetic stability (Table 4). Secondly, applying stress had significant effects on shifting microbial communities (Table 3) which varied between transient and persistent stresses (Figure 3). Thus, the nature of the stress could produce an increasingly strong deterministic selection on microbial assemblage towards a stable successional stage [52]. This could explain why microbial communities responded distinctly to Cu and heat (Figure 3). Thirdly, microbial growth and substrate utilization are phylogenetically deeply conserved. Low microbial abundance may not necessarily lead to low substrate utilization because of high overlap in substrate utilization among species [53]. In our study C mineralization, ammonia oxidation and denitrification were determined as microbial potential usages of certain added substrates. Lastly, the strategies for microbes to survive under unfavourable conditions could vary, such as shifting from an active to a dormant state and reversible transition between “non-growing” and “growing” status [54]. These transitions could reverse because intensity and persistence of the imposed stress could vary over time. However, the DNA captured in our study cannot discriminate between metabolically active and inactive bacteria, especially the inactive microbes that could compose a large fraction of the extracted DNA [54]. Testing this hypothesis requires further studies combining both RNA and DNA based microbial fingerprinting technologies to detect the changes of total and active microbial communities [50].

Interestingly, the resistance and resilience of C mineralization to Cu were both higher than to heat, which is in accordance with the response of the bacterial community structure to these stresses (Figure 1 and 3). This indicates that changes in the bacterial community structure may play a more important role than bacterial abundance in conferring the resistance and resilience of C mineralization to Cu and heat. For instance, Naveed et al. (2014) found that *Acidobacteria* were tolerant while *Proteobacteria* were vulnerable to a gradient of Cu contamination [26]. Our two soils are slightly acidic so they may favour the growth of *Acidobacteria*. Thus, the increases in the proportion of *Acidobacteria* to *Proteobacteria* may enhance the resistance and resilience of C mineralization to Cu without altering total bacterial abundance. During microbial succession, the presence of rare species can have a preponderant role in determining functional resistance and resilience [55]. Griffiths et al. (2004) [56] used progressive and irradiated-reinoculated methods to manipulate the microbial community, finding that the stability of decomposition of plant residues to Cu and heat was not consistent between these two methods of reducing microbial diversity. This suggests that future studies applying DNA-sequencing techniques could explain to which extent rare taxa play in conferring functional stability under environmental change.

Conclusion

Relatively higher resistance of C mineralization to Cu than to heat was related to the bacterial community structure rather than the copy number of 16S rRNA, implying rare taxa may play a paramount role in developing stability in the microbial community and securing functional resistance and resilience. The functional genes we targeted here (i.e., *amoA*, *nirK*, *nirS*, *nosZ-I* and *nosZ-II*) were not resistant to either Cu or heat. However, there was a significant recovery of denitrifying genes' copy number after heat. The degree of recovery from heat may vary from genes encoding nitrite reductase and nitrous oxide reductase which could alter the production ratio of N_2O to N_2, and thus contribute to lowering net emission of N_2O under warmer climate and during heat waves. The relationship between functional and genetic stability was not constant, which could be a result of interaction of soil physicochemical properties, the nature of stressor and microbial life history strategy. C mineralization was found to be significantly more resistant and resilient to Cu than ammonia oxidation and denitrification. However, this difference was not significant when soil was under heat stress. This challenges previous findings that a broad function such as C mineralization is more stable than narrower functions involved in N cycling under various stressors. Thus, our study suggests that functional resilience is tightly stress-dependent which is not merely reflected by the diversity of microbial communities carrying out the functions. Such differences in stress responses of the soil C and N processes and their underlying microbial communities contribute to understanding of soil buffering capacities against environmental challenges.

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References

[1] R.D. Bardgett, T. Caruso, Soil microbial community responses to climate extremes: Resistance, resilience and transitions to alternative states, Philos. Trans. R. Soc. B Biol. Sci. 375 (2020). https://doi.org/10.1098/rstb.2019.0112.

[2] D.G. Nimmo, R. Mac Nally, S.C. Cunningham, A. Haslem, A.F. Bennett, Vive la résistance: Reviving resistance for 21st century conservation, Trends Ecol. Evol. 30 (2015) 516–523. https://doi.org/10.1016/j.tree.2015.07.008.

[3] B.S. Griffiths, L. Philippot, Insights into the resistance and resilience of the soil microbial community., FEMS Microbiol. Rev. 37 (2013) 112–29. https://doi.org/10.1111/j.1574-6976.2012.00343.x.

[4] A. Shade, H. Peter, S.D. Allison, D.L. Baho, M. Berga, H. Bürgmann, D.H. Huber, S. Langenheder, J.T. Lennon, J.B.H. Martiny, K.L. Matulich, T.M. Schmidt, J. Handelsman, Fundamentals of microbial community resistance and resilience, Front. Microbiol. 3 (2012) 1–19. https://doi.org/10.3389/fmicb.2012.00417.

[5] A.S. Gregory, C.W. Watts, B.S. Griffiths, P.D. Hallett, H.L.

Kuan, A.P. Whitmore, The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England, Geoderma. 153 (2009) 172–185. https://doi.org/10.1016/j.geoderma.2009.08.002.

[6] W. Riah-Anglet, I. Trinsoutrot-Gattin, F. Martin-Laurent, E. Laroche-Ajzenberg, M.P. Norini, X. Latour, K. Laval, Soil microbial community structure and function relationships: A heat stress experiment, Appl. Soil Ecol. 86 (2015) 121–130. https://doi.org/10.1016/j.apsoil.2014.10.001.

[7] M. Komárek, E. Čadková, V. Chrastný, F. Bordas, J.C. Bollinger, Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects, Environ. Int. 36 (2010) 138–151. https://doi.org/10.1016/j.envint.2009.10.005.

[8] M. a Tobor-Kapłon, J. Bloem, P.F.A.M. Römkens, P.C. de Ruiter, Functional stability of microbial communities in contaminated soils near a Zinc smelter (Budel, the Netherlands), Ecotoxicology. 15 (2006) 187–197. https://doi.org/10.1007/s10646-005-0050-4.

[9] M.A. de Graaff, J. Adkins, P. Kardol, H.L. Throop, A meta-analysis of soil biodiversity impacts on the carbon cycle, Soil Discuss. 1 (2014) 907–945. https://doi.org/10.5194/soild-1-907-2014.

[10] J.P. Schimel, S.M. Schaeffer, Microbial control over carbon cycling in soil, Front. Microbiol. 3 (2012) 1–11. https://doi.org/10.3389/fmicb.2012.00348.

[11] J.I. Prosser, L. Hink, C. Gubry-Rangin, G.W. Nicol, Nitrous oxide production by ammonia oxidisers: Physiological diversity, niche differentiation and potential mitigation strategies, Glob. Chang. Biol. (2019) 1–16. https://doi.org/10.1111/gcb.14877.

[12] M.M.M. Kuypers, H.K. Marchant, B. Kartal, The microbial nitrogen-cycling network, Nat. Rev. Microbiol. 16 (2018) 263–276. https://doi.org/10.1038/nrmicro.2018.9.

[13] H.J. Di, K.C. Cameron, J.P. Shen, C.S. Winefield, M. O’Callaghan, S. Bowatte, J.Z. He, Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils, Nat. Geosci. 2 (2009) 621–624. https://doi.org/10.1038/ngeo613.

[14] Z. Jia, R. Conrad, Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil, Environ. Microbiol. 11 (2009) 1658–1671. https://doi.org/10.1111/j.1462-2920.2009.01891.x.

[15] S. Saggar, N. Jha, J. Deslippe, N.S. Bolan, J. Luo, D.L. Giltrap, D.G. Kim, M. Zaman, R.W. Tillman, Denitrification and N2O: N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts, Sci. Total Environ. 465 (2013) 173–195. https://doi.org/10.1016/j.scitotenv.2012.11.050.

[16] E.A. Paul, Soil microbiology, ecology, and biochemistry, Fourth Edi, Elsevier, 2015. https://www.elsevier.com/books/soil-microbiology-ecology-and-biochemistry/paul/978-0-12-415955-6.

[17] S. Yachi, M. Loreau, Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1463–1468. https://doi.org/10.1073/pnas.96.4.1463.

[18] B.S. Griffiths, K. Ritz, R.D. Bardgett, R. Cook, S. Christensen, F. Ekelund, S.J. Sorensen, E. Baath, J. Bloem,

P.C. de Ruiter, J. Dolfing, B. Nicolardot, Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship, Oikos. 90 (2000) 279–294. https://doi.org/10.1034/j.1600-0706.2000.900208.x.

[19] R. Jiang, M. Wang, W. Chen, X. Li, M. Balseiro-Romero, Changes in the integrated functional stability of microbial community under chemical stresses and the impacting factors in field soils, Ecol. Indic. 110 (2020) 105919. https://doi.org/10.1016/j.ecolind.2019.105919.

[20] P.C. Selmants, K.L. Adair, C.M. Litton, C.P. Giardina, E. Schwartz, Increases in mean annual temperature do not alter soil bacterial community structure in tropical montane wet forests, Ecosphere. 7 (2016) 1–10. https://doi.org/10.1002/ecs2.1296.

[21] F. Bastida, I.F. Torres, M. Andrés-Abellán, P. Baldrian, R. López-Mondéjar, T. Větrovský, H.H. Richnow, R. Starke, S. Ondoño, C. García, F.R. López-Serrano, N. Jehmlich, Differential sensitivity of total and active soil microbial communities to drought and forest management, Glob.

Chang. Biol. 23 (2017) 4185–4203. https://doi.org/10.1111/gcb.13790.

[22] A. Shade, J. Gregory Caporaso, J. Handelsman, R. Knight, N. Fierer, A meta-analysis of changes in bacterial and archaeal communities with time, ISME J. 7 (2013) 1493–1506. https://doi.org/10.1038/ismej.2013.54.

[23] B. Zhang, H. Deng, H. Wang, R. Yin, P.D. Hallett, B.S. Griffiths, T.J. Daniell, Does microbial habitat or community structure drive the functional stability of microbes to stresses following re-vegetation of a severely degraded soil?, Soil Biol. Biochem. 42 (2010) 850–859. https://doi.org/10.1016/j.soilbio.2010.02.004.

[24] M.R. Carter, E.G. Gregorich, Soil Sampling and Methods of Analysis, Second edi, CRC Press, 2007. https://doi.org/10.1201/9781420005271.

[25] H. Deng, X. Li, Y. Zhu, W. Cheng, Resistance and resilience of Cu-polluted soil after Cu perturbation, tested by a wide range of soil microbial parameters, FEMS Microbiol. Ecol. 70 (2009) 137–48. https://doi.org/10.1111/j.1574-6941.2009.00741.x.

[26] M. Naveed, P. Moldrup, E. Arthur, M. Holmstrup, M. Nicolaisen, M. Tuller, L. Herath, S. Hamamoto, K. Kawamoto, T. Komatsu, H.-J. Vogel, L. Wollesen de Jonge, Simultaneous Loss of Soil Biodiversity and Functions along a Copper Contamination Gradient: When Soil Goes to Sleep, Soil Sci. Soc. Am. J. 78 (2014) 1239–1250. https://doi.org/10.2136/sssaj2014.02.0052.

[27] X. Shu, P.D. Hallett, M. Liu, E.M. Baggs, F. Hu, B.S. Griffiths, Resilience of soil functions to transient and persistent stresses is improved more by residue incorporation than the activity of earthworms, Appl. Soil Ecol. 139 (2019) 10–14. https://doi.org/10.1016/j.apsoil.2019.03.008.

[28] P.M. Groffman, Nitrification and denitrification in conventional and no-tillage soils, Soil Sci. Soc. Am. J. 49 (1985) 329–334. https://doi.org/10.2136/sssaj1985.03615995004900020011x.

[29] J. Luo, R.E. White, P. Roger Ball, R.W. Tillman, Measuring denitrification activity in soils under pasture: Optimizing conditions for the short-term denitrification enzyme assay and effects of soil storage on denitrification activity,

Soil Biol. Biochem. 28 (1996) 409–417. https://doi.org/10.1016/0038-0717(95)00151-4.

[30] H. Deng, B. Zhang, R. Yin, H.L. Wang, S.M. Mitchell, B.S. Griffiths, T.J. Daniell, Long-term effect of re-vegetation on the microbial community of a severely eroded soil in sub-tropical China, Plant Soil. 328 (2010) 447–458. https://doi.org/10.1007/s11104-009-0124-9.

[31] T.J. Daniell, J. Davidson, C.J. Alexander, S. Caul, D.M. Roberts, Improved real-time PCR estimation of gene copy number in soil extracts using an artificial reference., J. Microbiol. Methods. 91 (2012) 38–44. https://doi.org/10.1016/j.mimet.2012.07.010.

[32] D.J. Lane, 16S/23S rRNA sequencing, in: E. Stackebrandt, M. Goodfellow (Eds.), Nucleic Acid Tech. Bact. Syst., John Wiley and Sons, New York, 1991: pp. 115–175.

[33] R Core Team, R: A Language and Environment for Statistical Computing, (2018). https://www.r-project.org/.

[34] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O’Hara, G.L.

Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner, vegan: Community Ecology Package, (2019). https://cran.r-project.org/package=vegan.

[35] S.F. Yanni, B.L. Helgason, H.H. Janzen, B.H. Ellert, E.G. Gregorich, Warming effects on carbon dynamics and microbial communities in soils of diverse texture, Soil Biol. Biochem. 140 (2020) 107631. https://doi.org/10.1016/j.soilbio.2019.107631.

[36] R.L. Barnard, C.A. Osborne, M.K. Firestone, Responses of soil bacterial and fungal communities to extreme desiccation and rewetting, ISME J. 7 (2013) 2229–2241. https://doi.org/10.1038/ismej.2013.104.

[37] C. Jacquet, I. Gounand, F. Altermatt, How pulse disturbances shape size-abundance pyramids, Ecol. Lett. 23 (2020) 1014–1023. https://doi.org/10.1111/ele.13508.

[38] A. Schaeffer, W. Amelung, H. Hollert, M. Kaestner, E. Kandeler, J. Kruse, A. Miltner, R. Ottermanns, H. Pagel, S. Peth, C. Poll, G. Rambold, M. Schloter, S. Schulz, T. Streck, M. Roß-Nickoll, The impact of chemical pollution on the

resilience of soils under multiple stresses: A conceptual framework for future research, Sci. Total Environ. 568 (2016) 1076–1085. https://doi.org/10.1016/j.scitotenv.2016.06.161.

[39] A.A. Malik, J.B.H. Martiny, E.L. Brodie, A.C. Martiny, K.K. Treseder, S.D. Allison, Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change, ISME J. 14 (2020) 1–9. https://doi.org/10.1038/s41396-019-0510-0.

[40] F. Degryse, E. Smolders, D.R. Parker, Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications - a review, Eur. J. Soil Sci. 60 (2009) 590–612. https://doi.org/10.1111/j.1365-2389.2009.01142.x.

[41] A. Kaurin, R. Mihelič, D. Kastelec, H. Grčman, D. Bru, L. Philippot, M. Suhadolc, Resilience of bacteria, archaea, fungi and N-cycling microbial guilds under plough and conservation tillage, to agricultural drought, Soil Biol. Biochem. 120 (2018) 233–245. https://doi.org/10.1016/j.soilbio.2018.02.007.

[42] J. Six, R.T. Conant, E.A. Paul, K. Paustian, Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils, Plant Soil. 241 (2002) 155–176. https://doi.org/10.1023/A:1016125726789.

[43] J.M. Smith, A. Ogram, Genetic and functional variation in denitrifier populations along a short-term restoration chronosequence, Appl. Environ. Microbiol. 74 (2008) 5615–5620. https://doi.org/10.1128/AEM.00349-08.

[44] A.J. Thomson, G. Giannopoulos, J. Pretty, E.M. Baggs, D.J. Richardson, Biological sources and sinks of nitrous oxide and strategies to mitigate emissions, Philos. Trans. R. Soc. B Biol. Sci. 367 (2012) 1157–1168. https://doi.org/10.1098/rstb.2011.0415.

[45] A. Black, P.C.L. Hsu, K.E. Hamonts, T.J. Clough, L.M. Condron, Influence of copper on expression of nirS, norB and nosZ and the transcription and activity of NIR, NOR and N2OR in the denitrifying soil bacteria Pseudomonas stutzeri, Microb. Biotechnol. 9 (2016) 381–388. https://doi.org/10.1111/1751-7915.12352.

[46] G.W. Nicol, S. Leininger, C. Schleper, J.I. Prosser, The

influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria, Environ. Microbiol. 10 (2008) 2966–2978. https://doi.org/10.1111/j.1462-2920.2008.01701.x.

[47] G. Subrahmanyam, H.W. Hu, Y.M. Zheng, A. Gattupalli, J.Z. He, Y.R. Liu, Response of ammonia oxidizing microbes to the stresses of arsenic and copper in two acidic alfisols, Appl. Soil Ecol. 77 (2014) 59–67. https://doi.org/10.1016/j.apsoil.2014.01.011.

[48] J. Mertens, S.A. Wakelin, K. Broos, M.J. Mclaughlin, E. Smolders, Extent of copper tolerance and consequences for functional stability of the ammonia-oxidizing community in long-term copper-contaminated Soils, Environ. Toxicol. Chem. 29 (2010) 27–37. https://doi.org/10.1002/etc.16.

[49] S.D. Jurburg, F.L. Assemien, L. Beaumelle, J.F. Salles, J.D. Van Elsas, X. Le Roux, A history of extreme disturbance affects the relationship between the abundances of nitrifiers in soil, Biol. Fertil. Soils. (2020). https://doi.org/10.1007/s00374-020-01491-8.

[50] E. Blagodatskaya, Y. Kuzyakov, Active microorganisms in soil: Critical review of estimation criteria and approaches, Soil Biol. Biochem. 67 (2013) 192–211. https://doi.org/10.1016/j.soilbio.2013.08.024.

[51] N. Eisenhauer, W. Schulz, S. Scheu, A. Jousset, Niche dimensionality links biodiversity and invasibility of microbial communities, Funct. Ecol. 27 (2013) 282–288. https://doi.org/10.1111/j.1365-2435.2012.02060.x.

[52] F. Dini-Andreote, J.C. Stegen, J.D. van Elsas, J.F. Salles, Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession, Proc. Natl. Acad. Sci. 112 (2015) E1326–E1332. https://doi.org/10.1073/pnas.1414261112.

[53] V. Kurm, W.H. Van Der Putten, W. De Boer, S. Naus-Wiezer, W.H. Gera Hol, Low abundant soil bacteria can be metabolically versatile and fast growing, Ecology. 98 (2017) 555–564. https://doi.org/10.1002/ecy.1670.

[54] S.E. Jones, J.T. Lennon, Dormancy contributes to the maintenance of microbial diversity, Proc. Natl. Acad. Sci.

107 (2010) 5881–5886. https://doi.org/10.1073/pnas.0912765107.

[55] A. Jousset, C. Bienhold, A. Chatzinotas, L. Gallien, A. Gobet, V. Kurm, K. Küsel, M.C. Rillig, D.W. Rivett, J.F. Salles, M.G.A. van der Heijden, N.H. Youssef, X. Zhang, Z. Wei, W.H.G. Hol, Where less may be more: how the rare biosphere pulls ecosystems strings, ISME J. 11 (2017) 853–862. https://doi.org/10.1038/ismej.2016.174.

[56] B.S. Griffiths, H.L. Kuan, K. Ritz, L.A. Glover, A.E. McCaig, C. Fenwick, The Relationship between Microbial Community Structure and Functional Stability, Tested Experimentally in an Upland Pasture Soil, Microb. Ecol. 47 (2004) 104–113. https://doi.org/10.1007/s00248-002-2043-7.

Table 1 Soil physicochemical properties. Mean (n = 4) ± standard deviation. Values in columns sharing the same letter do not differ significantly (*P* < 0.05) as determined by the post hoc Tukey's test.

Soil	Classification	Sand	Silt	Clay	pH	DOC	NO_3^{-1}	NH_4^+	MBC	TN	TC
Craibstone	Humic Podzol	58±5a	23±2a	19±2a	4.95±0.06b	970.21±76.82b	39.57±0.81b	0.47±0.04b	116.61±26.75b	0.30±0.00b	4.07±0.04b
Insch	Dystric Cambisol	54±4a	24±3a	22±3a	5.30±0.00a	457.64±13.72a	23.90±0.17a	0.37±0.04a	34.93±6.37a	0.21±0.00a	2.25±0.04a

Note: TN- total nitrogen (%); SOC- soil organic carbon (%); MBC- microbial biomass carbon (µg C soil g^{-1}); pH was measured by 0.01 M $CaCl_2$ extraction; NH_4^+- the amount of ammonia N (µg N soil g^{-1}); NO_3^-- the total concentration of nitrate (µg N soil g^{-1}); DOC- dissolved organic carbon (µg C soil g^{-1}).

Table 2 Two-way ANOVA significance testing of the functional and genetic stability to Cu and heat for the Craibstone and the Insch soil. DF is the degree of freedom (n = 4). *, **, * following F value represents significance at 0.05, 0.01, and 0.001 level.**

				Craibstone				
	Functional stability to Cu				**Functional stability to Heat**			
	DF	Sum of Squares	Mean Square	F value	DF	Sum of Squares	Mean Square	F value
Functions	2	108732	54366	293.54***	2	28190	14095	110.03***
Day	4	4475	1119	6.04***	4	24984	6246	48.76***
Functions*Day	8	19725	2466	13.31***	8	17153	2144	16.74***
	Genetic stability to Cu				**Genetic stability to Heat**			
	DF	Sum of Square	Mean Square	F value	DF	Sum of Square	Mean Square	F value
Genes	5	29498	5900	3.86**	5	615329	123066	50.52***
Day	4	51865	12966	8.48***	4	164285	41071	16.86***
Genes*Day	20	103869	5193	3.40***	20	296361	14818	6.08***
				Insch				
	Functional stability to Cu				**Functional stability to Heat**			
	DF	Sum of Squares	Mean Square	F value	DF	Sum of Squares	Mean Square	F value
Functions	2	35553	17777	94.32***	2	7669	3834	2.17
Day	4	8390	2098	11.13***	4	135531	33883	19.13***
Functions*Day	8	19343	2418	12.83***	8	49594	6199	3.50***
	Genetic stability to Cu				**Genetic stability to Heat**			
	DF	Sum of Squares	Mean Square	F value	DF	Sum of Squares	Mean Square	F value
Genes	5	95872	19174	7.48***	5	146640	29328	10.38***
Day	4	75053	18763	7.32***	4	210027	52507	18.58***
Genes*Day	20	82552	4128	1.61*	20	211266	10563	3.74***

Table 3 Two-way PERMANOVA on Bray-Curtis distance of Hellinger transformed T-RFLP 16S rRNA in each soil. DF is the degree of freedom (n = 4).

Craibstone

Parameter	DF	Sum of Squares	Mean Square	F value	R^2	P value
Stress	2	4.266	2.133	59.522	0.425	< 0.001
Day	4	1.378	0.344	9.611	0.137	< 0.001
Stress*Day	8	2.770	0.346	9.662	0.276	< 0.001

Insch

Parameter	DF	Sum of Squares	Mean Square	F value	R^2	P value
Stress	2	1.973	0.986	31.156	0.308	< 0.001
Day	4	0.769	0.192	6.070	0.120	< 0.001
Stress*Day	8	2.242	0.280	8.852	0.350	< 0.001

Table 4 Pearson correlations between stability of different indicators to Cu and heat at five time points after stress in the Craibstone and Insch soil, respectively (n = 4). *, ** and * means significant correlation at *P* < 0.05, 0.01, and 0.001.**

Correlation in the Craibstone soil	**Cu**					**Heat**				
	Day1	Day3	Day7	Day14	Day28	Day1	Day3	Day7	Day14	Day28
C mineralization ~ 16S rRNA	-0.218	-0.325	0.255	0.189	-0.141	-0.179	-0.031	-0.255	0.430	0.287
Ammonia oxidation ~ amoA	0.382	0.140	0.615*	0.032	-0.388	0.000	0.729**	-0.633**	-0.430	0.080
Denitrification ~ nirS	0.462	-0.357	0.401	0.079	-0.099	0.109	-0.377	-0.254	-0.065	0.042
Denitrification ~ nirK	0.145	0.226	0.553*	0.393	-0.131	0.291	-0.263	-0.478	-0.303	-0.118
Denitrification ~ nosZ-I	-0.174	-0.149	0.782***	0.426	0.140	-0.059	-0.596*	-0.121	-0.565*	0.305
Denitrification ~ nosZ-II	0.239	-0.156	0.547*	0.494	0.098	0.710**	-0.312	-0.372	-0.612*	0.284
Correlation in the Insch soil	**Cu**					**Heat**				
	Day1	Day3	Day7	Day14	Day28	Day1	Day3	Day7	Day14	Day28
C mineralization ~ 16S rRNA	-0.550*	-0.274	0.528*	0.161	-0.700**	-0.599*	0.418	0.041	-0.625*	-0.403
Ammonia oxidation ~ amoA	-0.106	-0.274	0.246	0.412	-0.294	0.000	0.000	0.000	-0.590*	-0.723**
Denitrification ~ nirS	0.300	-0.312	-0.196	0.631**	0.095	0.214	-0.309	-0.265	-0.032	0.037
Denitrification ~ nirK	0.529*	-0.359	-0.434	0.427	0.327	0.578*	-0.260	-0.442	-0.051	0.200
Denitrification ~ nosZ-I	0.779***	-0.507*	-0.330	0.306	-0.194	0.473	-0.580*	-0.599*	0.236	-0.214
Denitrification ~ nosZ-II	0.476	-0.339	-0.373	0.492	0.235	0.199	-0.543*	-0.273	0.493	0.198

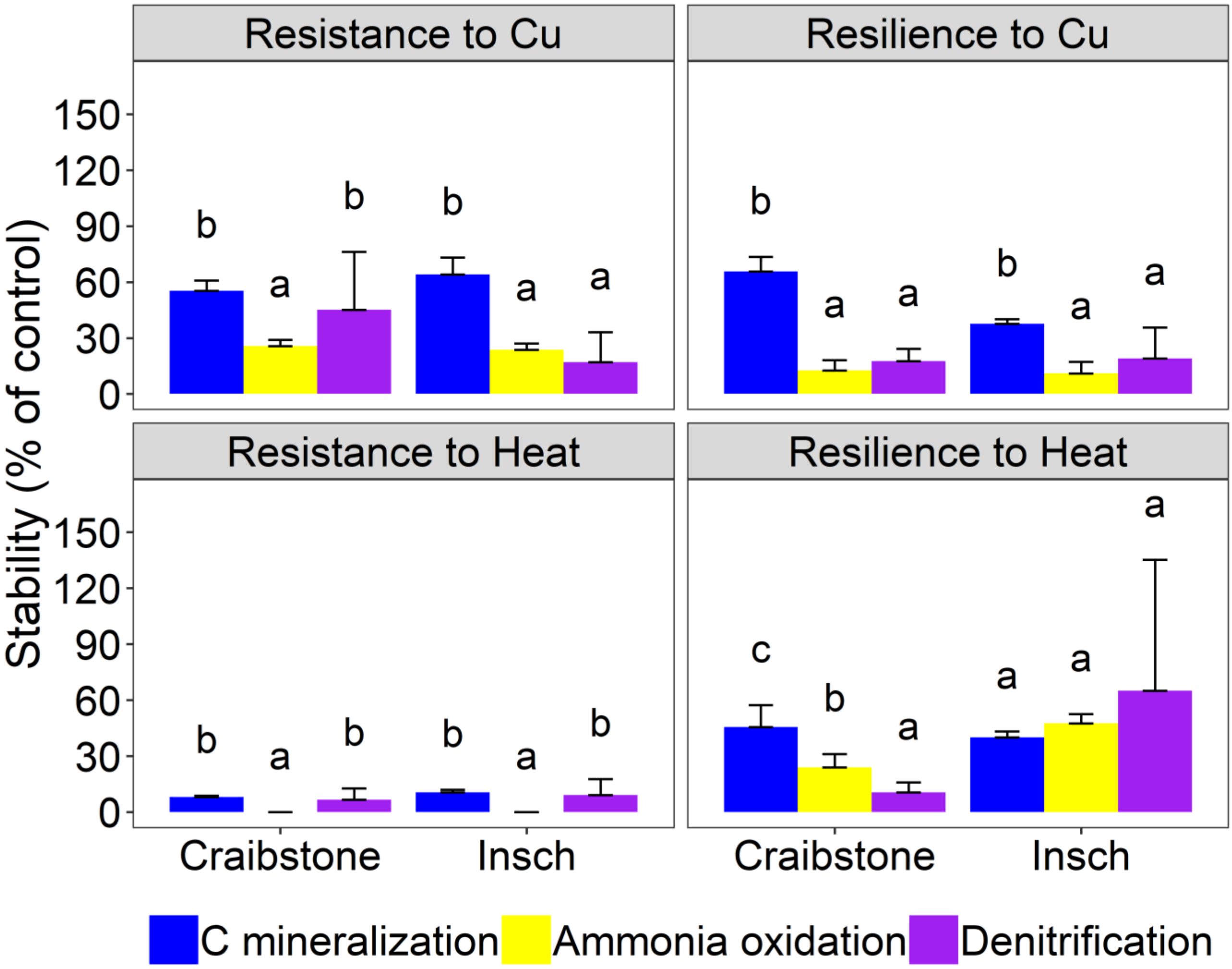

Figure 1 Resistance and resilience of C mineralization, ammonia oxidation and denitrification to Cu and heat in the Craibstone and the Insch soil. Means (n = 4) and standard deviation (error bar). Significant (*P* <0.05) differences are indicated by different lowercase letters within the same resistance or resilience in the same soil.

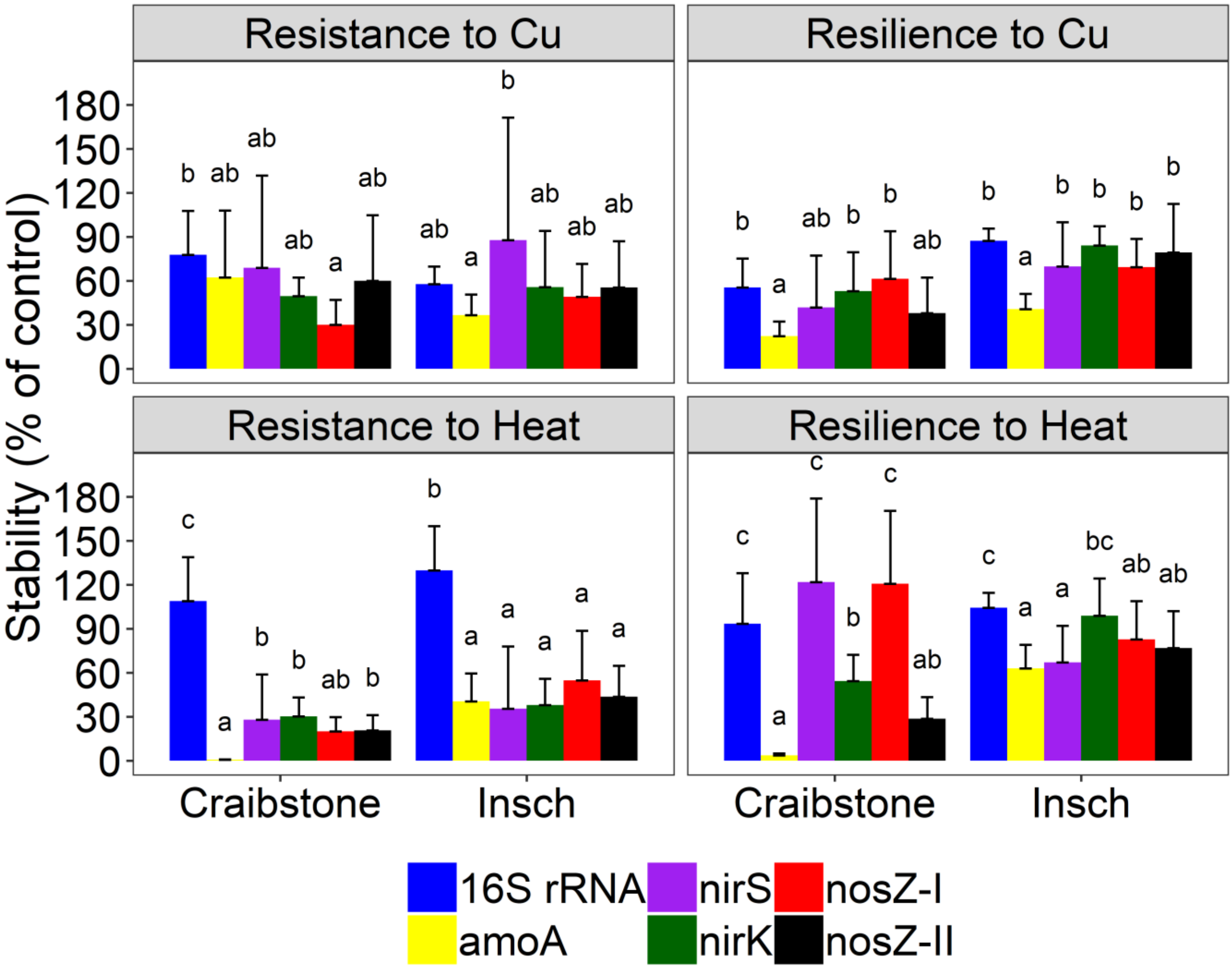

Figure 2 Resistance and resilience of gene abundance to Cu and heat in the Craibstone and the Insch soil. Means (n = 4) and standard deviation (error bar). Significant (*P* < 0.05) differences are indicated by different lowercase letters within the same resistance or resilience in the same soil.

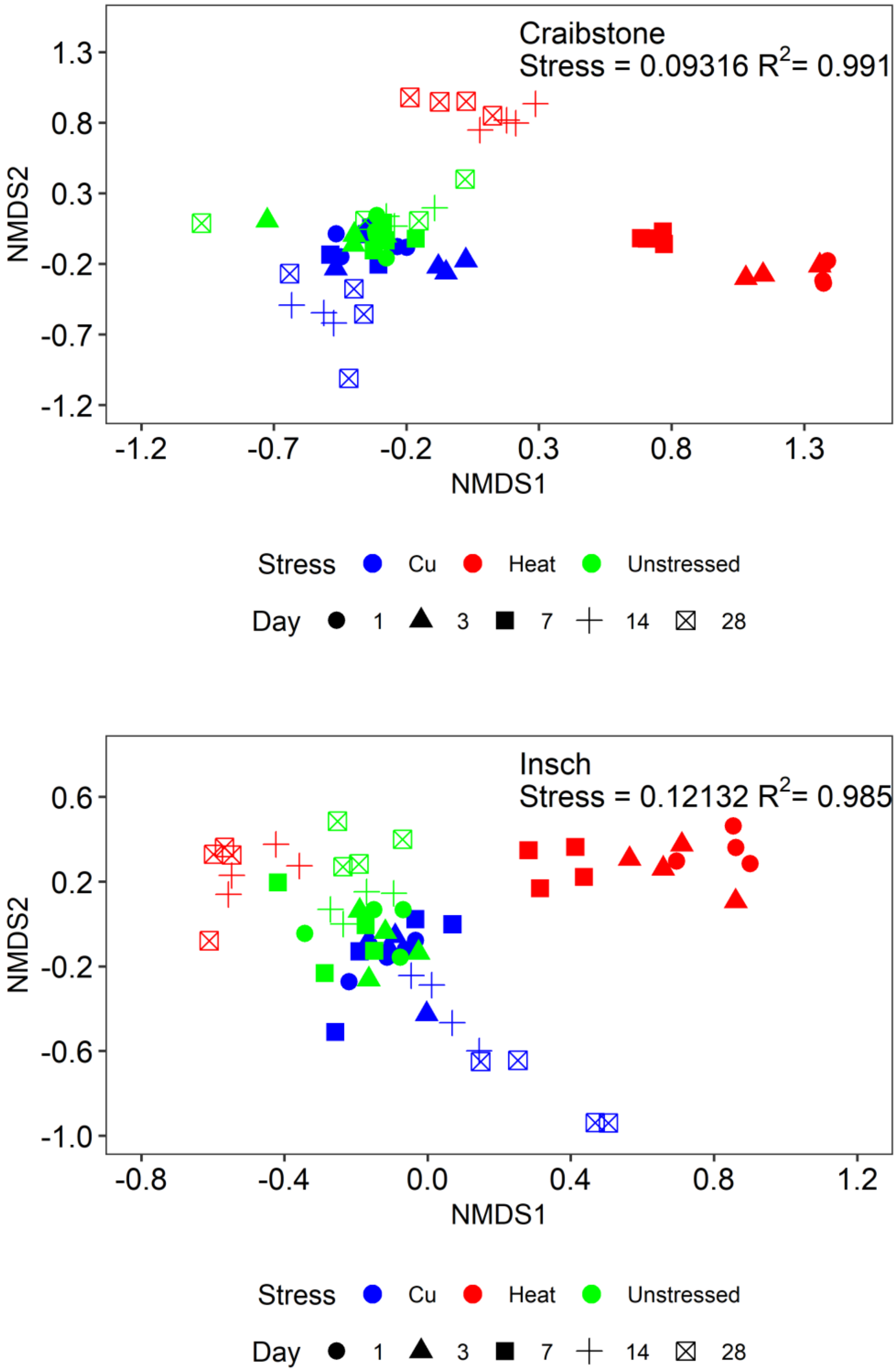

Figure 3 The bacterial community structure of two soils at 1, 3, 7, 14 and 28 days after being stressed with either Cu or heat. For each soil, individual non-metric multidimensional scaling (NMDS) was measured based on Bray-Curtis distance analysis of Hellinger transformed 16S rRNA T-RFLP data (n = 4). Different symbols represent samples at different time and under different stresses.