forests

MDPI

Article

Soil Nitrogen Responses to Soil Core Transplanting Along an Altitudinal Gradient in an Eastern Tibetan Forest

Li Zhang [1,2], Ao Wang [3], Fuzhong Wu [1,2], Zhenfeng Xu [1,2], Bo Tan [1,2], Yang Liu [1,2], Yulian Yang [1,2,4], Lianghua Chen [1,2] and Wanqin Yang [1,2,*]

1 Long-term Research Station of Alpine Forest Ecosystems, Institute of Ecology & Forestry, Sichuan Agricultural University, Chengdu 611130, China; zhangli19830116@hotmail.com (L.Z.); wufzchina@163.com (F.W.); sicauxzf@163.com (Z.X.); bobotan1984@163.com (B.T.); sicauliuyang@163.com (Y.L.); yangyulian2015@163.com (Y.Y.); sicauchenlh@126.com (L.C.)
2 Collaborative Innovation Center of Ecological Security in the Upper Reaches of Yangtze River, Chengdu 611130, China
3 Institute of Product Quality Inspection and Testing of Zunyi, Guizhou 563000, China; wangaocn@gmail.com
4 Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang 621000, China
* Correspondence: scyangwq@163.com; Tel.: +86-28-8629-1112

Received: 8 March 2018; Accepted: 27 April 2018; Published: 2 May 2018

Abstract: To understand the differential effects of altitudinal gradient on soil inorganic nitrogen concentration and associated ammonia-oxidizingbacteria (AOB) and archaea (AOA), intact soil cores from a primary coniferous forest were in situ incubated in an alpine forest at a 3582-m altitude (A1) and transplanted to subalpine forests at a 3298-m altitude (A2) and 3023-m altitude (A3) on the eastern Tibetan Plateau. Transplant cooled the soil temperature of A2 but warmed the A3 soil temperature. Both AOA and AOB were found at the three altitudes. Compared to A1, A2 had greater AOA and AOB abundance, but A3 showed lower AOA abundance in organic soil. The AOA abundance was negatively correlated with ammonium concentration at all three altitudes, but AOB showed the reverse trend. Our results suggested that the soil nitrogen process responded differentially to soil core transplanting at different altitudes.

Keywords: alpine forest; ammonia-oxidizing bacteria; ammonia-oxidizing archaea; ammonium; nitrate

1. Introduction

Ongoing climate change, characterized by warming winters, snow cover decline and extreme weather events, is changing the processes of terrestrial ecosystems in cold biomes. Until now, direct soil warming and snow removal experiments along latitudinal and altitudinal gradients have been widely used to understand the effects of climate warming on soil processes [1–4]. However, different soil processes have been observed during cold winters in many areas, and increasing air temperatures in the winter have led to soil cooling [5–7]. Therefore, direct soil warming experiments cannot fully reflect the impact of climate warming on soil processes in cold regions. In the alpine-gorge area, the duration and depth of seasonal snow cover, seasonal freeze-thaw cycles, and temperature vary along the altitudinal gradient within a small range [8], which provides an ideal platform for investigating the effects of warming, snow cover decline, and seasonal freeze-thaw cycles on soil processes.

As a main limiting factor for plant growth and net primary productivity, soil nitrogen availability and its responses to global environmental changes are crucial in terms of understanding how an

ecosystem will be affected by climate change [9,10]. The changes of environmental factors, such as temperature, water, and soil freezing, may consequently affect plant, soil and microbial processes and nutrient losses, and thus influence soil nitrogen process [6,11]. In high altitude and latitude regions, different elevations can lead to a continuous change in environmental factors, such as temperature, precipitation, and freeze-thaw characteristics [12–14], and the consequence of these factors may affect the soil nitrogen dynamics. To fully understand the nitrogen cycle under climate change scenarios, it is necessary to investigate the changes in soil nitrogen pools at different altitudes.

Ammonia oxidation is the first and rate-limiting step of nitrification, which plays a crucial role in the global nitrogen cycle [15]. Along with ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) have also been detected in extreme environments, such as deep marine areas, hot springs, and soils [16–19]. The active expression of AOA and AOB genes also has been detected in alpine areas [4,20–22]. In cold biomes, the seasonal soil freeze-thaw cycles create extreme conditions for soil microorganisms [23]. The dramatic temperature fluctuations lead to seasonal variations in the abundance and structure of AOA and AOB communities in alpine areas [4,23,24]. Although previous studies have documented that warming decreases the abundance of ammonia oxidizing bacteria and archaea under nitrogen fertilization [24] and the temperature influences the ammonia oxidizer population [25], the responses of AOA and AOB abundance to altitudinal gradient in both organic and mineral soils remain poorly understood.

The Tibetan Plateau is one of the most sensitive areas to global climate change [26]; it has experienced pronounced warming in recent decades that is expected to increase by 2.6–5.2 °C by 2100 [27]. Alpine forests in the upper reaches of the Yangtze River and the eastern Tibetan Plateau play important and irreplaceable roles in conserving water and soil, harboring biodiversity, sequestering atmospheric carbon dioxide, and indicating climate change [28]. As the area is affected by low temperatures and frequent geological disasters, the forest soils are characterized by a thick organic soil and a thin mineral soil [29]. However, how the inorganic nitrogen concentration and ammonia-oxidizing microbial community in both the organic and mineral soils respond to different altitudes remains unknown.

In this study, an altitudinal gradient experiment in combination with soil core transplanting was conducted to investigate the changes of soil nitrogen processes and the related ammonia-oxidizing microbial community (AOA and AOB) at different altitudes. We hypothesized that soil core transplanting might (1) increase the soil temperature at the two lower altitudes and (2) enhance the soil inorganic nitrogen concentration and related microbial abundance.

2. Materials and Methods

2.1. Site Description

This study was conducted at the Long-term Research Station of Alpine Forest Ecosystems in the Miyaluo Nature Reserve (102°53′–102°57′ E, 31°14′–31°19′ N, 2458–4619 m a.s.l.), which is located in Li County, western Sichuan, China (Figure 1). This is a transitional area situated between the Tibetan Plateau and the Sichuan Basin. The mean annual temperature is approximately 3 °C, with maximum and minimum temperatures of 23 °C (July) and −18 °C (January), respectively. The annual precipitation is approximately 850 mm. The forests consist of conifers and natural mixed hardwoods depending on the altitude and are mainly dominated by Minjiang fir (*Abies faxoniana* Rehd. et Wils.), Dragon spruce (*Picea purpurea* Mast.), and Red birch (*Betula albosinensis* Burk.). The forest soils are classified as Cambisols [30]. Seasonal soil freezing and thawing are observed in this area [31].

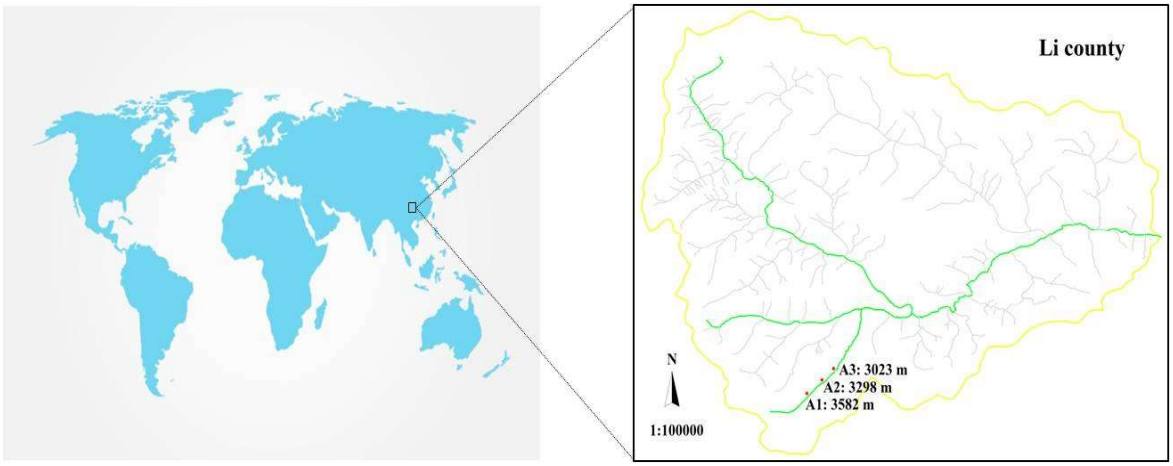

Figure 1. Location of experimental sites in this study.

2.2. Temperature Monitoring

Air temperature at 2 m height in the study site and soil temperatures (5 and 20 cm depths) were recorded at three locations using buried Thermochron iButton DS1923-F5 Recorders (Maxim/Dallas Semiconductor Corp, Sunnyvale, CA, USA) every hour between May 2010 and April 2011.

2.3. Soil Incubation

A 3 × 3 m sampling plot was randomly selected in a representative primary conifer alpine forest dominated by Minjiang fir at 3582 m. The basic properties of the organic and mineral soils are shown in Table 1. After clearing plants and fresh litter from the ground, Polyvinyl Chloride (PVC) cylinders (20 cm in length, 5 cm in diameter) were inserted into the soil to take undisturbed soil cores; forty-five soil cores were taken from this plot in May 2010. These soil cores were divided into three groups (fifteen cores in each group) and incubated in the 3582-m (A1, in situ incubate), 3298-m (A2) and 3023-m (A3) altitude sites.

Table 1. The basic properties of the soil organic layer and mineral soil layer in the eastern Tibetan forest.

	Organic Carbon ($g \cdot kg^{-1}$)	Total Nitrogen ($g \cdot kg^{-1}$)	NH_4^+ ($mg \cdot kg^{-1}$)	NO_3^- ($mg \cdot kg^{-1}$)	Bulk Density ($g \cdot cm^{-3}$)	pH
Organic soil	138.56 ± 4.04	7.28 ± 0.07	18.73 ± 0.36	140.75 ± 2.73	1.09 ± 0.05	5.6 ± 0.3
Mineral soil	25.03 ± 0.88	1.69 ± 0.03	11.32 ± 1.35	14.04 ± 1.45	1.2 ± 0.03	5.3 ± 0.2

2.4. Sample Collection

One incubated soil core was retrieved from each sampling plot during the early growing season (EGS, 24 May to 12 August 2010), late growing season (LGS, 12 August to 17 October 2010), onset of the freezing period (OFP, 17 October to 23 December 2010), freezing period (FP, 23 December 2010 to 3 March 2011), and thawing period (TP, 3 March to 19 April 2011). Soil samples of the organic and mineral soils were collected in each plot [32]. All soil samples were hand-sorted to remove gravel and coarse roots. The samples were stored in freezer boxes, transported to the laboratory within 24 h, and stored at −20 °C. Soils were sieved through a 2-mm mesh before chemical analysis.

2.5. Inorganic Nitrogen Concentration

Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the extract were measured using indophenol-blue and phenol disulfonic acid colorimetry. A 10-g soil sample from each soil core was taken, to which 50 mL 2 M KCl at room temperature was added. The mixture of soil and extractant

was shaken for 1 h. After shaking, the soil suspension was filtered (Whatman filter paper, 12.5 cm in diameter). Soil solutions were kept frozen prior to analysis for ammonium and nitrate using a TU-1901 Analyzer (Beijing Purkinje General Instrument Co. Ltd., Beijing, China).

2.6. DNA Extraction

DNA was extracted from 0.8 g to 1.0 g (fresh weight) of soil using the E.Z.N.A.® Soil DNA Kit (Omega Bio Inc., Norcross, GA, USA). The extracted DNA was checked on 1% agarose gel, and the concentration was determined using a Nanodrop® ND-1000 UV-Vis spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA).

2.7. Quantification of amoA Genes by Real-Time PCR

The *amoA* gene cloning and the method to create standard curves were as previously described [21]. The primer pairs Arch-amoAF/Arch-amoAR [33] and amoA-1F/amoA-2R [34] were used for real-time polymerase chain reaction (PCR) quantification of the archaeal and bacterial *amoA* genes, respectively. Real-time PCR was performed using the CFX96 System (Bio-Rad Laboratories Inc., Hercules, CA, USA) in 25 μL reactions containing 12.5 μL of SYBR® Premix Ex TaqTM (TaKaRa Biotechnology Co. Ltd., Dalian, China), 0.4 mg·mL^{-1} of bovine serum albumin, 200 nmol·L^{-1} of each AOA primer or 400 nmol·L^{-1} of each AOB primer, and 1 μL of DNA (1–10 ng) as the template. Three analytical replicates were performed for each soil sample. Amplifications were carried out as follows: 95 °C for 1 min followed by 40 cycles of 10 s at 95 °C, 25 s at 63 °C for AOA or 57 °C for AOB, and 1 min at 72 °C. The plates were read at 72 °C after each cycle. The product specificity was confirmed using a melting curve analysis (65–95 °C, 0.5 °C per read with a hold time of 5 s) at the end of each PCR run.

2.8. Statistical Analyses

All statistical tests were performed using the software Statistical Package for the Social Sciences (SPSS Inc., IBM, Armonk, NY, USA) version 16.0. Data were subjected to one-way analysis of variance, and significant differences between treatment means for each variable were compared by the LSD post hoc test at $p < 0.05$. The relationships between the abundances of AOA and AOB, ammonium and nitrate concentration at each altitude were tested by Pearson correlation analyses.

3. Results

3.1. Air and Soil Temperatures

The temperature dynamics of air and soil at the three altitudes from May 2010 to April 2011 are shown in Figure 2. Air temperature increased with a decrease in altitude; compared with A1, A2 and A3 increased by 1.39 °C and 2.64 °C, respectively. However, the soil annual temperature displayed different characteristics at different altitudes. Generally, compared to A1, A3 increased by 1.03 °C and 1.08 °C, but A2 decreased by 0.26 °C and 0.25 °C in the organic and mineral soils, respectively.

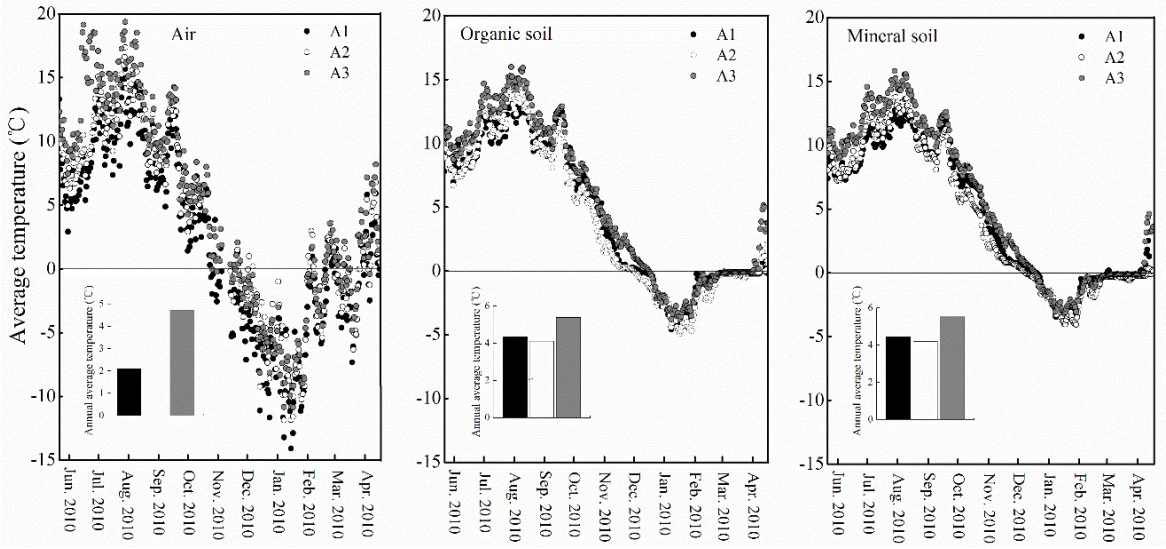

Figure 2. Daily and annual air and soil average temperatures at different altitudes. A1: 3582-m altitude; A2: 3298-m altitude; A3: 3023-m altitude. The inserts represent the mean values at the A1 (black), A2 (open) and A3 (gray) sites.

3.2. Inorganic Nitrogen Concentration

Transplanting soil significantly affected the soil inorganic nitrogen (ammonium and nitrate) concentration in both soil layers. With respect to organic soil, A3 had the highest ammonium and nitrate concentration during most of the sampling period except for the ammonium concentration during the onset of the freezing and thawing periods (Figure 3a,c). However, with respect to mineral soil, A3 showed the lowest ammonium concentration during the onset of the freezing period and during the freezing period (Figure 3a,c). In both soil layers, A2 had the highest ammonium and nitrate concentrations during the early growing season, but a lower ammonium concentration during the onset of the freezing period and the thawing period was found in the organic soil (Figure 3c). At all altitudes, the change in inorganic N concentration in the soil organic layer was more sensitive than that in the mineral soil (Figure 3c).

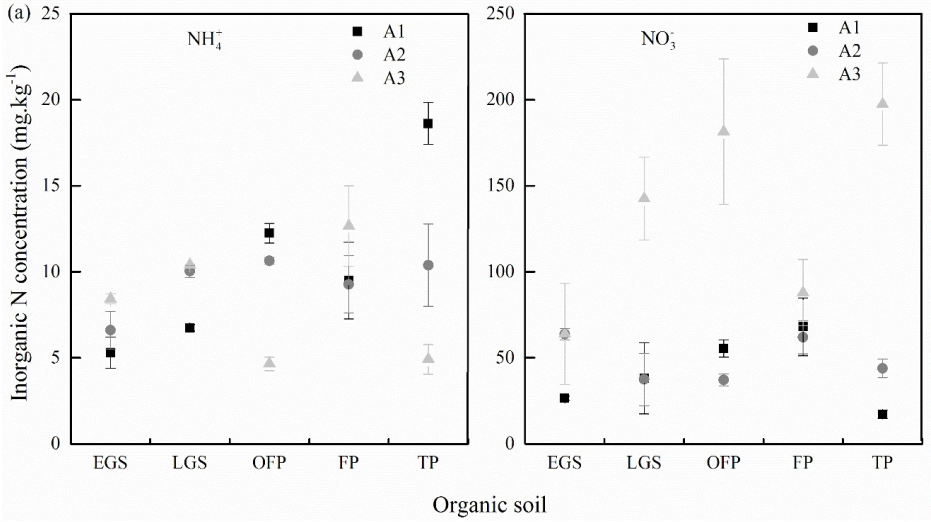

Figure 3. *Cont.*

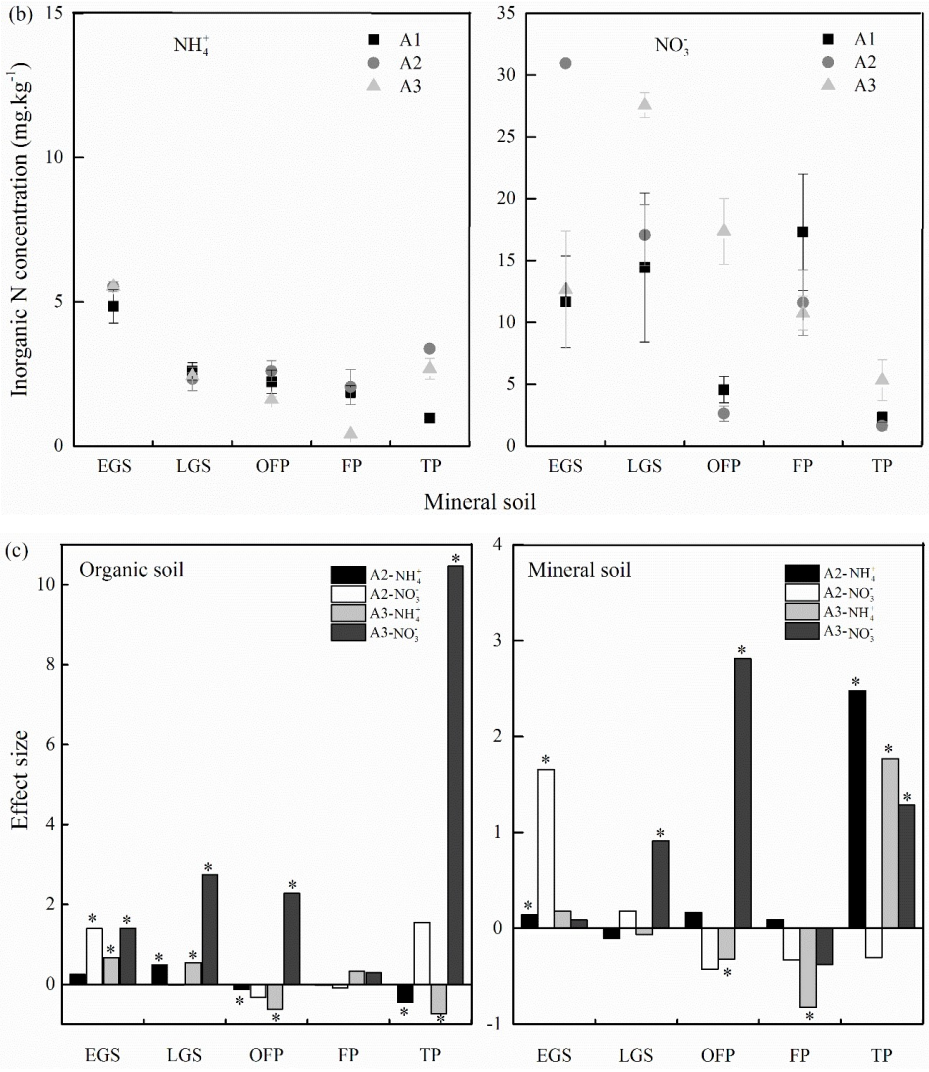

Figure 3. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in (**a**) organic and (**b**) mineral soils and (**c**) the effect size of soil core transplanting in different periods. The effect size is the average difference in the ammonium and nitrate concentration at A2 or A3 with respect to the original site (A1), A2 = (A2 − A1)/A1 and A3 = (A3 − A1)/A1. EGS: early growing season, LGS: late growing season, OFP: onset of freezing period, FP: freezing period, TP: thawing period. A1: 3582-m altitude; A2: 3298-m altitude; A3: 3023-m altitude. * indicates a significant difference ($p < 0.05$) between different altitudes.

3.3. Abundance of amoA Genes

Both archaeal *amoA* genes (8.15×10^4 to 1.50×10^9 g^{-1} soil) and bacterial *amoA* genes (1.11×10^5 to 1.20×10^8 g^{-1} soil) were detected in the soil at the three altitudes (Figure 4). Compared to A1, A2 had greater AOA and AOB abundance at most sampling times, except for the AOA abundance during the early growing season in the organic soil and the AOB abundance during the freezing stage in the mineral soil (Figure 4c). A3 had lower AOA abundance but greater AOB abundance in organic soil compared to A1. Regarding mineral soil, A3 had lower AOA and AOB abundance during the early growing season and at the onset of the freezing period, but greater abundance was observed during other sampling periods. The abundance of AOB and AOA showed significant correlations with the ammonium and nitrate concentration, respectively, at 3023 m altitude (Table 2).

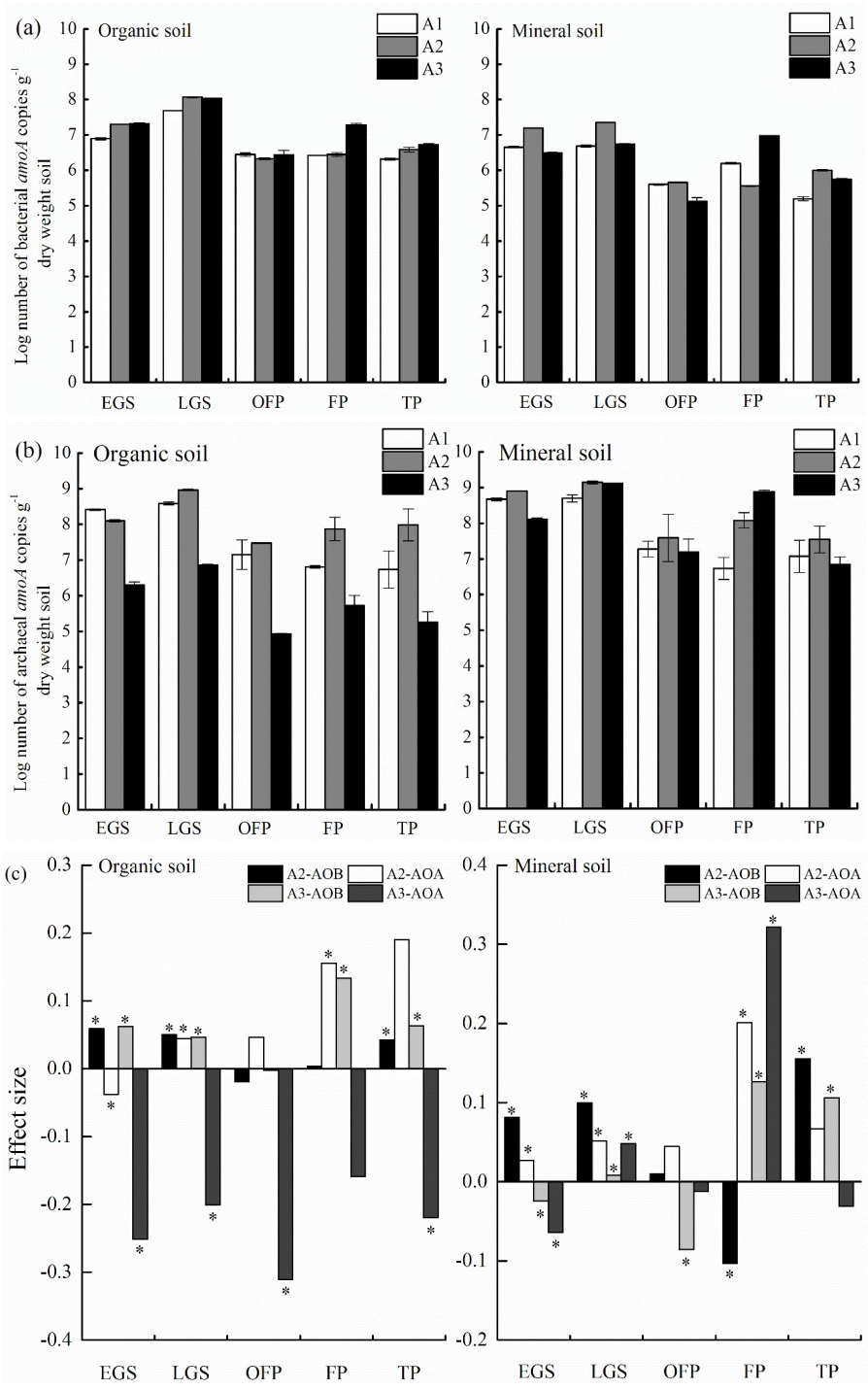

Figure 4. (**a**) Bacterial and (**b**) archaeal *amoA* gene copy numbers at different altitudes (mean ± SD, n = 3) and (**c**) the effect size of soil core transplanting in different periods. The effect size is the average difference in the ammonium and nitrate concentration at A2 or A3 with respect to the original site (A1). A2 = (A2 − A1)/A1 or A3 = (A3 − A1)/A1. A1: 3582-m altitude; A2: 3298-m altitude; A3: 3023-m altitude. EGS: early growing season; LGS: late growing season; OFP: onset of freezing period; FP: freezing period; TP: thawing period. * indicates a significant difference ($p < 0.05$) between different altitudes.

Table 2. Correlation analyses among the abundances of AOA, AOB and NH_4^+, NO_3^- at different altitudes.

Altitude		Abundance of AOB	Abundance of AOA	NH_4^+	NO_3^-
3582 m	Abundance of AOB	1	0.632 *	0.251	0.445
	Abundance of AOA		1	−0.323	−0.163
	NH_4^+			1	0.478
	NO_3^-				1
3298 m	Abundance of AOB	1	0.777 **	0.389	0.486
	Abundance of AOA			−0.093	0.076
	NH_4^+				0.741 *
	NO_3^-				1
3032 m	Abundance of AOB	1	−0.060	0.677 *	0.392
	Abundance of AOA			−0.493	−0.772 **
	NH_4^+				0.420
	NO_3^-				1

AOB is ammonia-oxidizing bacteria, AOA is ammonia-oxidizing archaea, NH_4^+ is ammonium, NO_3^- is nitrate. ** indicates significant difference at $p < 0.01$ (two-tailed). * indicates significant difference at $p < 0.05$ (two-tailed).

4. Discussion

Soil nitrogen cycling is one of the most important ecological processes in forest ecosystems [35]. Previous studies subdivided N cycling into decomposition processes, assimilative processes and dissimilative processes [36]. The uptake and utilization of ammonium or nitrate by plants and microorganisms for growth and replication were included in assimilative processes [36]. This soil core transplanting experiment was used to study the change and relationship between inorganic nitrogen (ammonium and nitrate) concentration and N-related (AOA and AOB) microorganisms. The observation in this study indicated that the soil transplant cooled A2 but warmed A3 winter soil temperature, respectively. This change may be related not only to the air temperature but also to the winter snow cover. The depth and duration of the snowpack is considered to be the important indirect effect of winter climate change [37,38]. Our previous study demonstrated that the snow depth decreased with decreasing altitude in this area [39]. The thickest snow cover in A1 may offer an ideal combination of moister and warmer soil conditions that can keep the soil warm [39,40]. However, the thinner and shorter duration of snow cover at A3 had a more sensitive response to solar radiation [41], which may produce a higher soil temperature.

Soil microorganisms are important drivers of soil quality and ecosystem function. Research on the spatial variations of AOB and AOA activity and their unique contributions to nitrification is needed [42]. Changes in environmental factors, such as elevation, N fertilization, temperature, and pH, may affect ammonia-oxidizing microorganisms in soil ecosystems. A previous study has pointed out that AOB were significantly higher than AOA during a soil warming and fertilization treatment [24], but the AOA were more abundant than AOB in a long-term fertilized soil [16]. This suggests that AOA may be more active than AOB in acidic soils, whereas this may be the opposite in alkaline soil [42]. However, the key factors are still difficult to assess [43]. A study at Mount Everest indicated that the AOA abundance increased along an altitudinal gradient decrease, whereas that of AOB did not shift significantly with altitude, suggesting that AOA may be more sensitive than AOB in response to elevated soil conditions [4]. In this study, we found that the samples at A2 had greater abundance of AOA and AOB than A1 at most sampling times, except for AOA in the early growing season and AOB in the freezing period in organic and mineral soils, respectively. Although previous studies have shown that a lower temperature may prevent microbial activity and even kill certain microbes [21,44], some tolerant or adaptive species may survive and replicate [45–47], to improve the microbial abundance of A2. At A3, the abundance of AOA and AOB was inconsistent in organic and mineral soils. In contrast to a previous study, temperature had a negative correlation with AOB but a positive correlation with AOA in a temperate beech forest soil [48]. However, AOA and AOB abundance was positively

correlated with temperature [49]. Increased temperature at A3 resulted in a lower abundance of AOA and a greater abundance of AOB in the organic soil. This may be affected by the different responses to the environmental variation of AOA and AOB in alpine areas [4,50,51].

Inorganic N, as a common substrate, influenced AOA and AOB abundance. The concentration of ammonium in soil has been identified as an important factor driving the relative distributions of AOA and AOB [52]. In the investigated alpine forest, the abundance of AOA varied almost inversely with the measured ammonium concentration at all sampling altitudes, while that of AOB varied positively with ammonium concentration (Table 2), suggesting that ammonium may drive the separation between AOA and AOB [53]. In this study, the abundance of AOB was higher at A2 and A3 during most of the sampling period (Figure 4c). Higher ammonium concentration was observed only in the early growing season and later growing season, likely due to N mineralization over the winter [41]. This result suggests that inorganic N may determine the distribution of AOA and AOB in alpine forest soils by providing the substrate for microbial mineralization.

5. Conclusions

In summary, AOA and AOB abundance was recorded in winter in this alpine forest. Although the soil temperature was cooled at A2, higher microbial abundance was observed at A2. The increased temperature at A3 decreased the AOA abundance in the organic soil. The concentration of ammonium was positively correlation with AOB abundance and negatively with AOA abundance.

Author Contributions: W.Y. and F.W. conceived and designed the experiments; A.W., Y.L. and L.C. performed the experiments; L.Z., B.T., Y.Y. and Z.X. analyzed the data, W.Y. contributed reagents/materials/analysis tools; L.Z., and W.Y. wrote the paper.

Funding: This project was financially supported by the National Natural Science Foundation of China (Nos. 31570445, 31500509, 31570601).

Acknowledgments: We are very grateful to the insightful comments and useful suggestions from the reviewers.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Tan, B.; Wu, F.Z.; Yang, W.Q.; He, X.H. Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest. *Appl. Soil Ecol.* **2014**, *76*, 34–41. [CrossRef]
2. Xu, Z.F.; Hu, R.; Xiong, P.; Wan, C.; Cao, G.; Liu, Q. Initial soil responses to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China: Nutrient availabilities, microbial properties and enzyme activities. *Appl. Soil Ecol.* **2010**, *46*, 291–299. [CrossRef]
3. Xu, Z.F.; Liu, Q.; Yin, H.J. Effects of temperature on soil net nitrogen mineralisation in two contrasting forests on the eastern Tibetan Plateau, China. *Soil Res.* **2014**, *52*, 562–567. [CrossRef]
4. Zhang, L.M.; Wang, M.; Prosser, J.I.; Zheng, Y.M.; He, J.Z. Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. *FEMS Microbiol. Ecol.* **2009**, *70*, 208–217. [CrossRef] [PubMed]
5. Edwards, A.C.; Scalenghe, R.; Freppaz, M. Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. *Quat. Int.* **2007**, *162*, 172–181. [CrossRef]
6. Groffman, P.M.; Driscoll, C.T.; Fahey, T.J.; Hardy, J.P.; Fitzhugh, R.D. Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* **2001**, *56*, 135–150. [CrossRef]
7. Williams, M.W.; Helmig, D.; Blanken, P. White on green: Under-snow microbial processes and trace gas fluxes through snow, Niwot Ridge, Colorado Front Range. *Biogeochemistry* **2009**, *95*, 1–12. [CrossRef]
8. Zhu, J.; Yang, W.; He, X. Temporal dynamics of abiotic and biotic factors on leaf litter of three plant species in relation to decomposition rate along a subalpine elevation gradient. *PLoS ONE* **2013**, *8*, e62073. [CrossRef] [PubMed]
9. Hungate, B.A.; Dukes, J.S.; Shaw, M.R.; Luo, Y.; Field, C.B. Atmospheric science. Nitrogen and climate change. *Science* **2003**, *302*, 1512–1513. [CrossRef] [PubMed]

10. Rustad, L.E.; Campbell, J.L.; Marion, G.M.; Norby, R.J.; Mitchell, M.J.; Hartley, A.E.; Cornelissen, J.H.C.; Gurevitch, J. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **2001**, *126*, 543–562. [CrossRef] [PubMed]
11. Groffman, P.M.; Hardy, J.P.; Fashu-Kanu, S.; Driscoll, C.T.; Cleavitt, N.L.; Fahey, T.J.; Fisk, M.C. Snow depth, soil freezing and nitrogen cycling in a northern hardwood forest landscape. *Biogeochemistry* **2011**, *102*, 223–238. [CrossRef]
12. Campbell, J.L.; Mitchell, M.J.; Groffman, P.M.; Christenson, L.M.; Hardy, J.P. Winter in northeastern North America: A critical period for ecological processes. *Front. Ecol. Environ.* **2005**, *3*, 314–322. [CrossRef]
13. Freppaz, M.; Williams, B.L.; Edwards, A.C.; Scalenghe, R.; Zanini, E. Simulating soil freeze/thaw cycles typical of winter alpine conditions: Implications for N and P availability. *Appl. Soil Ecol.* **2007**, *35*, 247–255. [CrossRef]
14. Kreyling, J. Winter climate change: A critical factor for temperate vegetation performance. *Ecology* **2010**, *91*, 1939–1948. [CrossRef] [PubMed]
15. Prosser, J.I. Autotrophic nitrification in bacteria. *Adv. Microb. Physiol.* **1989**, *30*, 125–181. [CrossRef] [PubMed]
16. He, J.Z.; Shen, J.P.; Zhang, L.M.; Zhu, Y.G.; Zheng, Y.M.; Xu, M.G.; Di, H.J. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environ. Microbiol.* **2007**, *9*, 2364–2374. [CrossRef] [PubMed]
17. Könneke, M.; Bernhard, A.E.; de la Torre, J.R.; Walker, C.B.; Waterbury, J.B.; Stahl, D.A. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **2005**, *437*, 543–546. [CrossRef] [PubMed]
18. Leininger, S.; Urich, T.; Schloter, M.; Schwark, L.; Qi, J.; Nicol, W.; Prosser, J.I.; Schuster, S.C.; Schleper, C. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **2006**, *442*, 806–809. [CrossRef] [PubMed]
19. Reigstad, L.J.; Richter, A.; Daims, H.; Urich, T.; Schwark, L.; Schleper, C. Nitrification in terrestrial hot springs of Iceland and Kamchatka. *FEMS Microbiol. Ecol.* **2008**, *64*, 167–174. [CrossRef] [PubMed]
20. Auguet, J.C.; Nomokonova, N.; Camarero, L.; Casamayor, E.O. Seasonal changes of freshwater ammonia-oxidizing archaeal assemblages and nitrogen species in oligotrophic alpine lakes. *Appl. Environ. Microbiol.* **2011**, *77*, 1937–1945. [CrossRef] [PubMed]
21. Wang, A.; Wu, F.Z.; Yang, W.Q.; Wu, Z.C.; Wang, X.X.; Tan, B. Abundance and composition dynamics of soil ammonia-oxidizing archaea in an alpine fir forest on the eastern Tibetan Plateau of China. *Can. J. Microbiol.* **2012**, *58*, 572–580. [CrossRef] [PubMed]
22. Wang, A.; Wu, F.Z.; He, Z.H.; Xu, Z.F.; Liu, Y.; Tan, B.; Yang, W.Q. Characteristics of ammonia-oxidizing bacteria and ammonia-oxidizing archaea abundance in soil organic layer under the subslpine/alpine forest. *Acta Ecol. Sin.* **2012**, *32*, 4371–4378. [CrossRef]
23. Henry, H.A.L. Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biol. Biochem.* **2007**, *39*, 977–986. [CrossRef]
24. Long, X.; Chen, C.; Xu, Z.; Linder, S.; He, J. Abundance and community structure of ammonia oxidizing bacteria and archaea in a Sweden boreal forest soil under 19-year fertilization and 12-year warming. *J. Soils Sediments* **2012**, *12*, 1124–1133. [CrossRef]
25. Avrahami, S.; Conrad, R. Patterns of community change among ammonia oxidizers in meadow soils upon long-term inubation at different temperatures. *Appl. Environ. Microbiol.* **2003**, *69*, 6152–6164. [CrossRef] [PubMed]
26. Liu, X.; Chen, B. Climatic warming in the Tibetan Plateau during recent decades. *Int. J. Climatol.* **2000**, *20*, 1729–1742. [CrossRef]
27. Chen, H.; Zhu, Q.; Peng, C.; Wu, N.; Wang, Y.; Fang, X.; Gao, Y.; Zhu, D.; Yang, G.; Tian, J.; et al. The impacts of chimate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. *Glob. Chang. Biol.* **2013**, *19*, 2940–2955. [CrossRef] [PubMed]
28. Yang, W.Q.; Feng, R.F.; Zhang, J.; Wang, K.Y. Carbon stock and biochemical properties in the organic layer and mineral soil under three subalpine forests in Western China. *Acta Ecol. Sin.* **2007**, *27*, 4157–4165. [CrossRef]
29. Feng, R.F.; Yang, W.Q.; Zhang, J. Review on biochemical property in forest soil organic layer and its responses to climate change. *Chin. J. Appl. Environ. Biol.* **2006**, *12*, 734–739. [CrossRef]

30. Gong, Z.T.; Zhang, G.L.; Chen, Z.C. *Pedogenesis and Soil Taxonomy*; Science Press: Beijing, China, 2007; ISBN 9787030196538.
31. Tan, B.; Wu, F.Z.; Yang, W.Q.; Liu, L.; Yu, S. Characteristics of soil animal community in the subalpine/alpine forests of western Sichuan during onset of freezing. *Acta Ecol. Sin.* **2010**, *30*, 93–99. [CrossRef]
32. Xu, W.; Li, W.; Jiang, P.; Wang, H.; Bai, E. Distinct temperature sensitivity of soil carbon decomposition in forest organic layer and mineral soil. *Sci. Rep.* **2014**, *4*, 6512. [CrossRef] [PubMed]
33. Francis, C.A.; Roberts, K.J.; Beman, J.M.; Santoro, A.E.; Oakley, B.B. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *PNAS* **2005**, *102*, 14683–14688. [CrossRef] [PubMed]
34. Rotthauwe, J.H.; Witzel, K.P.; Liesack, W. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl. Environ. Microbiol.* **1997**, *63*, 4704–4712. [CrossRef] [PubMed]
35. Binkley, D.; Hart, S.C. The Components of Nitrogen Availability Assessments in Forest Soils. In *Advances in Soil Science*, 1st ed.; Stewart, B.A., Ed.; Springer: New York, NY, USA, 1989; pp. 57–112. ISBN 978-1461387732.
36. Levy-Booth, D.J.; Prescott, C.E.; Grayston, S.J. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biol. Biochem.* **2014**, *75*, 11–25. [CrossRef]
37. Mellander, P.E.; Löfvenius, M.O.; Laudon, H. Climate change impact on snow and soil temperature in boreal Scots pine stands. *Clim. Chang.* **2007**, *85*, 179–193. [CrossRef]
38. Pederson, G.T.; Gray, S.T.; Woodhouse, C.A.; Betancourt, J.L.; Fagre, D.B.; Littell, J.S.; Emma, W.; Luckman, B.H.; Graumlich, L.J. The unusual nature of recent snowpack declines in the North American cordillera. *Science* **2011**, *333*, 332–335. [CrossRef] [PubMed]
39. Wu, Q. Effects of Forest Gap on Foliar Litter Decomposition in the Subalpine/Alpine Forest of Western Sichuan. Ph.D. Thesis, Sichuan Agricultural University, Chengdu, China, 2014. Available online: http://cdmd.cnki.com.cn/Article/CDMD-10626-1016050649.htm (accessed on 3 June 2014).
40. Freppaz, M.; Williams, M.W.; Seastedt, T.; Filippa, G. Response of soil organic and inorganic nutrients in alpine soil to a 16-year factorial snow and *N*-fertilization experiment, Colorado Front Range, USA. *Appl. Soil Ecol.* **2012**, *62*, 131–141. [CrossRef]
41. Yin, R.; Xu, Z.F.; Wu, F.Z.; Yang, W.Q.; Li, Z.P.; Xiong, L.; Xiao, S.; Wang, B. Effects of snow pack on wintertime soil nitrogen transformation in two subalpine forests of western Sichuan. *Acta Ecol. Sin.* **2014**, *34*, 2061–2067. [CrossRef]
42. Shen, J.P.; Zhang, L.M.; Di, H.J.; He, J.Z. A review of ammonia-oxidizing bacteria and archaea in Chinese soils. *Front. Microbiol.* **2012**, *3*, 296–302. [CrossRef] [PubMed]
43. Erguder, T.H.; Boon, N.; Wittebolle, L.; Marzorati, M.; Verstraete, W. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiol. Rev.* **2009**, *33*, 855–869. [CrossRef] [PubMed]
44. Edwards, K.A.; McCulloch, J.; Peter Kershaw, G.; Jefferies, R.L. Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. *Soil Biol. Biochem.* **2006**, *38*, 2843–2851. [CrossRef]
45. Walker, V.K.; Palmer, G.R.; Voordouw, G. Freeze-Thaw Tolerance and Clues to the Winter Survival of a Soil Community. *Appl. Environ. Microbiol.* **2006**, *72*, 1784–1792. [CrossRef] [PubMed]
46. Wilson, S.L.; Walker, V.K. Selection of low-temperature resistance in bacteria and potential applications. *Environ. Technol.* **2010**, *31*, 943–956. [CrossRef] [PubMed]
47. Drotz, S.H.; Sparrman, T.; Nilsson, M.B.; Schleucher, J.; Öquist, M.G. Both catabolic and anabolic heterotrophic microbial activity proceed in frozen soils. *PNAS* **2010**, *107*, 21046–21051. [CrossRef] [PubMed]
48. Rasche, F.; Knapp, D.; Kaiser, C.; Koranda, M.; Kitzler, B.; Zechmeister-Boltenstern, S.; Richter, A.; Sessitsch, A. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME J.* **2011**, *5*, 389–402. [CrossRef] [PubMed]
49. Cao, H.; Li, M.; Hong, Y.; Gu, J.D. Diversity and abundance of ammonina-oxidizing archaea and bacteria in polluted mangrove sediment. *Syst. Appl. Microbiol.* **2011**, *34*, 513–523. [CrossRef] [PubMed]
50. Cavicchioli, R. Cold-adapted archaea. *Nat. Rev. Microbiol.* **2006**, *14*, 331–343. [CrossRef] [PubMed]
51. Tan, B.; Wu, F.; Yang, W.; Yu, S.; Liu, L.; Wang, A. The dynamics pattern of soil carbon and nutrients as soil thawing proceeded in the alpine/subalpine forest. *Acta Agric. Scand. B-SP* **2011**, *61*, 670–679. [CrossRef]

52. Regan, K.; Stempfhuber, B.; Schloter, M.; Rasche, F.; Prati, D.; Philippot, L.; Boeddinghaus, R.S.; Kandeler, E.; Marhan, S. Spatial and temporal dynamics of nitrogen fixing, nitrifying and denitrifying microbes in an unfertilized grassland soil. *Soil Biol. Biochem.* **2017**, *109*, 214–226. [CrossRef]
53. Prosser, J.I.; Nicol, G.W. Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialization and differentiation. *Trends Microbiol.* **2012**, *20*, 523–531. [CrossRef] [PubMed]