INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0682/2020/24–5–1107–1114
DOI: 10.17957/IJAB/15.1539
http://www.fspublishers.org

Full Length Article

Nitrification Resilience and Response of Ammonia-Oxidizing Bacteria upon Heat-Drought Extremes across Three Soil Ecosystems in Lower Himalaya

Tahir Zaman[1], Wasila Qazi[1], Saeed Ahmad Asad[2], Muhammad Bilal[1], Akhtar Iqbal[1], Rashid Nazir[1], Sabir Hussain[3], Muhammad Irshad[1] and Farhan Hafeez[1*]

[1]*Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Tobe Camp University Road Abbottabad 22060 Pakistan*

[2]*Centre for Climate Research and Development (CCRD), COMSATS University, Park Road Islamabad-45550, Pakistan*

[3]*Department of Environmental Sciences & Engineering, Government College University, Allama Iqbal Road, 38000, Faisalabad, Pakistan*

[*]For correspondence: drfarhan@cuiatd.edu.pk

Received 25 April 2020; Accepted 04 June 2020; Published 31 August 2020

Abstract

Soil ecosystems despite of providing a vast range of value services are expected to face climatic extremes. Assessing the resilience degree of such ecosystems is integral in evaluating their functional stability. This study investigated the resilience and resistance of potential nitrification activity (PNA) and involved guilds (ammonia-oxidizing bacteria - AOB) upon two heat-drought extremes (low (40°C for 24 h) and high (40°C for 48 h)) at 1, 7, 14, 28 days' intervals across three different soil ecosystems (arable, grassland, forest) in lower Himalaya. Results demonstrated that three soils responded differentially to heat-droughts (~20–55% decrease PNA) with arable soil showing high resistance (withheld ~55.3% PNA) and low resilience (~82–85%). The gene abundances of AOB revealed that the community size was significantly reduced upon the heat-droughts (up to 50.1%) and could not ultimately recover showing an incomplete resilience. In contrast to arable, the grassland and forest soils were better in resilience with PNA at day 28 observed near to control (93.4 and 91.4%). The correlation analyses showed a strong positive relationship of AOB to PNA particularly in arable soil. The high resilience exhibited by grassland and forest soils implied that despite of reduction in AOB numbers, functional redundancy might have inferred an increased resilience showing that these soils can recover once the stress period is over. This work underlines the functional stability of different soils for nitrification potential highlighting the relevance of resilience and resistance perspectives in wake of predicted climatic extremes on soil N cycling and associated N losses in lower Himalaya. © 2020 Friends Science Publishers

Keywords: Soil services; Nitrification; AOB; Community gene; Heat-drought; Himalaya

Introduction

Soil ecosystems are important natural capital that not only perform various functions crucial for the life on earth but also regulate a vast range of value services (Starke *et al*. 2019) beside providing the physical support to human settlements and urban development (Pereira *et al*. 2017). These ecosystems actually host a wide diversity of microbial communities which in turn play vital roles in maintaining the overall functioning hence contribute in ecosystem stability (Liu *et al*. 2019). Nutrient cycling and their implications are one of the very important services provided by the soil ecosystems and the inhabited microbial communities. For example, cycling of nitrogen that carries various economic and ecological implications is occurred through various processes which are mainly driven by the microbial communities hosted in soil.

Nitrogen is among the vital elements in the soil ecosystem (Green *et al*. 2019) where it performs various important roles being as a component of enzymes, proteins and nucleic acids (Pajares and Bohannan 2016). It is necessary for plant productivity thus contributes at great scale to the food chain for humans (Zheng *et al*. 2020) by sustaining human existence. Cycling of nitrogen involved various important processes including nitrification, conversion of ammonia (NH_3) to nitrite (NO_2^-) and its subsequent conversion to nitrate (NO_3^-) (Purkhold *et al*. 2000) and ammonia oxidizers are considered as the key drivers in nitrification (Siljanen *et al*. 2019) because this ammonia oxidation is a rate limiting step where ammonia oxidizers are the main actors accountable in these transformations (Butterbach-Bahl *et al*. 2013). In soils, nitrification coupled with denitrification is crucial since these processes ultimately are the major contributors of

To cite this paper: Zaman T, W Qazi, SA Asad, M Bilal, A Iqbal, R Nazir, S Hussain, M Irshad, F Hafeez (2020). Nitrification resilience and response of ammonia-oxidizing bacteria upon heat-drought extremes across three soil ecosystems in lower Himalaya. *Intl J Agric Biol* 24:1107–1114

atmospheric nitrous oxide emissions (Li *et al.* 2017) that is the most important of the long-lived and powerful greenhouse gases involved in ozone-depleting (Brevik 2013) and collectively nitrification and denitrification may cause about 70% N_2O losses to overall environment (Paré and Bedard-Haughn 2012). Thus, nitrification is an important process in estimating the N cycling functioning and losses across various soil ecosystems since it carries various ecological implications (Ashraf *et al*. 2019).

During 21st century the frequency, intensity and duration of extreme climatic events are found to be increasing substantially (Yeni and Alpas 2017), such as those related to bringing the frequent and intense droughts. For example, soil heat-drought stresses have been observed in different regions (Jha and Srivastava 2018) where these may also effect the various processes in different ecosystems thus disrupt the associated functions. Pakistan also lies in the region which is among the fast temperature rising hotspots hence reported as the region that is vulnerable to changing climate events (Ullah *et al*. 2018). The greater risk of heat droughts have already witnessed the impact of climate change including the severe drought period of 2000 and 2009 with rainfall well below than that of the usually observed (Webster *et al*. 2011). As the frequencies of extreme heatwaves and droughts are increasing (Harrison *et al*. 2016) and the winter season showing increased warming trends, extent of the winter season has been reduced on both ends leaving the summer extended (Rasul *et al*. 2012).

These extreme events such as droughts and heat waves are among the major abiotic stresses that may reduce the overall crop productivity thus weaken the food security. Moreover, the high temperatures can bring the hydrological fluctuations particularly in the mountain ecosystems which in turn may disrupt the biogeochemical cycles (Kang *et al*. 2019). The mountainous regions comprising a range of soil ecosystems including arable, grassland and forest ecosystems are among the highly prone areas to be affected by the extreme events such as global warming which may ultimately lead to the changes in hosted microbial communities (Sharma *et al*. 2020) and thus the associated functions. Whether these various ecosystems can withhold such future predicted changes to the hosted communities and the associated services is yet rarely estimated.

In this regard, assessing the functional stability through investigating the resilience (ability to recover once the stress period is over) and resistance (ability to resist the change upon any perturbation) parameters (Wittebolle *et al*. 2009) in crucial to understand the extent to which various soil ecosystems, hosted microbial communities and the associated functions may withhold the stresses related to extreme climatic events. Uneven precipitations and increased temperatures are usually linked to the climate change events and thus can also be crucial to be investigated in scenario of functional stability perspectives.

Overall, the biological processes are reported to be influenced by increased temperatures where nitrification process has also been described as affected by increased temperature (Zeng *et al*. 2014). Moreover, nitrification is critical in estimating the overall fate of nitrogen crucial in N cycling (Li *et al*. 2018) where little has been reported how soil nitrogen processes respond to the temperature linked heat-drought stresses under changing climate (Chen *et al*. 2019), studying the nitrification process is crucial in these perspectives. This study aimed in assessing the resilience and resistance of nitrification potential and the involved functional guilds – ammonia oxidizing bacteria against the two levels of heat-drought stresses applied at soil microcosm level for three different soil ecosystems (arable, grassland, forest) in lower Himalaya. The assumption is that the arable, in contrast to grassland and forest soil ecosystems (the least disturbed in comparison to aforementioned ecosystem), being already under the influence of human perturbations (relative to other two) might have developed an increased microbial community resistance and the functional redundancy may help to cope with the applied disturbance but also the changing environmental factors. We estimated the impact of heat-droughts on resilience and resistance of potential nitrification activity and community gene abundances of the ammonia oxidizers across arable, grassland and forest soil ecosystems of lower Himalaya.

Materials and Methods

Site description and soil sampling

For this study, we collected the samples from arable, grassland and forest soil ecosystems located under similar pedoclimatic conditions in Abbottabad, lower Himalaya (34°14'00.74" N latitude, 73°17'00.68" E longitude). The assumption behind was the fact that arable soils are relatively more exposed to the anthropogenic perturbations in contrast to grassland and forest thus may have acquired relatively better capability to withhold the external disturbance. The mean maximum temperature recorded at studied area was ~22.76°C while the minimum temperature was ~11.41°C. The annual mean precipitation is 166.08 mm with annual total precipitation of 1,366.18 mm (Tahir *et al*. 2015). The soil sampling site topography was complex mountainous terrain with an average elevation of 1714 m. Forest soil (34°14'00.74" N latitude, 73°17'00.68" E longitude) mainly sandy clay loam was considered as an un-disturbed ecosystem, grassland soil (34°13'54.22" N latitude, 73°16'39.83" E longitude) mainly sandy loam was chosen as moderately disturbed by human activities while, arable soil (34°13'45.00" N latitude, 73°16'48.87" E longitude) mainly sandy loam was selected with the above described assumption. Soil samples in triplicate from all three arable, grassland and forest soils were randomly collected from the Ap horizon (0–20 cm).

Experimental setup and resilience measurements

The triplicate samples collected from soils were brought to

laboratory for setting the soil microcosm experiment. For every studied soil, 36 soil microcosms in plasma flasks (12 from every triplicate sample, 36 x 3 soils n=108), having 30 g of dry soil prepared with sieved at 5 mm size, were placed for incubation at room temperature (~25°C) for the time-period of 28 days (control). At day 0, from each of the three experimental units (three soils), 12 out of 36 samples were exposed to 40°C for 24 h (low level of heat-drought stress treatment) and 12 to 40°C for 48 h (high level of heat-drought stress treatment). The rest of 12 plasma flasks were not exposed to any perturbation and placed at room temperature hence considered as control. The replicate of such set-up was also prepared for other two soils hence samples from all three soil ecosystems were placed under low and high heat-drought treatments with control beside for a period of 28 days with water holding capacity maintained at 70% throughout the experimental time period. For assessing the resistance and resilience parameters, the samples from the experimental set-up were collected for further analyses at regular time intervals i.e. on day 1, 7, 14 and 28. After the stress period was over, analyses carried out at day 1 represented the resistance while the measurements recorded at day 7, 14 and 28 depicted the resilience with time as shown by the respective functions. The resistance and resilience were measured in terms of the percent changes, for studied parameters, in stressed samples with respect to control where control was considered as 100%.

Soil physicochemical analyses

Soil samples collected from the fields were processed for further analyses after homogenizing the soil by removing rocks, pebbles, roots, and stalks. Soil physicochemical properties including soil moisture, *pH*, organic matter content, soil NO_3-N and soil texture were determined. Soil *pH* was determined in 1:5 (w/v) soil: water suspensions with a *pH* meter, after equilibration for 1 h. Soil NO_3-N were measured following the procedure, briefly, stock solution of nitrate was prepared after drying potassium nitrate (KNO_3) in an oven at 105°C for 24 h. 1 g KNO_3 was dissolved in water and diluted to 1000 mL. From the stock solution, NO_3^- standards were prepared as 0, 2, 4, 6, 8 and 10 ppm. 10 g dry soil was mixed with 100 mL distilled water and shaken for 1 h. Then the sample was filtered, 50 mL clear sample was added with 1 mL 1N HCl and mixed properly. The absorbance from the UV visible spectrophotometer was read at 220 nm. A regression plot was drawn to determine the concentration of nitrate in soil samples. Soil organic matter contents were determined by loss on ignition method (Nelson and Sommers 1996) while soil texture was examined by hydrometric method (Gee and Or 2002).

Potential nitrification activity measurements

The potential nitrification activity (PNA) was determined using a widely adopted laboratory incubation method as described by Hoffmann *et al*. (2007). In brief, 4 g soil (fresh soil weighing around 2.5 g dry soil) was added to 10 mL solution of reagent while these suspensions were put on orbital shaker with conditions at 175 rpm. At regular time intervals of 1 h, 8 h and 24 h and 48 h, the suspension of 2 mL was sampled, and the oxidation of ammonium was blocked through adding KCl (2 mL) followed by centrifugation for 2 min at speed of 3000 rpm. The supernatant, about 0.8 ml, was collected and added with NH_4Cl buffer (1.5 mL) and Diazzo (0.4 mL). The absorbance was recorded after 30 minutes using known standards (0, 2, 4, 6, 8 and 10 ppm) at the wavelength 530 nm on Spectrophotometer and the PNA was quantified as μg NO_2-N per g dw (dry weight) soil per h.

Real-time Quantitative PCR of community gene abundances (*amoA*) of ammonia oxidizers

Soil DNA extractions were performed for each sample in triplicate using the PowerSoil DNA Isolation Kit (MO BIO Laboratories), according to the procedure as described by the manufacturer. The soil DNA concentrations were determined through gel quantification of the extracted DNA with known concentrations beside. The community size of the gene *amoA* in ammonia oxidizing bacteria was quantified from the extracted DNA through carrying out Real-time PCR and the quantitative PCR assays were carried out as described by Hafeez *et al*. (2012). The quantification of the genes (*amoA*) involved in coding the enzymes responsible for oxidation of ammonia in bacteria was conducted to understand the size of ammonia oxidizers (AOB) and the protocol and conditions of thermocycler were followed as described by Leininger *et al*. (2006). In this regard, we conducted the independent assays for every sample and the average was calculated for further analyses.

Statistical analysis

The obtained data were statistically analyzed adopting the analysis of variance (ANOVA) which was performed using STATISTICA 10.0. Separation of the means were performed using least significant difference of Fisher's test with $p<0.05$. The relationships among the various characteristics including PNA and AOB gene abundances were also calculated using Pearson's correlation. Resistance and resilience parameters by calculating the percent changes in stressed samples with respect to control where control was considered as 100%. The ratio of these stressed samples to control was calculated following the indices as explained by Souza (1980).

Results

Physicochemical characteristics of various soil ecosystems

Soil physicochemical properties including soil moisture, *pH*, NO_3-N, organic matter percent and soil texture were

Table 1: Soil physiochemical properties across various soil ecosystems

Soil parameter	Arable Soil	Grassland Soil	Forest Soil
pH (1:5)	7.6 ± 0.01	7.3 ± 0.01	7.1 ± 0.01
NO_3-N (mg kg^{-1})	8.26 ± 1.4	7.67 ± 0.76	7.92 ± 1.09
Organic matter (%)	4.15 ± 0.04	6.6 ± 0.30	9.9 ± 0.06
Sand (%)	68.1	72.7	61.0
Silt (%)	24.5	11.1	18.2
Clay (%)	7.4	16.2	20.8
Class for texture	Sandy-loam	Sandy-loam	Sandy-clay-loam

determined and the results are summarized in Table 1. Soil *pH* ranged from 7.8, 7.3, 7.1 for arable, grassland and forest soil ecosystems, respectively. The nitrate contents an indicator of net nitrification were higher for the arable soil with average as 8.26 mg kg^{-1} and a non-significant difference in soil nitrate contents was recorded for the grassland and forest soil ($P < 0.05$) with values as 7.67 and 7.92 mg kg^{-1}, respectively. Soil textural for the arable and grassland soils were sandy loam while forest soil was categorized as sandy clay loam and the description of various particles distribution deciding the textural classes are explained in Table 1. The organic matter content was significantly higher ($P < 0.05$) in forest soil with values as 9.9% followed by the grassland and arable soil ecosystems as 6.6 and 4.15%, respectively.

Comparison of PNA and community size of AOB across various soils

Results showed that the initial PNA was found to be high for the control treatment at day 1 and it ranged up to 3.6 μg NO_2-N per g dw soil per h being significantly higher ($P < 0.05$) in arable soil ecosystem. In contrast, these differences were non-significant ($P < 0.05$) both in grassland and forest soils with mean values as 0.73 and 0.57 μg NO_2-N per g dw soil per h, respectively (Fig. 1). The samples subjected to no-stress showed high PNA for arable soil throughout the incubation period followed by forest and grassland soil. The size of the AOB in samples from all three soil ecosystems was determined through Real-time quantification of bacterial *amoA* genes and the detailed are described in Fig. 3. The community gene abundances were not significantly different at day 1 for the control in all three soils ($P < 0.05$). The high number was recorded as 6.33 x 10^4 number of gene copies per ng DNA for the arable soil followed by forest and grassland soils as 4.38 x 10^4 and 4.03 x 10^4 number of gene copies per ng DNA, respectively. Pearson correlation analyses revealed a positive relationship between the PNA and size of the AOB communities which was stronger for arable soil (r=0.59, $P < 0.05$) as compared to grassland (r=0.28, $P < 0.05$) and forest soils (r=0.29, $P < 0.05$).

Resistance of nitrification potential and community gene abundances of AOB

The resistance of the PNA and AOB gene abundances were

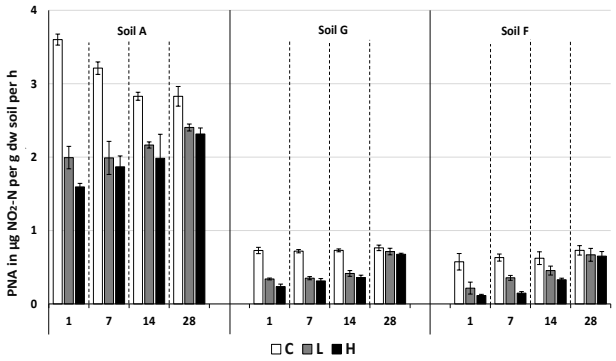

Fig. 1: Potential nitrification activity in control - without stress (white bars) and for the treatments under low (grey bars) and high (black bars) levels of perturbations quantified over a period of 28 days incubation where A stand for arable, G for grassland and forest soil denoted by F. Each bar represents the mean of three replicates in μg N_2O-N per g of dw soil per h

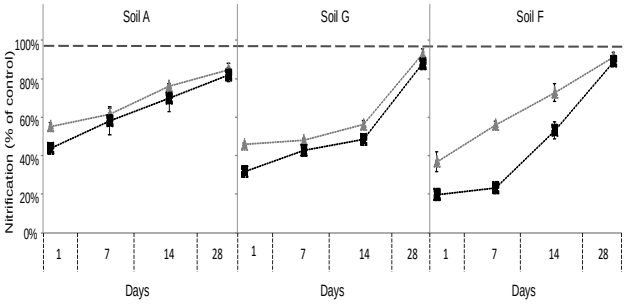

Fig. 2: Resilience and resistance shown through percent indices calculated for the potential nitrification activity over a period of 28 days incubation, for both levels of perturbation; low represented by (▲) while high level is shown by (▮) levels of heat-drought stresses in comparison with samples with no-stress, where A stand for arable, G for grassland and forest soil denoted by F

quantified by comparing soils under heat-droughts to the undisturbed controls and the ratio was calculated following the indices as explained by Souza (1980). Percent changes in PNA and AOB size at day 1 represented the resistance while at day 7, 14, and 28 it denoted the recovery over time (Fig. 2 and 4). We observed that at day 1, soils varied for their response heat-drought stresses. For example, PNA was found to be reduced up to 55.3, 46.7 and 37.2% of the control upon low level stress for arable, grassland and forest soils, respectively (Fig. 2). In contrast, the high level of stress resulted in a strong decrease in PNA for all three soils with reduction in PNA up to 44.3, 32.4 and 20.0% of the control at day 1 for arable, grassland and forest soils, respectively (Fig. 2). However, this effect was pronounced for the grassland and forest soils while arable soil showed better resistance both for low- and high-level stress.

The response shown by the nitrifying communities – AOB was in agreement to the PNA with decrease in number of gene copies of AOB communities upon applied stress.

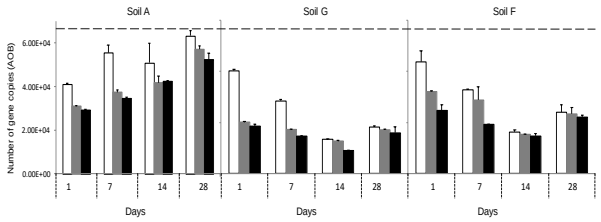

Fig. 3: The community gene abundance of nitrifying communities quantified through Real-time quantitative PCR of bacterial *amo*A (AOB) in number of gene copes per ng DNA, where A stand for arable, G for grassland forest soil denoted by F

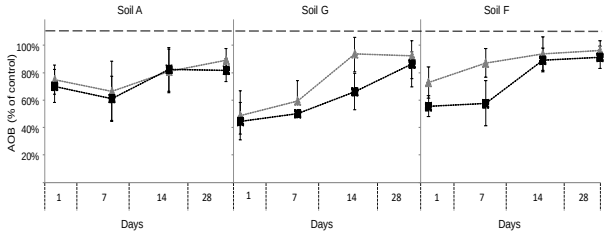

Fig. 4: Relative change in community gene abundances of AOB over a period of 28 days incubation, for both levels of perturbation; low represented by (▲) while high level is shown by (■) levels of heat-drought stresses in comparison with samples with no-stress, where A stand for arable, G for grassland and forest soil denoted by F

Low stress level caused a significant reduction in gene abundances of AOB ($P < 0.05$) which was relatively stronger for the grassland soil with reduction up to 50.1% in AOB community size at day 1. The arable and forest soils showed resistance to this change upon low level of stress however the numbers remained up to 76.0 and 73.6% of control, respectively. High-stress treatment strongly affected both forest and grassland soil and resulted in ~56.4 and ~46.0% of reduction in size of AOB, respectively. Results revealed a relatively strong impact of high-level heat-drought disturbances with severe reduction in number of AOB.

Resilience of nitrification potential and community gene abundances of AOB

Significant differences were observed for recovery in PNA and size of AOB among three different soil ecosystems ($P < 0.05$) and after the stress period PNA was found to variably recover at day 7, 14 and 28. Forest and grassland soils showed better recovery to both stress levels and recovered the PNA near to the initial level after the 28 days. The recovery rate for the forest and grassland soils was 91.4 and 89.20, and 93.4 and 88.6% for low and high level, respectively. Relatively low resilience observed in arable soil which and could recover up to 85.2 and 82.1% of initial for PNA upon the low and high stress, respectively. Overall, PNA was high in arable soil at all days and recorded as 1.99, 2.16 and 2.40 μg NO_2-N per g dw soil per h in contrast to grassland and forest soil which showed 0.35, 0.42 and 0.7 and 0.36, 0.46 and 0.67 μg NO_2-N per g dw soil per h, for the day 7 but also at intervals of 14 and 28 days, respectively (Fig. 1). The nitrification rates were strongly decreased upon high stress level and found to be 1.87, 1.98 and 2.32 μg NO_2-N per g dw soil per h for arable soil, 0.31, 0.36, 0.68 μg NO_2-N per g dw soil per h for grassland, and 0.15, 0.33 and 0.65 μg NO_2-N per g dw soil per h for forest soil at time interval of 7, 14 and 28 days, respectively ($P < 0.05$) (Fig. 1).

Conversely, the community size of AOB was less resilience in comparison to PNA. Though there was recovery in size of the AOB communities at various sampling intervals however the resilience could not be completed especially for the high stress. After 28 days of incubation, arable soil could recover 82.9% of initial AOB numbers however the grassland and forest soils showed a recovery of up to 87.7 and 92% AOB abundances.

Discussion

We have quantified the impact of two levels of heat-drought extremes in arable, grassland and forest soil ecosystems on potential nitrification activity which due to subsequent N losses is crucial from economic and ecological perspectives. We found that arable soil exhibited strong resistance toward both levels of heat-drought disturbances however it could not fully recover the initial functions at short term. Conversely, grassland and forest soils despite of facing a strong short-term reduction in nitrification and AOB abundances showed better recovery and forest soil recovered the PNA almost to unstressed treatment (Fig. 2 and 4). Arable soils are under long term N fertilization hence high nitrification was obvious (Alam *et al*. 2020) however the AOB might have not withheld the applied disturbance hence PNA was collapsed at short term. The conventional agricultural practices such as heavy fertilization in the arable soil might have caused an increased ammonium content that serves as substrate for ammonia oxidizers. Such increased nitrification rates in various soil ecosystems have already been reported previously (Alam *et al*. 2020). Applying stress to the soil system and then examining how it responds to the perturbation is a standard procedure of studying the stability of soil system (Hafeez *et al*. 2014). The reduction in nitrification activity upon heat-drought stress was obvious and such reduced N cycling enzyme activities have been reported in different soils (Beltz *et al*. 2020).

The AOB community size was reduced upon heat-droughts however the difference between the low and high stress was non-significant in arable soil ($P < 0.05$). The stability as observed showed that ammonia oxidizers have developed resistance to external perturbation. Earlier such resistance by the relevant microbial communities have also been reported for a range of studied functions (Beltz *et al*. 2020; Fakhraei *et al*. 2020). Arable soils are generally already under anthropogenic perturbations hence enrichment of resistant microbial communities is obvious. In addition,

various soil native physicochemical properties may also contribute toward this phenomenon. Earlier, Tan *et al.* (2020) unraveled the key drivers of bacterial community assembly in arable soils and suggested that the mean annual precipitation and soil *pH* are the major environmental factors that may shape the hosted soil bacterial communities. In addition, the increased PNA could also be associated to high soil nitrogen and organic matter in respective soils since many of these characteristics are found to influence the nitrification in a range of studies (Amoah-Antwi *et al.* 2020). Forest and grassland soils being least disturbed in comparison to arable soils showed low nitrification rates (Fig. 1). Earlier, net nitrification rates in the cultivated soils were recorded significantly higher than in the uncultivated soil (Wang *et al.* 2019) showing that the addition of fertilizer or input through cultivation associated organic matter contribute in increased microbial enzyme activities through provision of suitable substrates. High resistance exhibited by arable soil indicates the favorable micro-environment for AOB as compared to grassland and forest soils. These nitrification rates are described to be influenced both by abundance and composition of ammonia oxidizing communities but also due to the abiotic factors (Di *et al.* 2009).

Correlation analyses between the various soil edaphic factors, nitrification potential and AOB showed variable relationship among these parameters. For example, the correlation between the PNA and the AOB was relatively less strong for grassland and forest soils implying that beside AOB there were the factors contributing in observed resilience. Organic matter contents were significantly higher ($P < 0.05$) in forest and grasslands and were positively correlated to PNA (r=0.56, r=0.21, $P < 0.05$) which along with the community composition might have contributed to strong recovery in PNA. Since its not only the number but also the microbial diversity which is crucial in estimating a function (Liu *et al.* 2019) in agreement to functional redundancy concepts (Upton *et al.* 2018).

The relatively week correlation between AOB to nitrification for the forest soil indicates that the AOB were not solely driving the PNA. Similar observations were recorded by Taylor *et al.* (2019) whereas Hafeez *et al.* (2012) reported the inverse. Likewise, AOB contributed to nitrification in a different environment in paddy soil (Wang *et al.* 2019). Nitrification is better at moderate temperature however high temperatures inversely effects the specific function (Tan *et al.* 2018). For example, soil N contents stimulate the nitrification that may increase under moderate warming conditions and through promoting microbial growth in certain environments (Dan *et al.* 2019). In addition, increased temperatures to a certain range may have a positive correlation with nitrification (Troy and Tang 2011), and this may result in gradual increase in nitrification only up to ~30°C (Taylor *et al.* 2017) in certain environments. Beyond, it may shift the community selection and change the rate of function as assumed in this case. The optimum temperature recorded for nitrification can also be ~31°C with AOB being nitrifiers (Chen *et al.* 2019).

Diverse range of microbes drive ecosystem functions and are as targeted by specific genes encoding certain enzymes (Liu *et al.* 2019). Recovery in AOB size and PNA showed the degree of resilience in these soils and can be attributed to high stoichiometric flexibility of microorganisms as observed previously by Guillot *et al.* (2019) upon droughts. Such recovery to associated denitrification against similar heat-droughts is observed by Hafeez *et al.* (2014). Moreover, reduced AOB size may be linked to the observation where irreversible stoichiometric changes are found because of the combined drought and heat (Guillot *et al.* 2019) since the heat stress along with increasing temperature may cause reduced moisture, and the droughts may leave a prolonged effect on bacterial communities (Vries *et al.* 2018). Generally, community gene abundances and microbial community composition are supposed to be less resistant than specifically the AOB communities during drought (Thion and Prosser 2014). The recovery is also obvious through microbial adaptations in soils especially upon heat-droughts (Hafeez *et al.* 2014). The results validate the resistance and resilience of soil microbial communities against drought with and without heat stress (Guillot *et al.* 2019). The findings are based on short term perturbations and considering the functional redundancy concepts, the microbial communities may recover to initial state implying a strong resilience post disturbances (Huang *et al.* 2020).

Conclusion

The resilience and resistance of nitrification potential and ammonia-oxidizing bacteria upon two different levels of heat-droughts were investigated across arable, grassland and forest soils of lower Himalaya. Variable response of nitrification activity and community gene abundances of AOB were observed. Arable soil under anthropogenic perturbations showed high resistance but relatively low resilience. The grassland and forest soils exhibited high resilience and recovered PNA similar to unstressed soils despite the reduction in size of AOB. This study sets an ecological understanding about the soil functional stability for N cycling that is crucial in plant productivity and environmental quality perspectives under various climatic extremes in lower Himalaya.

Acknowledgements

This work was financially assisted by research grants provided by International Foundation for Science, Sweden (IFS - C/5429-1) and Higher Education Commission (HEC - No: PD-IPFP/HRD/HEC/2013/3003) of Pakistan.

Author Contributions

Funding acquisition: FH; Data curation; TZ, FH and AI;

Methodology: TZ, FH, WQ, Al, RN and MI; Project administration: FH and AI; Resources, FH, RN and MB; Supervision: FH and MB; Data analyses and technical input: TZ, FH and SAA; Writing – original draft: TZ, FH, SAA and AI; Writing–review & editing, FH, MB, RN, SH, SAA, MB and MI.

References

Alam MK, RW Bell, ME Haque, MA Islam, MA Kader (2020). Soil nitrogen storage and availability to crops are increased by conservation agriculture practices in rice–based cropping systems in the Eastern Gangetic Plains. *Field Crops Res* 250:1–14

Amoah-Antwi C, J Kwiatkowska-Malina, SF Thornton, O Fenton, G Malina, E Szara (2020). Restoration of soil quality using biochar and brown coal waste: A review. *Sci Total Environ* 722; Article 137852

Ashraf MN, T Aziz, MA Maqsood, HM Bilal, S Raza, M Zia, A Mustafa, M Xu, Y Wang (2019). Evaluating organic materials coating on urea as potential nitrification inhibitors for enhanced nitrogen recovery and growth of maize (*Zea mays*). *Intl J Agric Biol* 22:1102–1108

Beltz JK, H McMahon, IT Nunez, AE Bernhard (2020). Vegetation-dependent response to drought in salt marsh ammonia-oxidizer communities. *Microorganisms* 8:9–22

Brevik E (2013). The potential impact of climate change on soil properties and processes and corresponding influence on food security. *Agriculture* 3:398–417

Butterbach-Bahl K, EM Baggs, M Dannenmann, R Kiese, S Zechmeister-Boltenstern (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Phil Trans Soc B Biol Sci* 368:1–13

Chen L, Y Wen, J Zeng, H Wang, J Wang, B Dell, S Liu (2019). Differential responses of net n mineralization and nitrification to throughfall reduction in a *Castanopsis hystrix* plantation in southern China. *For Ecosyst* 6:14–24

Dan X, Z Chen, S Dai, X He, Z Cai, J Zhang, C Müller (2019). Effects of changing temperature on gross N transformation rates in acidic subtropical forest soils. *Forests* 10; Article 894

Di HJ, KC Cameron, JP Shen, JZ He, CS Winefield (2009). A lysimeter study of nitrate leaching from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea. *Soil Use Manage* 25:454–461

Fakhraei H, TJ Fahey, CT Driscoll (2020). The Biogeochemical Response of Nitrate and Potassium to Landscape Disturbance in Watersheds of the Hubbard Brook Experimental Forest, New Hampshire, USA. *In: Forest-Water Interactions*, pp:537–563. Springer International Publishing, Cham, Switzerland

Gee GW, D Or (2002). Particle-size analysis. *In: Methods of Soil Analysis Part 4- Physical Methods*, pp:255–294. Dane JH, GC Topp (Eds.). Soil Science Society of America, Incorporation, Madison, Wisconsin, USA

Green SM, JAJ Dungait, C Tu, HL Buss, N Sanderson, SJ Hawkes, TA Quine (2019). Soil functions and ecosystem services research in the Chinese karst Critical Zone. *Chem Geol* 527; Article 119107

Guillot E, P Hinsinger, L Dufour, J Roy, I Bertrand (2019). With or without trees: Resistance and resilience of soil microbial communities to drought and heat stress in a Mediterranean agroforestry system. *Soil Biol Biochem* 129:122–135

Hafeez F, L Philippot, A Spor, F Martin-Laurent (2014). Assessment of the resilience and resistance of remediated soils using denitrification as model process. *J Soils Sedim* 14:178–182

Hafeez F, A Spor, MC Breuil, C Schwartz, F Martin-Laurent, L Philippot (2012). Distribution of bacteria and nitrogen-cycling microbial communities along constructed Technosol depth-profiles. *J Hazard Mater* 231–232:88–97

Harrison MT, BR Cullen, RP Rawnsley (2016). Modelling the sensitivity of agricultural systems to climate change and extreme climatic events. *Agric Syst* 148:135–148

Hoffmann H, M Schloter, BM Wilke (2007). Microscale-scale measurement of potential nitrification rates of soil aggregates. *Biol Fert* 44:411–413

Huang L, J Bai, X Wen, G Zhang, C Zhang, B Cui, X Liu (2020). Microbial resistance and resilience in response to environmental changes under the higher intensity of human activities than global average level. *Glob Chang Biol* 26:2377–2389

Jha S, R Srivastava (2018). Impact of drought on vegetation carbon storage in arid and semi-arid regions. *Rem Sens Appl Soc Environ* 11:22–29

Kang W, G Chen, J Wang, L Huang, L Wang, R Li, JP Smol (2019). Assessing the impact of long-term changes in climate and atmospheric deposition on a shallow alpine lake from southeast Tibet. *Sci Total Environ* 650:713–724

Leininger S, T Urich, M Schloter, L Schwark, J Qi, GW Nicol, C Schleper (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442:806–809

Li T, X Chen, W Xu, S Chi, W Zhao, Y Li, C Zhang, D Feng, Z He, Z Wang (2018). Effects of coated slow-release fertilizer with urease and nitrification inhibitors on nitrogen release characteristic and uptake and utilization of nitrogen, phosphorus and potassium in cabbage. *Intl J Agric Biol* 20:422–430

Li W, Y Sun, G Li, Z Liu, H Wang, D Zhang (2017). Contributions of nitrification and denitrification to N_2O emissions from aged refuse bioreactor at different feeding loads of ammonia substrates. *Waste Manage* 68:319–328

Liu J, Z Meng, X Liu, XH Zhang (2019). Microbial assembly, interaction, functioning, activity and diversification: A review derived from community compositional data. *Mar Life Sci Technol* 1:112–128

Nelson DW, LE Sommers (1996). Total carbon, organic carbon, and organic matter. Methods soil Anal Part 3. *Chem Meth* 5:961–1010

Pajares S, BJM Bohannan (2016). Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Front Microbiol* 7; Article 1045

Paré MC, A Bedard-Haughn (2012). Landscape-scale N mineralization and greenhouse gas emissions in Canadian Cryosols. *Geoderma* 189:469–479

Pereira P, I Bogunovic, M Munoz-Rojas, EC Brevik (2017). Soil ecosystem services, sustainability, valuation and management. *Curr Opin Environ Sci Health* 5:7–13

Purkhold U, A Pommerening-Röser, S Juretschko, MC Schmid, HP Koops, M Wagner (2000). Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: Implications for molecular diversity surveys. *Appl Environ Microbiol* 66:5368–5382

Rasul G, M Afzal, M Zahid, SAA Bukhari (2012). *Climate Change in Pakistan: Focused on Sindh Province*. Pakistan Meteorological Department Technical Report No. PMD 25/2012,1214:61

Sharma V, D Dahiya, D Vasanth (2020). Characteristics of Microbial Community and Enzyme Activities in Higher Altitude Regions. *In: Microbiological Advancements for Higher Altitude Agro-Ecosystems and Sustainability*, pp: 201–226. Goel R, R Soni, DC Suyal (Eds.). Springer, Singapore

Siljanen HMP, RJE Alves, JG Ronkainen, RE Lamprecht, HR Bhattarai, A Bagnoud, C Biasi (2019). Archaeal nitrification is a key driver of high nitrous oxide emissions from arctic peatlands. *Soil Biol Biochem* 137:107539

Souza WP (1980). The responses of a community to disturbance: The importance of successional age and species' life histories. *Oecologica* 45:72–81

Starke R, N Jehmlich, F Bastida (2019). Using proteins to study how microbes contribute to soil ecosystem services: The current state and future perspectives of soil metaproteomics. *Proteomics* 198:50–58

Tahir AA, A Muhammad, Q Mahmood, SS Ahmad, Z Ullah (2015). Impact of rapid urbanization on microclimate of urban areas of Pakistan. *Air Qual Atmos Health* 8:299–306

Tan W, J Wang, W Bai, J Qi, W Chen (2020). Soil bacterial diversity correlates with precipitation and soil pH in long-term maize cropping systems. *Sci Rep* 10; Article 6012

Tan X, D Shao, W Gu (2018). Effects of temperature and soil moisture on gross nitrification and denitrification rates of a Chinese lowland paddy field soil. *Paddy Water Environ* 16:687–698

Taylor AE, DD Myrold, PJ Bottomley (2019). Temperature affects the kinetics of nitrite oxidation and nitrification coupling in four agricultural soils. *Soil Biol Biochem* 136:107523

Taylor AE, AT Giguere, CM Zoebelein, DD Myrold, PJ Bottomley (2017). Modeling of soil nitrification responses to temperature reveals thermodynamic differences between ammonia-oxidizing activity of archaea and bacteria. *ISME J* 11:896–908

Thion C, JI Prosser (2014). Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying–rewetting stress. *FEMS Microbiol Ecol* 90:380–389

Troy C, J Tang (2011). Effects of temperature and moisture stress on nitrous oxide production in agricultural soil. *In: Semester in Environmental Science*, 21 p. Villanova University, Villanova University, USA. (Available at: https://www.mbl.edu/ses/files/2014/09/Troy-Cynthia.pdf; Accessed 20 June 2020)

Ullah W, T Nihei, M Nafees, R Zaman, M Ali (2018). Understanding climate change vulnerability, adaptation and risk perceptions at household level in Khyber Pakhtunkhwa, Pakistan. *Intl J Clim Chang Strat and Manage* 10:359–378

Upton RN, EM Bach, KS Hofmockel (2018). Belowground response of prairie restoration and resiliency to drought. *Agric Ecosyst Environ* 266:122–132

Vries FTD, RI Griffiths, M Bailey, H Craig, M Girlanda, HS Gweon, S Hallin, A Kaisermann, AM Keith, M Kretzschmar, P Lemanceau, E Lumini, KE Mason, A Oliver, N Ostle, JI Prosser, C Thion, B Thomson, RD Bardgett (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nat Commun* 9; Article 3033

Wang Z, Y, Cao, AL Wright, X Shi, X Jiang (2019). Different ammonia oxidizers are responsible for nitrification in two neutral paddy soils. *Soil Till Res* 195; Article 104433

Webster PJ, VE Toma, HM Kim (2011). Were the 2010 Pakistan floods predictable? *Geophys Res Lett* 38:1–5

Wittebolle L, W Verstraete, N Boon (2009). The inoculum effect on the ammonia-oxidizing bacterial communities in parallel sequential batch reactors. *Water Res* 43:4149–4158

Yeni F, H Alpas (2017). Vulnerability of global food production to extreme climatic events. *Food Res Intl* 96:27–39

Zeng J, D Zhao, Z Yu, R Huang, QL Wu (2014). Temperature responses of ammonia-oxidizing prokaryotes in freshwater sediment microcosms. *PLoS One* 9; Article e100653

Zheng S, Q Wang, Y Yuan, W Sun (2020). Human health risk assessment of heavy metals in soil and food crops in the Pearl River Delta urban agglomeration of China. *Food Chem* 316; Article 126213