Wildfire effects on ecosystem nitrogen cycling in a Chinese boreal larch forest, revealed by ^{15}N natural abundance

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Abstract. Wildfire is reported to exert strong influences on N cycling, particularly during the early succession period immediately after burning (i.e., < 1 year). Previous studies have mainly focused on wildfires influences on inorganic N concentrations and N mineralization rates; but plant and soil ^{15}N natural abundance (expressed by $\delta^{15}N$), as a spatial-temporal integrator of ecosystem N cycling, could provide a more comprehensive understanding of wildfire on various N cycling processes at a relatively broader time scale. In this study, we attempted to evaluate legacy effects of wildfire on nitrogen cycling using $\delta^{15}N$ in a boreal forest of northeastern China, which is an important yet understudied ecosystem. We measured inorganic N concentrations (NH_4^+ and NO_3^-) and net N transformation rates (net ammonification, net nitrification, and net mineralization) of organic and mineral soil 4 years after a wildfire and compared with unburned area. We also measured $\delta^{15}N$ of plant and soil samples in 4 and 5 years after the fire. We found that even 4 years after burning, net mineralization and net ammonification in the organic soil were still higher than those in the unburned area. NH_4^+ and total inorganic N (TIN) concentrations in the organic soil of the burned area did not significantly differ from those of the unburned area. Organic soil and foliar $\delta^{15}N$ were significantly higher (by 2.2‰ and 7.4‰, respectively) in the burned area than those in the unburned area. Five years after fire, $\delta^{15}N$ of plant tissues such as foliar, branch, fine roots and moss in the burned area were significantly greater (by 1.7‰ to 6.4‰) than that in unburned area. $\delta^{15}N$ of Oi, Oa+e and 0-10 cm mineral soil were also significantly higher in the burned area than unburned area, but showed no significant difference in deeper layer of mineral soil. The observed soil ^{15}N enrichment might be attributed to various mechanisms such as NH_3 volatilization, combustion, litter return, and denitrification. Greater dependence of plant on deeper soil N and less dependence on mycorrhizal fungi in the burned area might also have contributed to the increase of the ^{15}N in plant and soil. Such ^{15}N enrichments in soil and plant suggest that N cycling could remain openness years after fire disturbance has occurred, with N supply exceeding demand, leading to a great amount of nitrogen loss from the system for a relatively long time.

1 Introduction

Wildfire-induced nitrogen (N) cycling changes can greatly alter ecosystem structure and functions, such as species composition and biodiversity (Gallant *et al*., 2003), biogeochemical cycles and productivity (Boerner, 1982; Chorover *et al*., 1994; Woodmansee and Wallach, 1981), as N is likely to be the most essential element limiting plant growth in terrestrial ecosystems (Elser *et al*. 2007, Harpole *et al*. 2011; Lebauer and Treseder, 2008; Vitousek and Howarth 1991). In response to recent global climate changes, wildfire frequency and extent in temperate and boreal forests are projected to be enhanced (Flannigan *et al*., 2009; Lucash *et al*., 2014; Westerling *et al*., 2006). Therefore, a better understanding of wildfire effects on N dynamics is of growing importance. Many studies have attempted to examine the effects of fire on N cycles through the analysis of available N concentrations and N mineralization rates (e.g., Turner *et al*., 2007; Koyama *et al*., 2010; Deluca *et al*., 2006). However, two principal limitations have greatly challenged this objective. First, these two indices vary significantly in space and time (Cain *et al*., 1999; Hu *et al*., 2013). Second, soil available N concentrations and N mineralization rates only account for a fraction of N cycling processes. Other N-related processes such as denitrification and leaching are also important in forest ecosystems (Fang *et al*., 2015), yet these processes are difficult to measure directly, thus constraining our ability to generalize the response of N cycle to wildfire.

The natural abundance of $^{15}N/^{14}N$ of plant and soil is considered as a more time- and space- integrator of N cycling than available N concentration and N mineralization rate, and could reflect the openness of ecosystem N cycling (Robinson, 2001). Soil processes, such as N mineralization, nitrification, denitrification and NH_3 volatilization, discriminate against ^{15}N and lead to soil N pool with different $\delta^{15}N$ signatures (Craine *et al*., 20015), which further express in the values of plant $\delta^{15}N$ that utilize these N pools for their N demands. Higher values of ^{15}N in soil and plant generally indicate larger N losses through ammonia volatilization, nitrification or denitrification (Craine *et al*., 2009; Houle *et al*., 2014; Matsushima *et al*., 2012). Thus, the ecosystems with a more open N cycle tend to be isotopically enriched in ^{15}N (Martinelli *et al*., 1999). In fact, although the responses of available N concentration and N mineralization to wildfire could vary by time and space, we can expect higher values of $\delta^{15}N$ in plant and soil as a legacy of longer or short term opening in the N cycles when available N supply exceeds demand, resulting in an increase in N loss. Therefore, $\delta^{15}N$ could provide us a promising and comprehensive tool to detect the legacy effect of wildfire on N cycling openness years after the fire disturbance has occurred.

Boreal forests are typical N limited ecosystems where N cycling is slow and a large proportion of total N capital is tied up in undecomposed organic matter (Hyodo *et al*., 2013; Metcalfe *et al*., 2013; Popova *et al*., 2013; Sah *et al*., 2006). This is consistent with low $\delta^{15}N$ values of soil and plant (Hogberg, 1997). Wildfire is a primary agent of disturbance in boreal forests and has a profound impact on N cycling (Baird *et al*., 1999; Bond-Lamberty *et al*., 2006). Wildfires consume N from vegetation and the upper surface soil layer, resulting in a reduction of N storage in burned forest (Hogberg, 1997; Hyodo *et* *al*., 2013). Estimates of post-fire inorganic N concentrations and mineralization rates vary, but most studies show an immediate increase in inorganic nitrogen (Deluca and Sala, 2006; Koyama *et al*., 2012; Turner *et al*., 2007). Nevertheless, the immediately increased NH_4^+ can decline to the pre-fire level within the first year and the elevated NO_3^- generally returned to pre-fire level within 5 years (Wan *et al*. 2001). Studies have also shown a wildfire-induced pulse in foliar $\delta^{15}N$ (LeDuc *et al*., 2013). However, this wildfire-induced pulse in foliar $\delta^{15}N$ is shown short-lived followed by a rapid decline after the first few years (LeDuc *et al*., 2013; Szpak, 2014). Compared to much attention paid to these changes in N dynamics at the 0-3 years after a fire event in boreal forest (Baird *et al*., 1999; DeLuca and Zouhar, 2000; White, 1986), little is known about the legacy effects of fires on N cycling over a long time period following this pulse, particularly through the use of natural abundance of N isotopes to detect the responsible processes.

The Great Xing'an Mountains of northeast China are located on the southern extension of the larch forests of the eastern Siberia. It has been reported that the boreal forests in Northeast China stored 1.0 - 1.5 Pg C and provided approximately 24 - 31% of the total timber production in China (Fang *et al*., 2001). This region experiences frequent wildfires with historical fire return intervals of 30-120 years (Xu *et al*., 1997). Despite the knowledge that fires can significantly influence soil N dynamics elsewhere, the understanding on the influence of post-fire N cycling in this region is limited. Previous studies have investigated the responses of soil inorganic N concentration and N mineralization to wildfire in this region (Kong *et al.*, 2015), and showed that a pulse of inorganic nitrogen concentration appeared in one year post-fire. However, there are few studies of response of soil inorganic N and mineralization to fire in this boreal larch forest over a relatively longer post-fire time period, and even less regarding the fire effects on plant and soil $\delta^{15}N$.

In this study, we compared the N status of burned area (4 and 5 years after a large wildfire) with unburned area through comprehensive analysis of $\delta^{15}N$ for foliage and soil, inorganic nitrogen concentrations and N mineralization rates. Our overarching goal was to determine the legacy effect of the wildfire on N cycling in boreal forest of Greater Xing'an Mountains and explore the underlying mechanism. Specifically, we expected that:

1) The inorganic nitrogen concentration and N mineralization rate in the burned area would be similar with the unburned area since most studies have shown that available N concentrations and mineralization rates declined to the pre-fire level within 5 years after fire;

2) Soil $\delta^{15}N$ would be higher in the burned area than unburned area, especially in the organic soil, because live biomass in recently burned area are still largely reduced, which would result in a larger inorganic nitrogen loss than its demand;

3) Plants in the burned area would be more enriched in ^{15}N than unburned forest because plants utilized

the N resources that were ^{15}N-enriched due to N losses.

2 Methods

2.1 Study area

Our study area was in Huzhong National Natural Reserve (51°17′42″ N to 51° 56′ 31″ N, 122° 42′ 14″ E to 123° 18′ 05″ E), which is located in the Great Xing'an Mountains of northeastern China (Fig. 1). This reserve encompassed 167,213 ha and experienced a terrestrial monsoon climate, characterized by a long and severe winder. The average annual temperature was -4.7 ℃ and mean annual precipitation was ca 500 mm. More than 60% of the annual precipitation fallen in the summer season from June to August (Liu *et al*. 2012; Zhou, 1991).

Dahurian larch (*Larix gmelinii*), a typical boreal conifer species, dominate the late successional forests. Other tree species, such as pine (*Pinus sylvestris* var. *mongolica*), spruce (*Picea koraiensis*), birch (*Betula platyphylla*), two species of aspen (*Populus davidiana, Populus suaveolens*), willow (*Chosenia arbutifolia*), are interspersed with larch forest and have a small area of distribution (<2%) (Cai *et al*., 2013). Understory communities in the Great Xing'an mountains include *Vaccinium vitis-idaea*, *Ledum palustre*, *Carex schmidtii*, *Vaccinium uliginosum*, *Rhododendron dauricum*, and *Rubus sachalinensis*. *Vaccinium vitis-idaea* and *Ledum palustre* are the mostly widely distributed understory species.

The historical fire regime in this region is described as frequent, surface fires mixed with infrequent, stand-replacing crown fires, with fire-free interval ranged from 30 to 120 years (Xu *et al*., 1997; Liu *et al*., 2012). However, climate change, forest management and human activities have altered fire regimes in this region (Jackson *et al*., 1997; Wang *et al*., 2007). Although the dominant tree species Dahurian larch is regarded as a fire-tolerant species with thick bark near the stem bottom, its post-fire mortality rate is still high, mainly due to a horizontal shallow-distributed root system (Fang *et al*., 2015; Vijayakumar *et al*., 2016). A stand-replacing wildfire，which was ignited by lighting, burned 600 ha of Huzhong National Natural Reserve on June 26th, 2010. This fire provided an ideal opportunity to study the effects of fire on soil N dynamics in this ecosystem.

2.2 Experimental design and field sampling

In early June 2014, we randomly selected 12 plots (each 10 m ×10 m) in the burned area with six plots at northern and southern slopes, respectively. In the unburned area, we also randomly set six plots (each 20 m × 20 m, Fig. 1) with three plots at each slope (northern and southern slope). To mitigate edge effects, we located these plots at least 100 m away from the roads. In addition, each plot was 200 m away from each other in order to minimize samples’ spatial autocorrelation.

Soil samples were taken from two layers (organic layer and 0-20 cm mineral layer) at five random locations within each plot and were composited. We also recorded the temperature of organic layer by soil thermometer at the soil depth of 5 cm (whenever applicable). The soil temperature was measured between 10am and 4pm. To account for the inherent hourly and daily temperature variations, we also measured soil temperatures at two fixed places at the hourly basis and used them as the baseline temperature data to correct such sources of uncertainty. The corrected values would be used to compare the difference in mean soil temperature between burned and unburned areas. On the same day the soil samples were collected, 7.5 g fresh organic soil passed through 5 mm sieve and 30 g fresh mineral soil passed through 2 mm sieve were extracted by 75 ml 2 M KCl solution, shaking for 1 h at 160 r/m and then filtered. The extracts were frozen and maintained at -20 ℃ until later laboratory analysis. For each plant species, the foliage was sampled from at least 5 separate individuals within the same plot.

In early June 2015, we further collected plant and soil samples in the same plots. In the unburned area, the dominant overstory species is *Larix gmelinii*, and the dominant understory species include *Vaccinium vitis-idaea*, *Ledum palustre*, *Rhododendron dauricum*, and *Pinus pumila*. In the burned area, the dominant species include seedlings of *Larix gemlinii* and some shrubs and herbs, such as *Vaccinium vitis-idaea*, *Ledum palustre*, *Carex schmidtii* and *Rubus sachalinensis*. Different moss species were observed in unburned and burned area, *Hypnum spp*. was observed in the unburned area, whereas *Polytrichum piliferum* was the common moss species in the burned area. For plant tissues, foliage and branch were sampled from at least 5 separate individuals. Mosses were collected at five random locations within each plot and were composited. Fine roots were separated from forest floor (Oa+e layer) and different mineral soil profiles (0-10 cm, 10-20 cm). For soils, forest litter (Oi), forest floor (Oa+e layer) and three mineral soil profiles (0-10 cm, 10-20 cm and 20-30 cm) samples were collected using the same method as the one used in year 2014.

2.3 Laboratory treatment and chemical analysis

Subsamples of organic and mineral soils were air-dried, crushed and sieved through 5 mm and 2 mm mesh, respectively, for chemical analysis. Soil pH was measured in H_2O employing a soil: solution ratio of 1:10. Soil samples were dried at 105 ℃ for 48 h to measure soil water content. Ammonium in the extract was determined by the indophenol blue method followed by colorimetry, and NO_3^- was determined colorimely using the same autoanalyser in the form of NO_2^- after reduction of NO_3^- in a Cd-Cu column followed by the reaction of NO_2^- with N-1-napthylethylenediamine to produce a chromophore (Rivas *et al*., 2012).

Plant and soil samples were dried at 60 ℃ to constant weight and ground into powder using a ball mill and used to analyze ^{15}N natural abundance (expressed as $\delta^{15}N$), N and C concentrations by elemental analyzer (vario MICRO cube; Elementar Analysensysteme GmbH, Hessen Hanau, Germany) coupled to an IsoPrime100 continuous flow IRMS instrument. Calibrated glycine ($\delta^{15}N$ = 1.6‰), D-glutamic ($\delta^{15}N$ = -5.7‰), L-histidine ($\delta^{15}N$ = -7.6‰), and acetanilide ($\delta^{15}N$ = 1.4‰) were used as the internal standards. The $\delta^{15}N$ of the sample relative to the standard (atmospheric N_2) was expressed as the following:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000;$$

where R_{sample} represents the isotope ratio ($^{15}N/^{14}N$) of sample and $R_{standard}$ is the $^{15}N/^{14}N$ for atmospheric N_2. The analytical precision for $\delta^{15}N$ was in general better than 0.2‰.

In order to examine the net N mineralization rate, we collected soil from the same plots in early August of 2014 with the same sampling method. We used the soil samples collected in the late growing season for this purpose because we didn't have low-temperature sample transportation facilities during the first-round soil collection in early June. Net N mineralization rates were estimated using laboratory soil incubations. 7.5 g fresh organic soil passed through 5 mm sieve and 30 g fresh mineral soil passed through 2 mm sieve were put into a plastic cups with polyethylene film to minimize moisture evaporation and incubated at 20 °C for 1 week without light. Incubated soil mineral N (NH_4^+ and NO_3^-) was extracted and measured as above mentioned. On the same day, the soil samples were extracted by 75 ml 2 M KCl solution by the same method as used in the June. The extracts were frozen and maintained at -20 °C until later laboratory analysis. Net N mineralization potentials were calculated as the difference between final and initial inorganic N (NH_4^+ + NO_3^-) concentrations divided by the number of incubation days. The expression “N mineralization potential” is used to designate soil samples that produced net amounts of inorganic N.

2.4 Statistical analysis

We used one-way analyses of variance (ANOVA) to test whether wildfire significantly affected soil N availability and examine the differences of $\delta^{15}N$ (‰) of foliage, organic soil and mineral soil in burned and unburned area. Significance level was set at a *P* value of 0.05 unless otherwise stated. Significant differences among treatment means of soil properties were analyzed using One- way ANOVA. Data were statistically analyzed in R (R Core Team, 2014).

3 Results

3.1 Basic soil properties

The alteration of soil basic properties in the burned area 4 years after the wildfire was mainly found in the organic soil, not in the mineral soil (Table 1). The soil water content (SWC), total nitrogen (TN), total carbon (TC), C:N were lower in the burned soil, but only the reduction of SWC and TC reached the significant level ($p <= 0.05$). Mean soil water content at the organic layer was significantly lower in the burned area when compared to the unburned area (41.2% vs. 117.6%). Mean TC at the organic layer in the burned area was 9.2%, which was significantly lower than that in the unburned area (29.2%). In contrast of those properties that were reduced after fire, pH and temperature were increased. The mean organic soil temperature in the burned area was 10.0 ^{o}C and was significantly higher than that in the unburned area (2.9 ^{o}C).

3.2 Soil inorganic nitrogen concentrations

At the beginning of growing season (early June), total inorganic N pools were greater in the organic soil than the mineral soil both in burned and unburned area (Fig. 2A). However, the significant increases in soil inorganic N concentrations in response to wildfire were only observed in the mineral soil. Mean total inorganic N concentration in the mineral soil in the burned area (5.55 mg N kg^{-1}) was significantly higher than that (2.22 mg N kg^{-1}) in the unburned area. Compared to unburned area (1.6 mg N kg^{-1}), the amount of NH_4^+ was significantly higher (5.0 mg N kg^{-1}) in mineral soil of burned area (Fig. 2B). NO_3^- concentrations were consistently low in both organic and mineral soil, and had no difference between burned and unburned area (Fig. 2C).

At the end of growing season (early August), there were no significant differences in total inorganic N, ammonium and nitrate concentrations between burned and unburned area (Figs. 2D and 2F). However, the significant decreases in soil inorganic N concentrations in response to wildfire were only observed in the mineral soil. Mean total inorganic N and ammonium concentrations in the organic soil were 5.86 and 4.27 mg N kg^{-1} in the burned area, which were significantly lower than those (12.07 and

10.35 mg N kg^{-1}, respectively) in the unburned area (Figs. 2D and 2E).

3.3 Nitrogen transformation rates

The response pattern of N transformation after the fire was similar to that of soil inorganic N concentrations. Both mean net mineralization and ammonification rates (0.056 mg N kg^{-1}d^{-1} and 0.029 mg N kg^{-1}d^{-1}) were significantly higher in the organic soil of the burned area compared to the unburned area of -0.653 mg N kg^{-1}d^{-1} and -0.579 mg N kg^{-1}d^{-1}, respectively (Figs. 3A and 3B). In contrast, ANOVA test revealed no significant differences on mineralization and ammonification rates between burned and unburned mineral soil. There was no significant difference in net nitrification either in the organic or in the mineral soil between burned and unburned soil (Fig. 3C).

3.4 Plant and soil δ^{15}N

Mean foliar δ^{15}N in the burned sites was 3.7‰ and was significantly higher compared to the mean foliar δ^{15}N in unburned site (-3.7‰) in the 4 years after fire (Appendix 1). The species occurring on both the burned and unburned sites such as *Vaccinium vitis-idaea*, *Ledum palustre* and *Deyeuxia angustifolia* had significantly higher foliar δ^{15}N values in the burned area than the unburned area. The values for *Vaccinium vitis-idaea*, *Ledum palustre* and *Deyeuxia angustifolia* were 0.2‰, 2.6‰ and 1.8‰, respectively, in the burned area. In the unburned area, their corresponding values were -3.7‰, -3.4‰

and -2.4‰, respectively (Table 2). A significant difference of $\delta^{15}N$ between the burned (3.6‰) and unburned (1.3‰) area was also found in the organic soil (Appendix 1). However, there was no significant difference in the mineral soil between burned (4.9‰) and unburned (4.8‰) area (Appendix 1).

The effects of wildfire on $\delta^{15}N$ were also detected in plants' aboveground parts in the burned area 5 years after the fire (Fig. 4). Mean foliar and branch $\delta^{15}N$ were 2.3‰ and 1.5‰, respectively, in the burned area, and were significantly (p<0.001) greater than those in the unburned area (both -4.1‰). The moss $\delta^{15}N$ ranged from 0.9‰ to 1.7‰, and the mean was 0.7‰, which was significantly (p<0.001) higher than the moss collected in the unburned area. Fine root $\delta^{15}N$ was 0.7‰ in burned area and was significantly (p<0.001) higher than that in unburned area (-0.9‰). As the various sub soil organic layers, the Oi was more depleted in ^{15}N (-3.6‰) in unburned area than in burned area (-2.4‰). The wildfire also significantly increased the $\delta^{15}N$ of Oa+e and 0-10 cm mineral soil, but had no significant effects on the deeper mineral soil layers.

4 Discussions

4.1 The effects of fire on soil inorganic nitrogen concentrations and transformation rates

We initially expected that the inorganic N concentrations and N mineralization rates in the burned area would have recovered to the pre-fire level 4 years after fire. However, our data didn't fully support this hypothesis. In contrast, our study showed that wildfire still had a strong effect on inorganic nitrogen concentrations and N mineralization rates. Specifically, we observed higher than the control level NH_4^+ and TIN concentrations in the mineral soil in June, and higher net mineralization and ammonification rates in the organic soil in August. Although the N mineralization rates were high in the organic soil of the burned area, we found that TIN and NH_4^+ concentrations in the organic soil of the burned area were significantly lower than those in the unburned area. Such lower amount of TIN and NH_4^+ may be due to plant uptake or N loss through gases and leaching. Large decreases in TIN and NH_4^+ concentrations in the mineral soil of burned area after a growing season (from June to August) might be contributed to plant uptake as the fine roots were mainly distributed in the mineral soil after fire (Appendix 2).

The significantly increased rates of net mineralization and net ammonification in organic soil after fire might be attributed to following three reasons: (1) increased available organic matter to microbes (Dannenmann *et al*., 2011), as shown in our results that C:N ratio decreased from 27.5 to 20.8 (Table 1), may enhance microbial activities to decompose litter. (2) post-fire abiotic environments such as increased soil pH resulted from increased base cation availability and temperature (Table 1) tend to be more suitable for microbial activities (Smithwick *et al*., 2005). Increased temperature might have played a key role in N transformation (Klopatek *et al*.,1990) because decomposition rates may increase by 50% - 100% when soil temperatures increase 5 ^oC – 10 ^oC (Richter *et al*., 2000). In this study, organic soil temperature was increased (by 7.1 ^{o}C) significantly after fire, mainly due to combustion of thick organic layer (the thickness was decreased from 22.2 cm to 5.3 cm) and the removal of overstory tree, leading to more solar radiation reaching ground surface (Christensen and Muller, 1975). On the contrary, net nitrification rate remained unchanged after fire, despite increased soil net ammonification (Fig. 3). This could be because nitrifier population size may be too low after fire and it may take some time to increase (Turner *et al*., 2007). In addition, fire might have an adverse effect on nitrifying microbes, as some previous studies have suggested nitrifiers are more susceptible to fire than other soil microbial groups (Hart *et al*., 2005).

Other studies have also observed increase in inorganic N after fire (Certini, 2005; Gomez-Rey and Gonzalez-Prieto, 2013; Koyama *et al*., 2012). Turner (2007) studied inorganic N pools and mineralization rates in the first 3 years after a stand-replacing wildfire in the Greater Yellowstone ecosystems and found that soil NH_4^+ concentration increased and followed by increases in soil NO_3^-, but fire had a net negative influence on N mineralization due to microbe immobilization. Koyama *et al*. (2010) found soil NO_3^- concentrations elevated in the 2 years after wildfire in the coniferous forests of central Idaho resulted from reduced microbial NO_3^- uptake capacity, but NH_4^+ concentrations between the treatments were not significantly different. They also suggested that reduced available C was the key factor regulating soil N cycling after fire. On the contrary, Deluca and Sala (2006) showed recurrent, low-severity fire had a different effect on N in ponderosa pine forests. In their study, post-fire soil total N concentrations and potential mineral N (PMN) rates using the 14-day anaerobic incubation procedure decreased, and the concentrations of NH_4^+ and NO_3^- were not in line with the changes of total N pool and PMN rate. These studies collectively showed fire severity, time after fire, vegetation type and soil sampling depth may be responsible for the inconsistency of the reported findings (Wan *et al.*, 2001; Wang *et al.*, 2014).

4.2 The effect of fire on soil δ^{15}N

Our results showed that ^{15}N natural abundance in organic soil was significantly higher in the burned area than unburned area (Fig. 4). These results are consistent with our expectation that soil δ^{15}N would be higher in the burned area than unburned area, especially in the organic soil. Similar results were reported in other forest ecosystems (LeDuc *et al.*, 2013; Schafer and Mack, 2010). Combustion of the upper δ^{15}N-depleted surface soil layer and enhanced nitrification are the two widely-recognized mechanisms to explain ^{15}N enrichment in organic soil (Hogberg, 1997; LeDuc *et al.*, 2013; Schafer and Mack, 2010; Szpak, 2014). However, other mechanism such as NH_3 volatilization, combustion, litter return, nitrate leaching, denitrification can also contribute to the observed ^{15}N enrichment in our study.

Our results showed higher net mineralization and net ammonification didn't lead to higher ammonium concentrations in burned organic soils (Figs. 2-3). On the contrary, the ammonium and total inorganic nitrogen in the organic soil of the burned area were significantly lower than those in the unburned area (Fig. 2). Such lower NH_4^+ and TIN concentrations in the burned soil were likely due to NH_3 volatilization -- although several other mechanisms such as surface run-off and filtration to mineral soil might also contribute to this observed pattern. Higher soil temperature and pH values in the burned area as observed in the present study could enhance NH_3 volatilization (Nelson and Conrad, 1982; Raison, 1979). NH_3 volatilization is associated with strong fractionation against ^{15}N and higher gaseous losses of ^{15}N-depleted NH_3, and leads to the remaining soil NH_4^+ to be enriched in ^{15}N (Hobbie and Ouimette, 2009). Therefore, fire-stimulated NH_3 volatilization, associated with strong isotopic fractionation and subsequent export of ^{15}N-deplted NO_3^-, is considered as one likely being responsible for ^{15}N enrichment of organic soil.

Combustion of surface soil layer could also cause the upper soil to be enriched in ^{15}N since high, sustained fire temperatures cause a greater loss of ^{14}N compared to ^{15}N (Huber *et al*., 2013; Schafer and Mack, 2010). In our study, wildfire, characterized with high temperature, combusted the thick organic layer, leading to a significant higher $\delta^{15}N$ in the burned organic soil. For the mineral soil, the significant higher $\delta^{15}N$ was only observed in 0-10cm mineral soil but not in deeper mineral soil. This pattern may have resulted from the insulation of underlying mineral soil from heating and limited downward conduction of heat from soil surface to deep soil (Smithwick, *et al*., 2005).

The ^{15}N enriched litter return, to some extent, might have an effect on ^{15}N enrichment in the upper soil in the burned area. Plant tissues fallen onto the surface soil, resulting in litter with a similar value of $\delta^{15}N$. In mature larch boreal forest where N is limited, the ^{15}N-depleted leaf could lead to a lower $\delta^{15}N$ in litter. In the burned area, Oi was supposed to have a similar $\delta^{15}N$ with the leaf. However, the Oi was composed of a large number of ^{15}N-depleted coarse woody debris and a small number of recently added litter with a higher $\delta^{15}N$ value, which contribute to a relatively lower $\delta^{15}N$ in Oi than that in leaf in the burned area.

Fang *et al.* (2015) reported that denitrification was an important N loss pathway and could account for 48% to 86% total NO_3^- loss in forest ecosystems. Although we didn't measure this N process directly, we also considered denitrification as a potential mechanism for the higher $\delta^{15}N$ in the organic soil. On one hand, lower plant and microbial biomass in the burned area would result in lower N need and more NO_3^- loss through denitrification. On the other hand, the lack of increase in net nitrification of the burned soil resulted from 7-day laboratory incubation might be due to an enhanced denitrification, which is associated with strong fractionation against ^{15}N and higher gaseous losses of ^{15}N -depleted N_2 or N_2O, remaining soil NO_3^- to be enriched in ^{15}N (Hobbie and Ouimette, 2009; Robinson, 2001).

4.3 The effect of fire on plant $\delta^{15}N$

Foliar $\delta^{15}N$ values were significantly higher in the burned area, which supports our initial expectation that plant $\delta^{15}N$ in the burned forest would be enriched in ^{15}N. Three complementary processes are likely responsible for this ^{15}N enrichment. First, fire consumed the ^{15}N-depleted surface layers of litter, forcing plants to take up the N from deeper horizons which are more enriched ^{15}N than the surface soil (Hogberg, 1997; Sah *et al*., 2006). This assumption is supported by our field experiment in 2015. We found the root was significantly lower in the organic layer and fine roots were mainly distributed in the 0-20 cm mineral soil in the burned area; while in the unburned area, fine roots were mainly distributed in the organic soil layer (Appendix 2). Secondly, part of ^{15}N-enriched NH_4^+ and NO_3^- infiltrated into the deeper mineral soil with rainfall from the organic layer, which leads to the remaining soil N pool to be enriched in ^{15}N and further expressed in the values of $\delta^{15}N$ in plant that utilized these N pools for their N demand. Thirdly, increased N availability could lead to a lower dependence of plant N nutrition upon mycorrhizal fungi, which provide their host plants with ^{15}N-depleted N relative to the soil N sources (Craine *et al*., 2009; Hobbie *et al*., 2008). Boreal forest is a typical N-limited ecosystem and plants usually associated with mycorrhizal fungi to meet their N demand (Craine *et al*., 2009; Hobbie *et al*., 2008; Nasholm *et al*., 2013). Larch is the dominant species in the unburned area and is often associated with ECM. *Ledum* spp. and *Vaccinium* spp. are the main understory species and are often associated with ERM (Michelsen *et al*., 1998). Numerous studies have suggested that mycorrhizal fungi preferentially transfer isotopically depleted nitrogen to their host plants (Hobbie and Agerer, 2010; Högberg 1997; Whiteside *et al*., 2012). Thus we considered the mycorrhizal fungi would play a key role in N supply for plant in the unburned area and lead to lower foliar δ^{15}N values of their host plants.

Vaccinium vitis-idaea, Ledum palustre and *Deyeuxia angustifolia* were species occurring in both burned and unburned area. Nevertheless, there were significant differences in their foliar δ^{15}N values between burned and unburned area. For example, *Vaccinium vitis-idaea* δ^{15}N values were 0.2‰ and -3.7‰, respectively, in burned and unburned area (Table 2). Different N resources and change of fine roots distribution induced by fire could contribute these differences. Moreover, compared to the unburned area (mature larch boreal forest), which is a typical N limited ecosystem and has a negative foliar δ^{15}N (-3.7‰), the plant has a higher δ^{15}N in burned area, suggesting that this ecosystem has shifted from N limited to N open.

5 Conclusions

In this study we demonstrated that wildfire had a profound influence on N cycles in the boreal forests of the Great Xing'an Mountains. The ecosystem N cycle was still open in 4 and 5 years after fire. However, the wildfire effects were mainly limited in organic layer and 0-10cm mineral soil. The fire-induced increases in net mineralization rate and net ammonification rate were only exhibited in the organic soil, not in the mineral soil. The increased organic layer temperature and pH, decreased moisture and C:N could be the primary mechanism determining inorganic N transformation rates. We suggest that the observed ^{15}N enrichment in soil might be attributed to various mechanisms such as NH_3 volatilization, combustion, litter return and denitrification. Greater dependence of plant on deeper soil N and less dependence on mycorrhizal fungi might increase the ^{15}N of plant in the burned area. The $\delta^{15}N$ of plant and soil could be considered as a comprehensive indicator for explore the responses of N processes to wildfire in forest ecosystems.

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Table 1. Basic soil properties at two soil layer in unburned (n=12) and burned area (n=24). Values presented are means with the standard error in parentheses. Means in a row that have the same letter are not significantly different at alpha level is 0.05 (ANOVA, $p \leqslant 0.05$).

Layer	SWC (%)		pH		TN (%)		TC (%)		C:N		T(℃)	
	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned
Organic layer	117.6 (32.0)a	41.2 (18.6)b	4.4 (0.5)a	5.2 (0.5)a	0.8 (0.5)a	0.4 (0.2)a	29.2 (10.2)a	9.2 (4.0)b	27.5 (5.0)a	20.8 (5.4)a	2.9 (1.1)a	10.0 (2.5)b
Mineral layer (0-20cm)	36.2 (8.0)a	35.2 (7.7)a	5.2 (0.4)a	5.3 (0.3)a	0.2 (0.0)a	0.2 (0.0)a	4.3 (1.1)a	3.1 (0.5)a	24.1 (4.1)a	20.3 (2.4)a	NA	NA

Table 2. Foliar stable N isotope ratio ($\delta^{15}N$), N concentration, C concentration and C:N ratios for each sampled species in burned and unburned area.

Site location	Species	$\delta^{15}N$ (‰)	N conc.(%)	C conc.(%)	C:N ratio
Burned area	*Vaccinium vitis-idaea*	0.2	1.4	50.1	36.1
	Ledum palustre	2.6	2.1	51.8	25.2
	Deyeuxia angustifolia	1.8	2.6	43.7	17.1
	Carex schmidtii	3.4	2.1	42.9	21.4
	Chamerion angustifolium	5.2	3.9	46	12
	Betula platyphylla	2.4	3.1	48	15.7
	Rubus sachalinensis	3.4	2.8	44.5	16
	Mean ±SE	3.7 ±1.9	2.9 ±0.9	45.5 ±2.6	17.3 ±5.5
Unburned area	*Vaccinium vitis-idaea*	-3.7	1.2	49.4	40.9
	Ledum palustre	-3.4	2	51.5	26.3
	Deyeuxia angustifolia	-2.4	2.3	42.7	18.3
	Pinus pumila	-4.2	1.3	49.3	39.8
	Larix gmelini	-4.6	1.6	48.2	31.2
	Rhododendron dauricum	-2.9	2.1	48	22.6
	Mean ±SE	-3.7 ±1.3	1.7 ±0.5	48.7 ±2.0	31.5 ±9.0

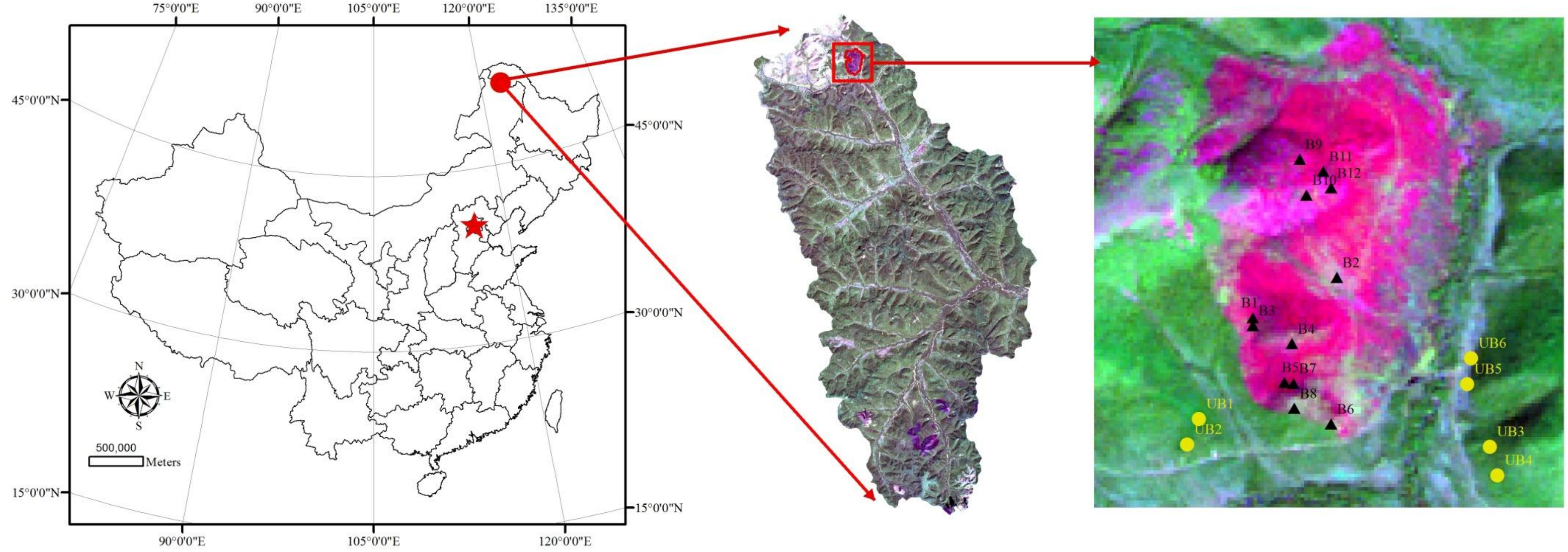

Figure 1. Map of research (burned and unburned) sites in the Huzhong Natural Reserve (HNR), China**.** A large wildfire burned almost 600 ha mature larch forest within the HNR in the summer of 2010. The red boundary represents the burned area. Unburned area is chosen in nearby burned area as a control. The black triangles represent burned plots, the yellow circles represent unburned plots.

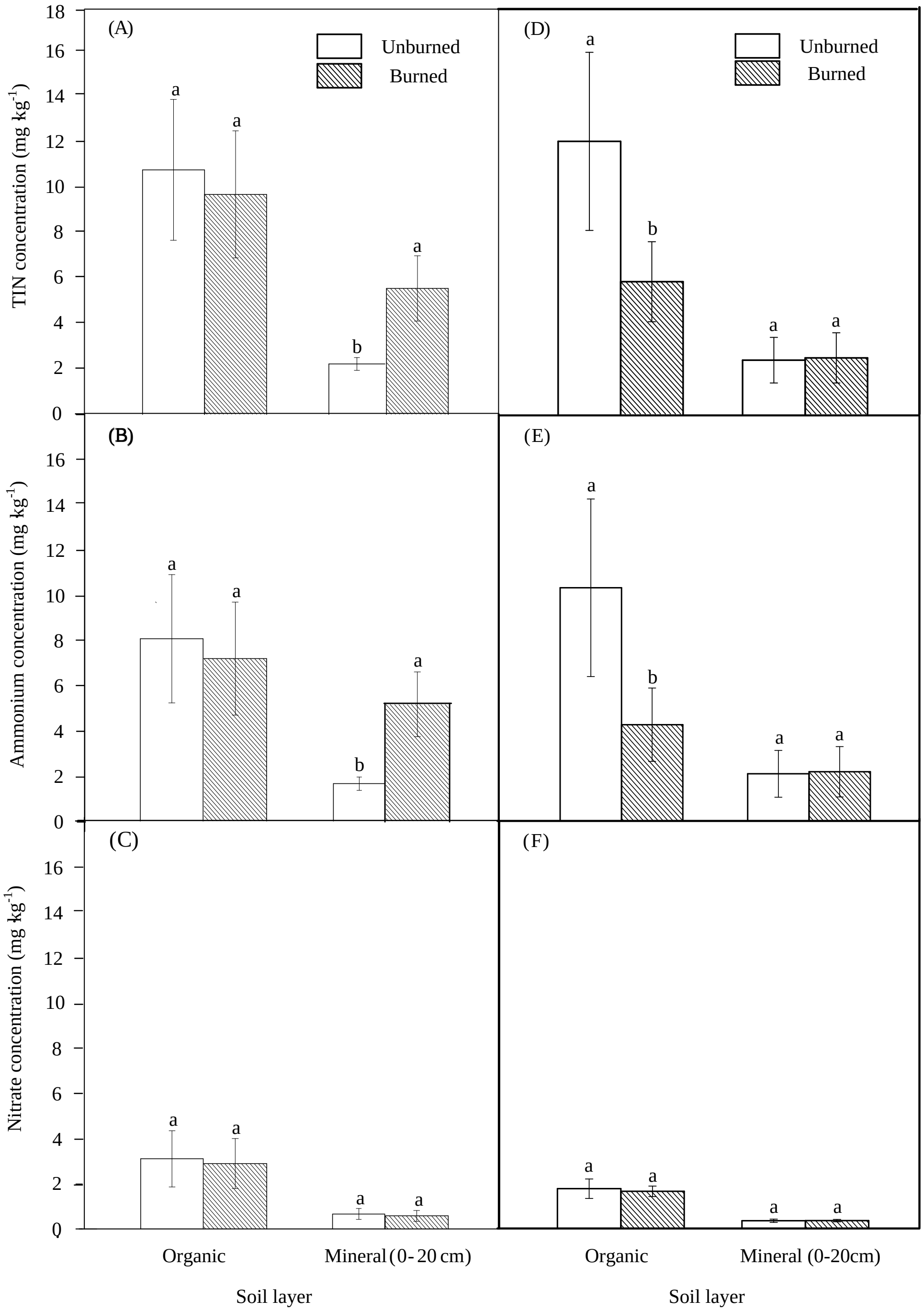

Figure 2. Soil inorganic N concentrations (TIN, NO_3^--N and NH_4^+-N)) of organic and 0-20cm mineral soils sampled in June, 2014 (A, B,C) and in August, 2014 (D, E,F). Bars show means ± standard error. Different letters indicate significant difference between burned and unburned plots.

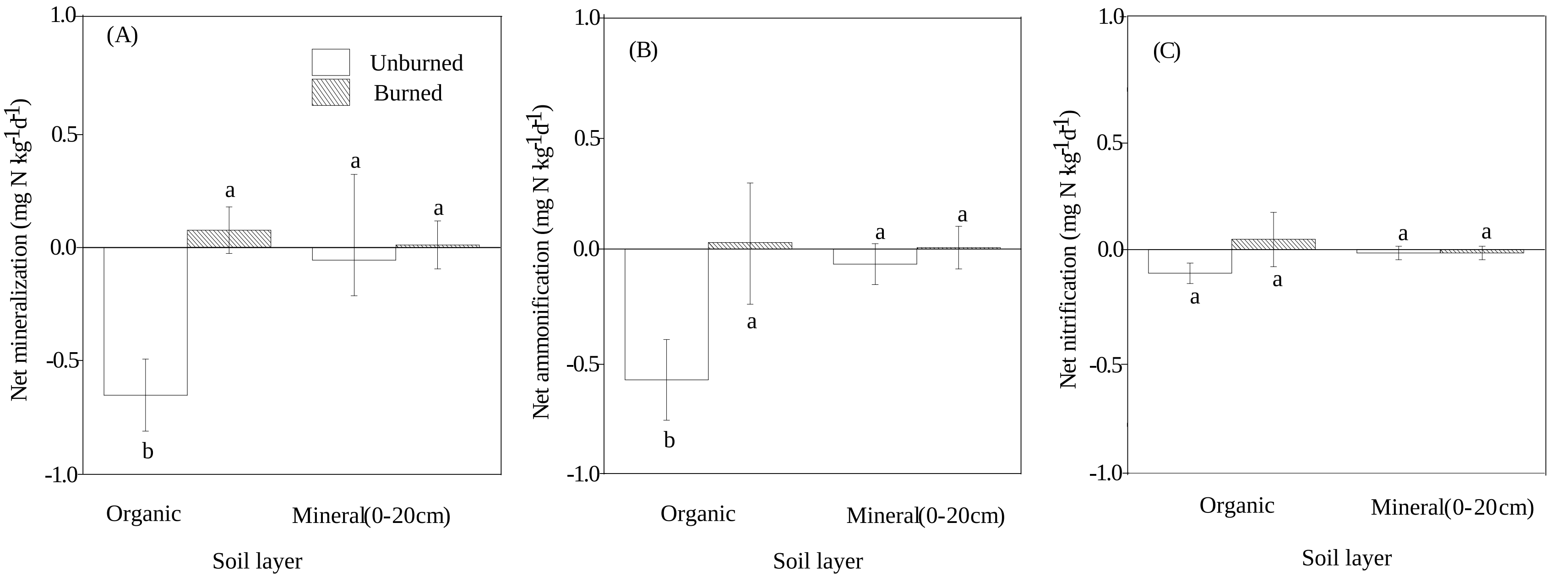

Figure 3. N transformation rates (net mineralization, ammonification and nitrification) of organic and mineral soils (0-20 cm) sampled in August, 2014. Bars show means ± standard error. Different letters indicate significant difference between burned and unburned plots.

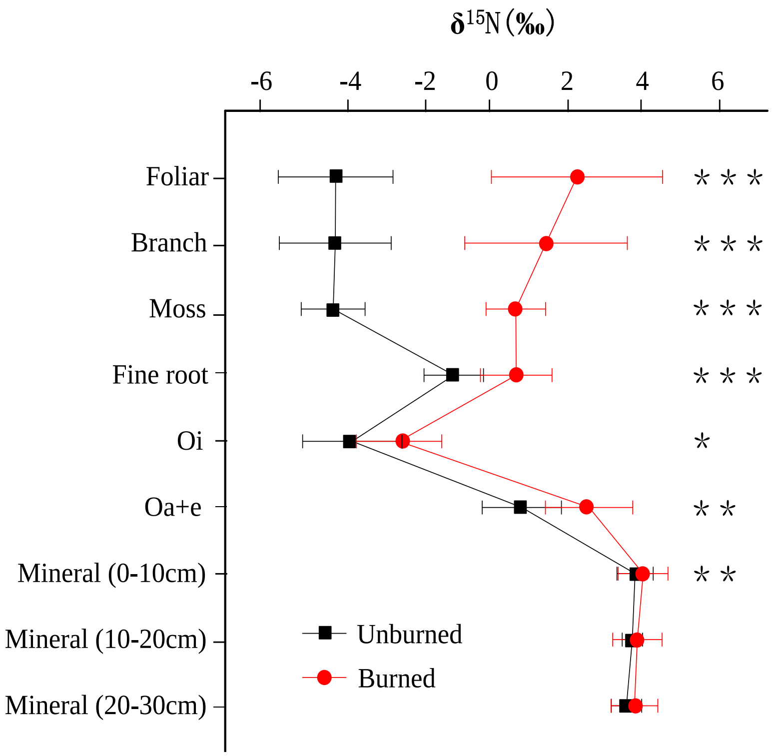

Figure 4. $\delta^{15}N$ (‰) for plant and soil in unburned and burned systems 5 years after wildfire. Solid red circles represent burned plots, solid black squares represent unburned plots, respectively. Data show means ± standard error. One asterisk indicate significant difference among forests at $p \leq 0.05$, two asterisks indicate significant difference among forests at $p \leq 0.01$, three asterisks indicate significant difference among forests at $p \leq 0.001$.

Appendix 1. $\delta^{15}N$ values (‰) of foliage, organic soil and mineral soils 4 years after wildfire. Values presented are means with the standard error in parentheses. Means in a row that have the same letter are not significantly different at alpha level is 0.05 (ANOVA, $p \leqslant 0.05$).

N pool	$\delta^{15}N$ (‰)	
	Unburned	Burned
Foliar	-3.7(1.3)b	3.7(1.9)a
Organic soil	1.3(1.2)b	3.6(0.8)a
Mineral soil(0-20cm)	4.8(0.3)a	4.9(0.6)a

Appendix 2. The fine roots biomass in two soil layers of both burned and unburned area. Means in a row that have the same letter are not significantly different at alpha level is 0.05 (ANOVA, $p \leqslant 0.05$).

Layer	Fine root (kg ha^{-1})	
	Unburned	Burned
Organic layer	24 405(13 503)a	1 054(1 824)b
Mineral layer(0-20cm)	4 826(9 037)a	6 275(1 595)a