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Responses of Soil Dynamics under Two Contrasting Oak and Pine Stands in a Korean Temperate Forest

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박 지 숙

A Dissertation for the Degree of Doctor of Philosophy

Responses of Soil Dynamics under Two Contrasting Oak and Pine Stands in a Korean Temperate Forest

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Responses of Soil Dynamics under Two Contrasting Oak and Pine Stands in a Korean Temperate Forest

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사랑하는 부모님께

이 논문을 바칩니다.

This dissertation is dedicated to

my beloved parents

ABSTRACT

Recently, forest environments have been continuously changed by the impacts of human activities such as industrialization and land-use changes. In forest ecosystems, maintaining a proper nutrient circulation system is essential for increasing forest productivity because organic matter properties, such as different plant residues, nutrient quality, and microbial activity, affect nutrient processes. The main environmental problems impacting forest soils are soil nitrogen deposition and deforestation. Therefore, the purpose of this study is to evaluate forest soil problems by assessing tree species, soil carbon, pH, and phosphorus. To do so, I developed four hypotheses.

First, experiments using ^{15}N-labeled urea were conducted for three years to assess the effect of nitrogen deposition on soil carbon and nitrogen dynamics under oak and pine forest stands in natural field conditions. Through this study, I revealed that the increases in total carbon and nitrogen contents due to nitrogen deposition were greater under coniferous forest stands than those under deciduous forest stands as a result of the greater mixing of new carbon substrates into the soil profile in this temperate forest. Second, I evaluated the effects of nitrogen treatment on the soils of two different forest floors on different sizes of soil aggregates and on soil organic carbon decomposition patterns. Through this study, I revealed that N treatments affect the decomposition of organic matter and soil aggregate distributions and that these effects depend on the tree species. Third, I evaluated the effects of nitrogen treatments on the soils of two different forest floors on humic substances and soil organic functional group patterns by soil aggregate size fraction. Through this study, I revealed that N treatment affects humic substances and soil organic functional

groups and that these effects depend on the tree species. Fourth, I challenged the widely held notion that phosphorus availability increases due to liming by hypothesizing that an increase in soil pH induced by liming would instantaneously disturb the chemical equilibria among inorganic P species through extensive interactions with increased Ca^{2+} due to the dissolution of $CaCO_3$ and with displaced Al^{3+} and Fe^{3+}. Through this study, I revealed that the application of lime has an unexpected opposite impact on Al-, Fe- and Ca-P compounds, at least in the early stage of equilibrium disturbances.

These results suggest that the soil carbon and phosphorus of forest soils are responsive to nitrogen application and liming and provide a foundation for investigating the impacts of tree species, soil aggregates, humic substances, soil organic functional groups, and phosphorus speciation.

Keywords: *soil carbon, nitrogen, phosphorus speciation, forest soil, soil aggregate, tree species, soil organic functional group*

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INTRODUCTION

In forest soils, physical and chemical properties develop under natural conditions due to the natural distribution of vegetation and the environment over a long period of time. These properties of forest soils may be almost permanent properties unless artificially modified by deforestation, cultivation, forest fires, and environmental pollution. However, recently, the forest environment has been continuously changed by the impacts of human activities such as industrialization and land-use changes. The main environmental problems impacting forest soils are soil nitrogen (N) deposition and deforestation.

Among these problems, in relation to N, increases in atmospheric N deposition and the overuse of N fertilizer are major concerns for forests soil (Nair et al., 2012; Vermeulen et al. 2012; Udaqatta, 2017). The current global concentrations of greenhouse gases in the atmosphere have reached their highest levels in at least the past 800,000 years (Pachauri et al., 2014). Meanwhile, N deposition from the atmosphere to forest soils has accordingly increased relative to preindustrial levels and is predicted to exceed 10 to 60 kg N ha^{-1} yr^{-1} by 2050 (Tietema and Wessel, 1992). However, in forests, either a high N fertilizer application or large atmospheric N deposition is required to ensure high forest productivity. For example, mature temperate forest stands require an annual N fertilization of approximately 500 kg N ha^{-1} yr^{-1} to meet a high demand of approximately 100 kg N ha^{-1} yr^{-1} (Rennenberg and Dannenmann, 2015), which is similar to those of many agricultural systems (Christ et al., 1995; Zhu et al., 2017; Schlesinger, 2009). Although most forest ecosystems are N limited, such high amounts of N inputs may become available in the N transformation processes of mature forests, affecting the productivity of these

forest ecosystems (Nowinski et al., 2008; Fog, 1988). In addition, the application of N fertilizer in forest soils is often used not only to increase yields but also to prevent crop damage. These N applications on the soil surface tend to move downward through the soil profile, affecting the aboveground and belowground biogeochemical processes that regulate the decomposition of tree litter and soil organic carbon (SOC) pools (Cotrufo et al., 2013).

In terrestrial ecosystems, carbon (C) and N are major elements, and their cycling processes are strongly coupled and linked together from photosynthesis to decomposition (Asner et al., 1997). Nitrogen application to plants increases the rate of CO_2 fixation through photosynthesis, thereby increasing plant organic matter (OM) (Farquhar et al., 1989; Rennenberg and Dannenmann, 2015). Therefore, N availability is a critical constraint that determines the size and composition of SOC pools in terrestrial ecosystems (Nowinski et al., 2008). According to previous research, N application leads to an increase in soil microbial activity, increasing the soluble and insoluble organic C pools in the soil (Christopher and Lal, 2007; Abraha et al., 2018). In addition, N application increases productivity in ecosystems, and large amounts of C are released into the atmosphere as CO_2 during the decay of OM by microorganisms, but a significant portion remains behind as soil organic matter (SOM) and microbial components. Therefore, the biological differences in SOC pools are dependent on features such as soil type, vegetation, and climate (Coleman et al., 1989; Lorenz and Lal, 2018).

In general, forest soils are important C pools (Goodale et al., 2002). Because tree species alter the organic material composition of forest soils, the storage functions of these systems may be different for different organic materials and tree species (Vesterdal et al., 2008). For example, because deciduous trees have higher nutrient levels and lower levels of lignin and polyphenols than coniferous trees, the leaf litter of the former decomposes faster than that of the latter (Prescott et al., 2000; Zhang

et al., 2008). The differences in tree species diversity and the biochemical compositions of leaf and root litter vary with the type (deciduous and coniferous) of tree species, and the composition of tree communities predominantly determines the size and quality of SOC pools (Zhang et al., 2008). Therefore, these substantial differences in biochemical composition between the litter composition of different tree species can affect residue decomposability and soil physical and chemical properties (Chiti et al., 2012; Gurmesa et al., 2013). Although many soil studies have compared different tree species, most have focused solely on the aboveground portion of the soil, particularly on soil microbial activity and litter decomposition. Therefore, differences in soil C dynamics across tree species type have not yet been clearly determined. Because the quality and decomposition of SOM is affected by the type of tree species and environmental conditions, these two major factors will in turn affect the soil C stocks in the forest. For this reason, to clarify these differences, it is important to understand how different tree species might affect SOC dynamics.

Soil organic matter consists of partially decayed animal and plant residues, soil microorganisms and the byproducts of decomposition that lead to the production of humic substances (HSs). Humic substances are relatively stable in soils and are very important in SOM dynamics. In general, HSs are divided into three fractions based on their solubility, and each has a variety of molecular components and chemical characteristics (Stevenson, 1994). In particular, the HSs within soil aggregates are very stable for a long time because of complexation with soil minerals (Pettie, 2006). Soil aggregates are formed by the interaction and binding of SOM, microorganisms, and soil mineral particles, and these aggregates are arranged in various configurations and occur in numerous sizes, varying the volume and size of soil pores (Horn et al., 1994; Brady and Weil, 2010). Because the OM bound within soil aggregates may be physically or chemically protected from decomposition, the

actual rate of SOM decomposition depends not only on chemical qualities but also on availability to microorganisms (Pulido-Moncada et al., 2018). Some researchers have confirmed that temporary and transient binding agents are more easily mineralized, while humified OM, which stabilizes microaggregates, is fairly recalcitrant (Tisdall and Oades, 1982; Beare et al., 1994; Ghabbour and Davies, 2014). Therefore, the C contents generally increase with increasing soil aggregate size. Because macroaggregates are composed of microaggregates held together by organic material, macroaggregates contain more SOC than microaggregates (Jastrow et al., 1996). Therefore, HSs and soil aggregates are particularly important in understanding SOC dynamics

Deforestation, a primary environmental problem facing forest soils, is the removal of trees in a forest where the land then becomes nonforested. This transition represents a potential threat to the health of terrestrial and aquatic ecosystems. The removal of trees without sufficient reforestation has led to unfavorable consequences, including the loss of habitat, loss of biodiversity, and poor soil quality (Hossain et al., 2011). Changes in soil chemical properties due to deforestation considerably decreases the soil nutrients, pH, and available phosphorus because decreasing the soil pH alters nutrient availability and thereby affects plant growth and productivity in highly acidic soils (Zaman et al., 2010). Phosphorous (P) has been recognized as a major nutrient limiting the productivity and resilience of terrestrial ecosystems (Poeplau et al., 2016), and the availability and chemical form of P is very sensitive to changes in soil pH. In acidic soils, Al^{3+} and Fe^{3+} react extensively with phosphates ($H_2PO_4^-$ and HPO_4^{2-}) to form insoluble Al and Fe phosphate compounds (Ro and Cho, 2000; Verma et al., 2005), while in neutral and alkaline soils, Ca^{2+} readily reacts with phosphates to form less insoluble Ca-P compounds (Lindsay, 1979). Therefore, most of these P forms have a very low solubility, and thus, plants have difficulty with the uptake of these compounds (Brady and Weil, 2010). Liming is known to increase

the availability of P in acidic soils by stimulating the mineralization of soil organic P pools (Haynes, 1982; Sharma et al., 2013). However, liming can reduce the availability of P in soils due to the extensive interaction of P with Ca^{2+}, Al^{3+} and Fe^{3+} ions (Machado and Silva, 2001), thus causing a shift in ionic composition. For these reasons, the mechanisms responsible for variations in P chemical speciation in soils due to liming are still not fully understood.

The purpose of this study is to evaluate the forest soil problems associated with soil N deposition and deforestation. I developed four hypotheses concerning the effects of N deposition, including 1) the effect of different SOC pool distribution patterns, 2) the effect of different sizes of soil aggregates on different SOC decomposition patterns, 3) the effect of different distribution patterns of HSs and the decomposition of the SOC functional groups, and 4) the effect of liming on inorganic P species.

LITERATURE REVIEW

1. Land-use changes

Land-use changes has been actively discussed around the globe since the mid-1970s and plays an important role in sustainable use as an environmentally friendly form of agriculture and forestry. Land-use change is a land use management system in which trees or shrubs interact with physical, biological, ecological, economic, and social dimensions. Land-use change is actively promoted globally to reduce harm to environmental features such as C stocks, plant nutrients, soils, and forests and to improve adaptation to and mitigation of climate change (Udaqatta, 2017). Deforestation, a type of land-use changes, is the removal of trees in a forest where the land then becomes nonforested, entailing a potential threat to the health of terrestrial and aquatic ecosystems. The removal of trees without sufficient reforestation has led to unfavorable consequences, including the loss of habitat, loss of biodiversity, and poor soil quality (Hossain et al., 2011). Changes in soil chemical properties due to deforestation have considerably decreased the soil nutrients, pH, and available phosphorus in these areas because decreased soil pH alters nutrient availability and thereby affects plant growth and productivity in highly acidic soils (Zaman et al., 2010). Because of these problems, many studies are currently evaluating the aboveground and belowground impacts of forest management with regard to soil nutrients, vegetation, plant growth, and fertilizer use (Nair et al., 2012; Vermeulen et al. 2012; Udaqatta, 2017; Lorenz and Lal, 2018).

Fertilizer is used to increase the productivity of forest ecosystems. However, because the effect does not always appear to be clearly linked to increases in forest productivity, the application of fertilizer in forest soil is more carefully implemented than in other soils (Schlesinger, 2009; Rennenberg and Dannenmann, 2015). In the forest, it is essential to maintain proper nutrient cycling to increase forest productivity because OM properties, such as various plant residues, nutrient quality,

and microbial activity, affect the nutrient processes of forest ecosystems (Nair et al., 2012; Zaehle et al., 2010; Udaqatta, 2017; Lorenz and Lal, 2018). Currently, the fertilizer is often applied in forest soils not only to increase yields but also to prevent crop damage. Previous studies have reported that the input of OM, such as fertilizer, affects the promotion of plant growth in forests and results in increased soil C content (Zaehle et al., 2010). In addition, the application of organic materials such as manure and compost affects soil aggregates, which increases the stability of SOC (Nair et al., 2010; Zhang et al., 2018), and chemical fertilizers such as N, phosphorus (P), and potassium (K) increase the stabilization of soil macroaggregates and the silt+clay fraction (Yu et al., 2012).

2. The general characteristics of soil organic carbon (SOC)

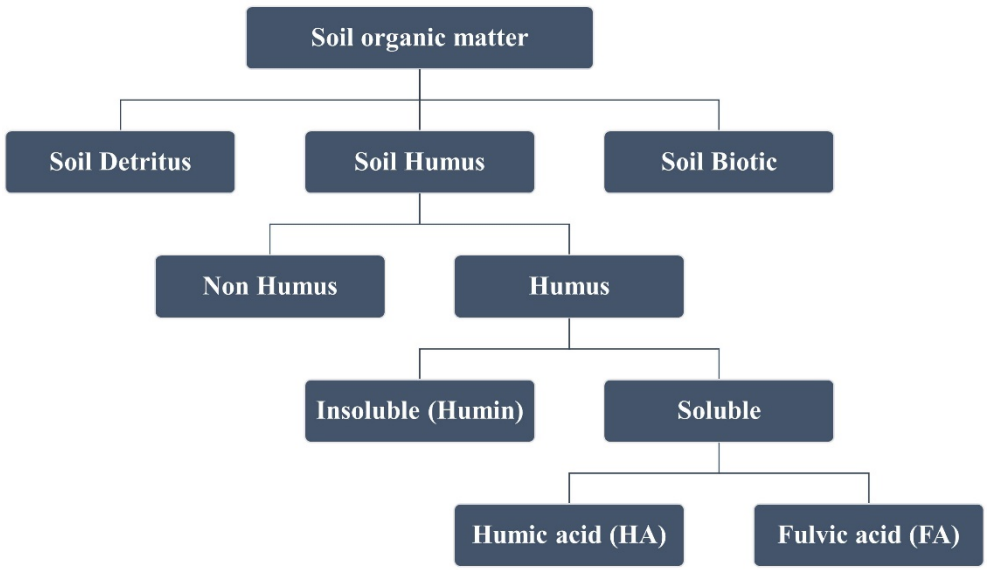
The earth C cycle is a multicompartment system with terrestrial, aquatic, and atmospheric compartments that are stored in five major pools, namely, the oceanic (38,400 Pg), geologic (4,130 Pg), pedologic (2,500 Pg), atmospheric (760 Pg), and biotic (560 Pg) pools (Lal, 2008; Lorenz and Lal, 2018). Despite being representing relatively small amount of the total pedologic pool, the C contained in the OM of terrestrial soils is a major reservoir of C that exists in various complex forms in the soil. In general, SOC originates from remains of organic materials such as litter, light-fraction SOC, microbial biomass, faunal biomass, belowground plant constituents, water-soluble organics, and stable humus (Figure 1). According to Allison (1973), SOM consists of plant and animal remains at different stages of decomposition, and these stages can be categorized by nine broad chemical compounds, namely (1) carbohydrates; (2) protein and amino acids; (3) fats, oils, and waxes; (4) alcohols, aldehydes, and ketones; (5) organic acids; (6) lignin; (7) compounds with cyclic or ring structures; (8) alkaloids and compounds with organic

bases; and (9) miscellaneous substances that are important but present in small amounts (Parton et al., 1987). These organic materials release a large amount of C into the atmosphere as CO_2 during decay by microorganisms, but a significant portion remains behind as SOM and microbial components. Therefore, the biological differences in the SOC pools are dependent on features such as soil type, vegetation, and climate (Coleman et al., 1989; Lorenz and Lal, 2018). Furthermore, SOM is an important determinant of soil quality, and these qualitative and quantitative changes in SOM are determined by terrestrial ecosystem characteristics, such as the physical (soil crust, moisture retention, and aggregate stability), chemical (pH, cation exchange capacity, and nutrient cycling), and biological (vegetation, soil enzymes, and microorganisms) properties of the soil (Doran and parkin, 1996). For example, in forest ecosystems, SOC originates from plant litterfall and woody debris in surface soil, and the subsoil acquires OM from the death and decay of tree roots. However, because the OM composition of leaf and root litter varies with the type (deciduous or coniferous) of tree species, the composition of tree communities predominantly determines the OM decomposition rates and the size and quality of SOC pools (Hobbie, 2015).

3. Effects of organic matter on soil properties in the forest soil

There are various sources of OM in forest soils that are accumulated continuously in the soil layer by the native forest vegetation (Finzi et al., 1998). In general, forest soils are important C stores (Goodale et al., 2002). Because tree species alter the composition of OM, the storage function of forest soils may differ with different organic materials and tree species (Vesterdal et al., 2008). In organic tree materials,

Figure 1. Scheme for the fractionation of soil organic matter.


Soil organic matter
Soil Detritus
Soil Humus
Soil Biotic
Non Humus
Humus
Insoluble (Humin)
Soluble
Humic acid (HA)
Fulvic acid (FA)

aliphatic compounds derived from roots have been reported to be more resistant to degradation than those from shoots because roots have more complex C compounds than shoots (Balesdent and Balabane, 1996; Crow et al., 2009). Therefore, these substantial differences in chemical composition in tree species litter composition can affect residue decomposability (Chiti et al., 2012; Gurmesa et al., 2013). In addition, these differences in tree species diversity and biochemical composition affect the soil's chemical and physical properties (Loreau et al., 2001). For example, because deciduous trees have higher nutrient levels and lower levels of lignin and polyphenols than coniferous trees, the leaf litter of the former decomposes faster than that of the latter (Prescott et al., 2000; Zhang et al., 2008). In particular, even under similar environmental conditions (climate region, temperature, rainfall, and soil moisture), it is well known that soil CO_2 efflux is highest from deciduous forests, followed by that from mixed forests and then that from coniferous forests (Finn et al., 2015; Lettens et al., 2005; Kim et al., 2010) as a result of the higher lignin content of their leaf litter (Laganière et al., 2010; Qualls, 2016) and lower SOM degradation (Kime et al., 2010). Therefore, these differences in tree species result in different soil chemical properties under these two different forest stands.

4. Soil organic functional groups

The organic functional groups in soils are chemically reactive molecular units that are attached to the surfaces of solids, and these compounds play significant roles in biochemical processes (Brady and Weil, 2010). During the decomposition of SOM, organic functional groups can be protonated or deprotonated by the adsorption of hydrogen and hydroxyl ions, and this process mainly depends on reactive groups, including carboxyl, hydroxyl, and phenolic groups (Haberhauer et al., 1998; Brady and Weil, 2010;). Previous research has reported that the major organic functional

groups that are influenced by microbial degradation are aliphatic C–H and polysaccharides (Swift et al. 1979; Haberhauer et al., 1998). Hsu and Lo (1999) reported that the decomposition and mineralization of SOM entail changes in functional group chemistry, such as the conversation of aromatic groups to aliphatic (carbon chains) groups during decomposition. However, as decomposition proceeds (not the decomposition of fresh OM), among the soil organic functional groups, the aromatic and polysaccharide groups increase and the aliphatic group decreases (Inbar et al., 1989; Hsu and Lo, 1999). Despite the many studies on soil organic functional groups, the changes in soil functional groups during SOM decomposition remain poorly understood (Parikh et al., 2014; Margenot et al., 2015).

Fourier transform infrared (FTIR) spectroscopy can be used to help explain SOM transformations and stabilization and enables the rapid characterization of relative changes in SOM functional groups (e.g., carboxylic and phenolic groups) (Martin-Neto et al., 2009) because a variety of infrared bands are characteristic of molecular structure and functional groups (Ellerbrock and Kaiser, 2005). Many previous studies have reported that FTIR spectroscopy can be used to describe decomposition processes through the reduction of carbohydrate markers as quantitative indicators of SOM in different soil horizons (Haberhauer et al., 2000; Artz et al., 2008). Therefore, FTIR is a very useful tool for observing structural variations in soil due to chemical and microbial activity changes resulting from SOM dynamics.

5. Humic substances (HSs)

Humic substances, which comprise 70–80% of SOM, are relatively stable in soils and are very important in SOM dynamics. Humic substances determine the soil structure, water holding capacity, cation and anion exchange, porosity, and the chelation of mineral elements (Pettie, 2006). In general, the HSs are divided into

three fractions based on their solubility, and each has a variety of molecular components and chemical characteristics (Stevenson, 1994) (Figure 1). Humins are insoluble in water under any pH condition, and the molecular weights range of these compounds from approximately 100,000 to 10,000,000 (Pettie, 2006). Humins are the most resistant to decomposition within the soil and play important roles in soil structure, stability, and fertility. Humic acids (HAs) consist of weak aliphatic (C chains) and aromatic (C rings) organic acids that are soluble in alkaline conditions but are insoluble under acid conditions (pH 1–2), and their molecular weights range from approximately 10,000 to 100,000. Elemental analyses of HAs reveal that these compounds are primarily composed of C 50–60%, oxygen (O) 30–35%, hydrogen (H) 3–5%, N 1.5–6%, sulfur (S) 1%, and P 0.3% (Stevenson, 1994). Fulvic acids (FAs) also consist of weak aliphatic (C chains) and aromatic (C rings) organic acids that are soluble in water under all pH conditions. Elemental analyses of FA reveal that they are primarily composed of C 49%, O 45%, H 5%, N 2%, S 2%, and P 0.3% (Stevenson, 1994). Moreover, FAs are more chemically reactive because of an approximately 1.5 to 2–fold greater O content than that of HA, and because of the relatively small size (approximately 1,000 to 10,000) of FAs, these compounds are the key ingredients of high-quality foliar fertilizers in the soil. Complex C compounds containing HSs are key components of soil structure and aggregates and are synthesized by microorganisms, and HSs along with clay and silt form soil aggregates (Stevenson, 1994; Pettie, 2006). Therefore, soil HSs are particularly important in soil structure.

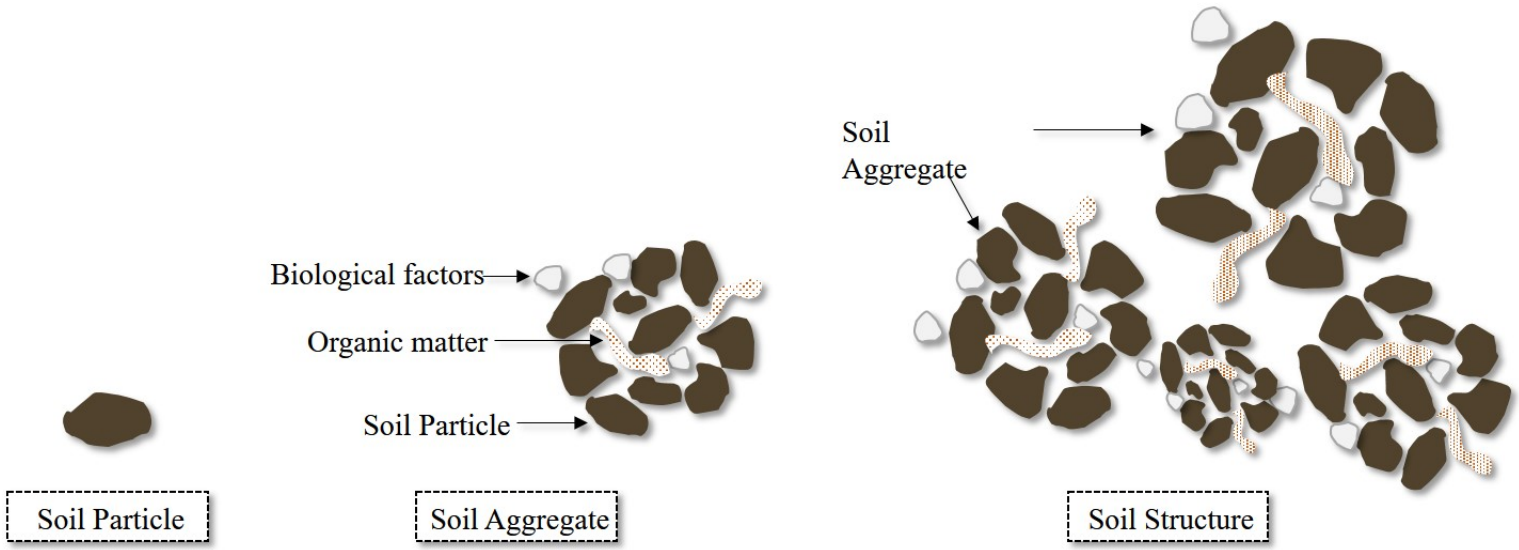
6. Soil aggregates

Soil aggregates are a basic unit of soil structure and are a heterogeneous mix of soil aggregates of various sizes held together by inorganic and organic binding agents

(Figure 2). Soil aggregates are formed by the interaction and binding of SOM, microorganisms, and soil mineral particles and are arranged in different configurations and have numerous sizes, varying the volume and size of soil pores (Horn et al., 1994; Brady and Weil, 2010). In addition, soil aggregates are influenced by the physical, chemical and microbiological characteristics of soils, which are in turn affected by OM, clay minerals, cation type, plant roots, and biological factors (Dorioz et al., 1993; Chenu et al., 2000). The different formation mechanisms of these soil aggregates depend on the different size soil aggregate size classes (Amézketa, 1999), which break down in a hierarchical model: macroaggregates (>250 μm) break down into microaggregates (20– to 250 μm) before particles (<20 μm) are released (Oades and Waters, 1991).

Tisdall and Oades (1982) found that the organic stabilization agents of soil aggregates can be categorized in several ways based on the age and degradation of the OM into transient, temporary and persistent binding agents. Transient binding agents are rapidly degraded by microorganisms and include microbial and plant-derived polysaccharides. Temporary binding agents include roots; hyphae, particularly vesicular-arbuscular mycorrhizal hyphae; and some fungi. Persistent binding agents consist of recalcitrant aromatic humic material combined with polyvalent metal cations and strongly adsorbed polymers (Tisdall and Oades, 1982). Some researchers have confirmed that temporary and transient binding agents are more easily mineralized, while humified OM, which stabilizes microaggregates, is fairly recalcitrant (Tisdall and Oades, 1982; Beare et al., 1994; Ghabbour and Davies, 2014). Because the OM bound within soil aggregates may be physically or chemically protected from decomposition, the actual rate of SOM decomposition depends not only on chemical properties but also availability to microorganisms (Pulido-Moncada et al., 2018). In general, when the OM content of the soil is increased, microbial activity increases, and as a result, microbial secretions released

Figure 2. Scheme for the structure of soil aggregates


Biological factors
Organic matter
Soil Particle
Soil
Aggregate
Soil Particle
Soil Aggregate
Soil Structure

into the soil form soil aggregates, and the secretions promote OM humification (humic, humic acid and fulvic acid)(Chaney and Swift, 1984). According to previous studies, in macroaggregates (>250 μm), OM acts as a binding agent through plant roots and hyphae (Tisdall and Oades, 1982), whereas in microaggregates (<250 μm), OM affects particle charge (Goldberg et al., 1990). Therefore, the C contents generally increase with increasing soil aggregate size. Because macroaggregates are composed of microaggregates held together by organic material, macroaggregates contain more SOC than microaggregates (Jastrow et al., 1996). Therefore, understanding soil aggregates is particularly important in understanding SOC dynamics.

7. Effects of N addition on SOC

Recently, increases in atmospheric N deposition and the overuse of N fertilizer have become major environmental concerns for forests (Nair et al., 2012; Vermeulen et al. 2012; Udaqatta, 2017). In terrestrial ecosystems, C and N are important elements with strongly coupled cycling processes that are linked together from photosynthesis to decomposition (Asner et al., 1997). The application of N to plants increases the rate of CO_2 fixation through photosynthesis, increasing plant OM (Farquhar et al., 1989; Rennenberg and Dannenmann, 2015). Therefore, N availability is a critical constraint that determines the size and composition of SOC pools in terrestrial ecosystems (Nowinski et al., 2008). According to previous research, N application leads to an increase in soil microbial activity, increasing the soluble and insoluble organic C pools in the soil (Christopher and Lal, 2007; Abraha et al., 2018). These increases are because N deposited to the soil surface increases the formation and degradation of SOM, resulting in an increased residence time of C in the soil (Fog, 1988). In addition, N application increases productivity in ecosystems, and the

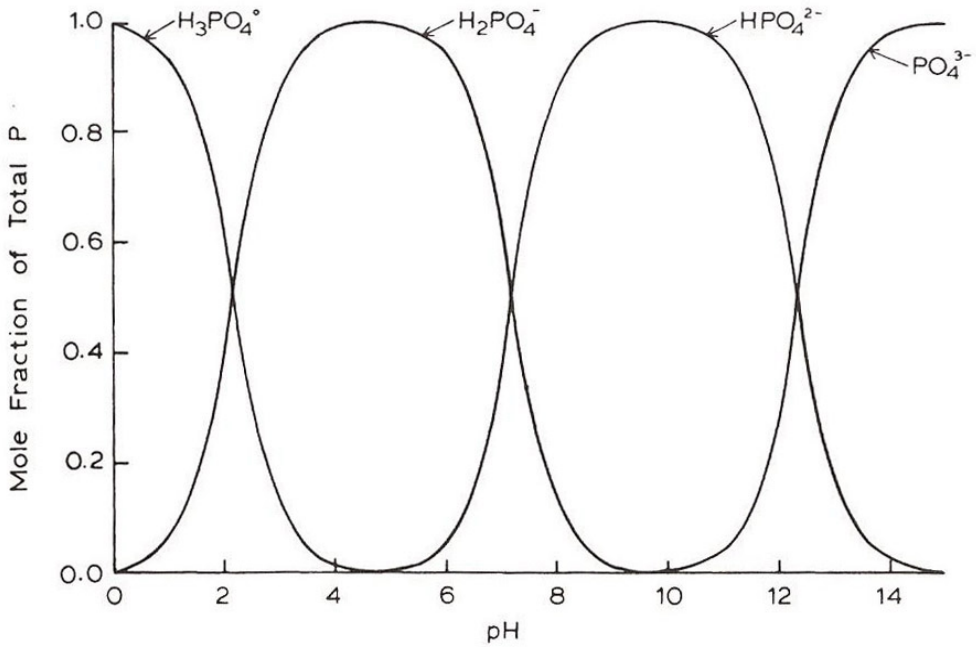
difference is dependent on the chemical composition and OM qualities of the plant species (Tonitto et al., 2014; Finn et al., 2015). In contrast, many researchers have reported that N application to soil promotes the respiration and decomposition of SOM, which has various effects on C loss, such as CO_2 emissions. However, although many studies have been conducted in this area, the effect of N application on increases and decreases in SOC is not fully understood (Lorenz and Lal, 2018; Zhang et al., 2018).

In addition, N applied to the soil surface tends to move downward through the soil profile, affecting the aboveground and belowground biogeochemical processes that regulate the decomposition of tree litter and SOC pools (Cotrufo et al., 2013). Therefore, understanding the interactive effects of N deposition in the soil and tree species on SOC decomposition dynamics along the soil profile is essential for determining the residence time and size of an ecosystem's SOC pools (Bradford et al., 2008; Hobbie, 2015; Lorenz and Lal, 2018).

8. The general characteristics of soil phosphorus (P)

Phosphorus is the most limiting nutrient for primary productivity in plants and soil microorganisms (Poeplau et al., 2016). In the pedosphere, almost all P occurs as phosphate in an oxidized form as orthophosphate; the P ions are complexed with calcium (Ca), iron (Fe), aluminum (Al) and silicate minerals; and the availability and chemical form of P is very sensitive to changes in soil pH (Jones et al., 1993). The influence of pH on the distribution of orthophosphate ions is described in Figure 3. As seen in Figure 3, in the pH range of most soils (5 to 8), $H_2PO_4^-$ and HPO_4^{2-} are the predominant species. Meanwhile, H_3PO_4 and PO_4^{3-} are predominant in soils with pH <2 and >12, respectively. Because these inorganic P forms are likely to be strongly adsorbed between mineral surfaces, various fractions, such as labile P,

Figure 3. Effect of pH on the distribution of orthophosphate ions in solution (Lindsay, 1979).


1.0
0.8
0.6
0.4
0.2
0.0
Mole Fraction of Total P
$H_3PO_4^\circ$
$H_2PO_4^-$
HPO_4^{2-}
PO_4^{3-}
0
2
4
6
8
10
12
14
pH

reductant P, and metal bound P, exist in the soil (Chang and Jackson, 1957; Williams et al., 1976). Therefore, most of these P forms have very low solubility, and plants have difficulty uptaking these compounds (Brady and Weil, 2010). In general, P is limited in acid soils, and P sorption is positively correlated with the number of free metal ion oxides in acid sulfate soils (Jugsujinda et al., 1995; Verma et al., 2005). The binding of these P forms to metals and free cations are affected by changes in soil pH, which can lead to a shift in the availability of nutrients such as calcium, hydrolytic iron, and aluminum species, which can in turn regulate the availability of phosphorus (House, 1999; Ro and Cho, 2000). Recently, many studies have evaluated P deficiency and soil nutrient availability in forest soils (Xu and Hirata, 2005; Pent et al., 2018). Soil phosphorous deficiency has a great influence on the decomposition of SOM and nutrient circulation, which is similar to N deficiency in forest ecosystems (Hobbie and Vitousek, 2000; Luo et al., 2017; Pent et al., 2018). Therefore, research on improving the effectiveness of P application without affecting forest ecology is critical (Hobbie and Vitousek, 2000; Vitousek et al., 2010).

CHAPTER I

Temporal variations in soil profile carbon and nitrogen during three consecutive years of ^{15}N addition to temperate oak and pine forest stands

Abstract

Experiments using ^{15}N-labeled urea were conducted for three years to assess the effect of N-deposition on soil C and N dynamics under oak (Qa) and pine (Pk) forest stands in natural field conditions. Throughout the experiment, an increase in total-C, mineral N and total-N due to N deposition was greater in coniferous forest soils than in deciduous forest soils, while decreasing the pH of both soils as a result of nitrification. Natural ^{13}C abundance of soil samples was interpreted to reveal the physical mixing of new C substrates from leaf-litter with old C substrates. The $\delta^{13}C$ of the upper soil layers became more negative, with greater decreases in the Pk soil. However, with time, the lowering of $\delta^{13}C$ was maintained greater in the Pk soil than in the Qa soil, indicating greater incorporation of new C substrates from leaf-litter decomposition into old SOC pools in the Pk soil than in the Qa soil. I revealed that an increase in total-C and N contents due to N deposition was greater under coniferous forest stands than under deciduous forest stands as a result of greater mixing of new C substrates into the soil profile in this temperate forest.

Keywords: *Natural isotope abundance; N-deposition; Tree litter; Forest floor; ^{15}N-isotope dilution*

1. Introduction

The current global concentrations of greenhouse gases in the atmosphere have reached their highest levels in at least the last 800,000 years (Pachauri et al.,2014) and nitrogen (N) deposition from the atmosphere to forest soils has accordingly increased relative to pre-industrial levels and is predicted to exceed 10 to 60 kg N ha^{-1} yr^{-1} by 2050 (Tietema and Wessel, 1992). However, in forests, high N fertilizer application or large atmospheric N deposition is required to ensure high forest productivity. For example, mature temperate forest stands require an annual N fertilization of about 500 kg N ha^{-1} yr^{-1} to meet high demand of about 100 kg N ha^{-1} yr^{-1} for mature forests (Rennenberg and Dannenmann, 2015), which is similar to many agricultural systems (Christ et al., 1995; Zhu et al., 2017; Schlesinger, 2009). Even though most forest ecosystems are N limited, such high amounts of N inputs may become available in the N transformation processes in mature forests, affecting the productivity of forest ecosystems (Fog, 1988; Nowinski et al., 2008). Nitrogen availability is a critical constraint that determines the size and composition of soil organic carbon (SOC) pools in terrestrial ecosystems (Nowinski et al., 2008). This is because N deposited into the soil surface increases the formation and degradation of soil organic matter (SOM), thereby resulting in increased residence time of carbon (C) in the soil (Fog, 1988). In forest ecosystems, an increase in N deposition has had a positive effect on net primary production (NPP), nutrient supply, and SOM decomposition (Kim and Kang, 2011; de Vries et al., 2014). However, several recent investigations have raised concerns regarding such unfavorable phenomena as soil acidification, biodiversity loss, unexpected modification of biogeochemical cycles, and retardation of biodegradation of persistent organic pollutants potentially resulting from increased N deposition (de Vries et al., 2014; van Diepen et al., 2015).

Nitrogen deposited in the soil surface tends to move downward through the soil profile, affecting aboveground and belowground biogeochemical processes that regulate the decomposition of tree litter and SOC pools (Cotrufo et al., 2013). However, despite the long history of N input to the soil, the response of soil C dynamics to external N deposition remains poorly understood (Bradford et al., 2008; Cotrufo et al., 2013). Therefore, understanding the interactive effects of N deposition in the soil and tree species on SOC decomposition dynamics along the soil profile is essential for determining the residence time and size of an ecosystem's soil organic C pools (Bradford et al., 2008; Hobbie, 2015).

In forest ecosystems, since the biochemical composition of leaf and root litter varies with the type (deciduous and coniferous) of tree species, the composition of tree communities predominantly determines the size and quality of SOC pools, and their decomposition characteristics are further changed by increased N availability due to N deposition in the forest floor (Hobbie, 2015). Previous studies have reported that the functional activities of soil microorganisms are related to tree species composition and soil properties (Gartzia-Bengoetxea et al., 2016), where the tree species affect the microbial community composition, thereby resulting in changes in the soil N mineralization rate, labile C availability, and C/N ratio (Huang et al., 2013). Even though many soil studies have been conducted by comparing different tree species, most of them have focused solely on the aboveground portion of the soil, particularly on soil microbial activity and litter decomposition. Therefore, differences in soil C dynamics due to the type of tree species were not clearly determined yet. In general, the degree of stabilization of SOC depends on the biochemical composition of organic matter in the soil, and increases with soil depth (Bradford et al., 2008; Cotrufo et al., 2013; Huang et al., 2013; Hobbie, 2015; Gartzia-Bengoetxea et al., 2016;; Gregory et al., 2016). Since the quality and decomposition of SOM is affected by the type of tree species and environmental

conditions, these two major factors will in turn affect the soil C stocks in the forest. Consequently, in order to clarify these differences it is necessary to investigate the changes in SOM decomposition with soil depth. However, since soil organic matter decomposes over a long time period, it is not sufficient to fully evaluate its variation within a short time frame, so long-term studies are needed.

Natural ^{13}C abundance is a useful tool for assessing organic matter turnover in the soil, which is used in many studies as an index to interpret the effects of various factors on the plant and soil environment (Farquhar et al., 1989; Gregory et al., 2016). Particularly, natural abundances of the stable C isotopes ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) of leaves are about –26‰ for C3 plants, as a result of the C isotope discrimination during photosynthesis (Farquhar et al., 1989). Therefore, natural abundances of the stable C isotopes of plant litters can vary depending on the isotopic C discrimination determined by photosynthetic pathways and environmental conditions, such as nutrient availability, vegetation type, temperature, moisture availability, and salinity (Farquhar et al., 1989; Gregory et al., 2016). On the other hand, natural ^{15}N abundances in soils are commonly higher than those in the atmospheric N_2 and may increase with depth in the soil profile during decomposition of plant materials (Turner et al., 1983). Therefore, stable C and N isotopes in soils can serve as a traceable proxy for the study of SOM dynamics. In addition, ^{15}N-isotope dilution technique has been used frequently to identify the fate and retention of N in terrestrial ecosystems. Particularly, it is used to trace the material balance and circulation through soil N cycles because it can identify influence of various factors affected by chemical and biological process (Turner et al., 1983; Farquhar et al., 1989; Perakis et al., 2005; Gregory et al., 2016). Therefore, to our knowledge, the natural abundance of stable C and N isotopes has the potential to provide important information for interpreting changes in the decomposition of the soil organic C pools.

It is a commonly-held belief that an increase in soil C pools due to N deposition is greater in deciduous forest soils than in coniferous forest soils. However, i noticed that the litter from deciduous leaves contains more decomposable components than that of coniferous leaves (Fog, 1988; Nowinski et al., 2008; Kim and Kang, 2011; de Vries et al., 2014; van Diepen et al., 2015), thus leading to greater C loss due to faster litter decomposition (Kim and Kang, 2011; Cotrufo et al., 2013; Gartzia-Bengoetxea et al., 2016). Therefore, I hypothesized that N deposition on the soil surface of two different forest (deciduous trees and coniferous trees) floors would result in different distribution patterns of the SOC pools in these forest soil systems. I tested this hypothesis with soil profile samples taken from two different forest floors in Mt. Taewha, Korea. To this end, I conducted a 3-year field experiment by applying ^{15}N-labeled urea once a year in a natural forest. During the experimental period, I measured the time-course patterns of total-C and N content, natural abundances of C isotopes along the soil profile.

2. Materials and Methods

2.1. Experimental sites and soil description

The experimental sites were located in a relatively mild hilly area (200 m above sea level), in a mixed coniferous-broadleaf forest that formed part of the Seoul National University Forest in Mt. Taewha, Geonggi-do, (37°18'N, 127°17'E), Korea (Figure 1). The annual mean air temperature and precipitation here are 11°C and 1389 mm, respectively. Since a mix of deciduous oak (*Quercus acutissima* Carruth., Qa) and coniferous pine (*Pinus koraiensis* Siebold & Zucc., Pk) tree species were predominant in the canopy of this forest, one stand each for these two tree species of a similar forest stand age (about 35–40 years old) were chosen for comparison. This forest did not receive any NPK fertilizations for 35–40 years since its establishment. Surface soils (0–20 cm) were collected from each of Qa soil and Pk soil using a soil auger, and the samples were then composited, air-dried at room temperature, passed through a 2–mm sieve and mixed homogeneously for physicochemical analyses. Soils under both experimental stands were classified as Dystrudepts (Great Group) and were texturally loam (USDA classification scheme): 443 g kg^{-1} sand, 323 g kg^{-1} silt and 235 g kg^{-1} clay for the Qa soil, and 517 g kg^{-1} sand, 294 g kg^{-1} silt and 189 g kg^{-1} for the Pk soil. Some relevant soil chemical properties under each forest stand are shown in Table 1.

2.2. Natural field ^{15}N experiments

Field experiments using ^{15}N-labeled urea were conducted for three consecutive years to assess the cumulative effect of N deposition on the soil C and N dynamics for the oak (Qa) and pine (Pk) forest stands under natural field conditions. Three locations

(15 m × 15 m each) spaced 20 m apart from each other were randomly chosen as replicates for each forest stand, and seven stainless steel soil retrieval profile cores (7.6 cm and 50.0 cm deep) per location were vertically installed in June (summer) 2011 after the removal of tree leaf-litter from the surface. One soil profile core per location was taken and dissected into six sections (0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm), and the soils in each section were analyzed for the initial characterization of chemical properties of the soil profile. In each experiment location, half of the remaining profile cores were treated individually with 0.28 g urea (= 0.136 g N) (^{15}N-urea at 300 kg N ha^{-1} yr^{-1}, 5 atom% excess) on the surface of each profile, while the other half was left untreated (control). Subsequently, 5 ml of distilled water was carefully applied to the surfaces to prevent loss of ^{15}N-urea. In June (summer) 2012, two profile cores per location were taken from each of the N-treated and control groups for soil chemical analyses (the first year sampling), and the same amount of ^{15}N-urea was applied to the soil surface of the remaining two profile cores assigned to the N-treatment groups. The same procedures were repeated in June 2013–2014 to take soil samples for the second and third year soil chemical analyses.

2.3. Sampling and chemical analyses

Soil samples were collected from the 0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm layers of each soil profile core. Soil bulk density was determined by gravimetry using intact cores (Blake and Hartge, 1986). Each soil sample was air-dried at room temperature, passed through a 2-mm sieve, mixed homogeneously, and analyzed for soil pH, mineral N (NH_4^+–N + NO_3^-–N), total-C, δ^{13}C, total-N and ^{15}N atom%. The soil pH was measured potentiometrically in a 1:5 (w/v) soil-to-water suspension using a pH meter (Orion 3 Star, Thermo Scientific, USA). Approximately 15 g of

fresh soil (8 g on an oven-dry basis) was extracted with 60–mL of 2 M KCl, and the extract was filtered through a Whatman No. 42 filter paper followed by a 0.45–μm nylon membrane. The filtrate was analyzed for NH_4^+–N and NO_3^-–N by steam distillation. A 30-mL aliquot of each filtrate was added to a distillation flask and steam-distilled with MgO for NH_4^+–N determination; thereafter, the sample in a flask was steam-distilled again after addition of Devarda's alloy for NO_3^-–N determination (Mulvaney, 1996). During each distillation, the liberated NH_3 was collected in a 0.005 M H_2SO_4 solution, and then titrated with a 0.01 M NaOH solution using an automatic titrator (702 SM Titrino, Metrohm, Switzerland) for the determination of each mineral N concentration. The total mineral N was calculated as the sum of NH_4^+–N and NO_3^-–N concentrations. The dried soil samples were ground into very fine powder using a ball mill (MM400, Retsch, Germany) to determine total-C (TC) and N (TN), and $\delta^{13}C$, $\delta^{15}N$, and ^{15}N atom%. The values of TC, TN, $\delta^{13}C$, $\delta^{15}N$, and ^{15}N atom% were analyzed with a continuous-flow stable isotope ratio mass spectrometer linked to a CN elemental analyzer (IRMS, IsoPirme-EA, Micromass, UK).

2.4. Calculation and Statistical analysis

Natural abundances (δ) of the stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were calculated as:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} is either the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for the samples and $R_{standard}$ is the ratio for a standard [Pee Dee Belemnite for C and atmospheric N_2 (= 0.0036765) for N]. In ^{15}N-isotope dilution, the ^{15}N recovery (%) in each soil receiving ^{15}N inputs can be obtained using the follows equations (Ro et al., 2018).

$$^{15}N \text{ Recovery } (\%) = NDFI / Nf$$

$$NDFI = T \times (AS / AF)$$

where NDFI is the N derived from ^{15}N-labeled inputs, Nf is the amount of N input, T is the total amount of N in the N-treated soil, AS is the atom % excess ^{15}N in the soil sample, and AF is the atom % excess ^{15}N in the N inputs treated.

All statistical analyses were performed with General Linear Model (GLM) procedures in SAS software (SAS Institute, Version 9.3, Cary, NC, USA). The effects of four factors (tree species, N treatment, time, and soil depth) and their interactions on soil pH, NH_4^+–N, NO_3^-–N, TN, NDFI, TC, and $\delta^{13}C$ were evaluated. A four-way analysis of variance (ANOVA) for a completely randomized design with three replications per treatment was performed to test for significant differences among the treatment means within each factor and for interactions among factors. Tukey's least significant difference (LSD) test at the level of $p < 0.05$ was used to test for significant differences among means. The calculated *p*-values for the three main factors and the interaction are shown in Table 2.

Figure 1. A map of the Mt. Taewha and location of experimental sites.

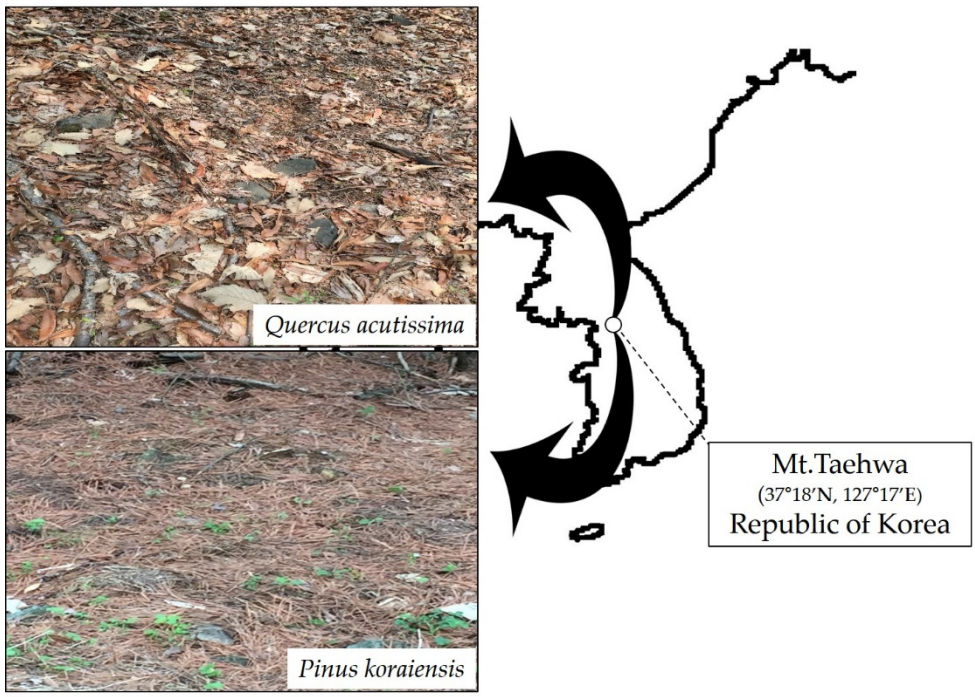

Quercus acutissima
Pinus koraiensis
Mt.Taehwa
(37°18′N, 127°17′E)
Republic of Korea

Table 1. Some chemical characteristics of the soils collected from two different oak (*Q. acutissima*) and pine (*P. koraiensis*) forest floor soils.

Soil properties	*Q. acutissima*	*P. koraiensis*
pH (soil:water = 1:5[a])	4.8 ± 0.0	4.6 ± 0.0
Mineral N (mg kg^{-1})	5.5 ± 1.1	12.1 ± 3.0
Total-C (g kg^{-1})	39.4 ± 1.4	22.6 ± 0.8
$\delta^{13}C$ (‰)	–29.6 ± 0.1	–24.9 ± 0.4
Total-N (g kg^{-1})	2.8 ± 0.1	1.4 ± 0.1
$\delta^{15}N$ (‰)	1.8 ± 0.1	2.1 ± 0.2
C/N ratio	14.2 ± 0.2	16.1 ± 0.3

[a] Soil-to-suspension ratio of 1:5

[b] The values are given as mean ± standard deviation (n = 3)

3. Results

3.1. Initial chemical properties of two different forest floor soils

The chemical properties of Qa and Pk soils are shown in Figure 2. Mineral N contents and pH were higher in the Pk soil than in the Qa soil at soil depth 0–20 cm, but the inverse results at soil depth 20–50 cm ($p < 0.05$) (Figure 2a and 2b). Mineral N contents decreased with soil depth from 11.6 mg kg^{-1} (0–5 cm) to 1.5 mg kg^{-1} (40–50 cm) for the Qa soil, and from 16.7 mg kg^{-1} to 7.8 mg kg^{-1} for the Pk soil ($p < 0.01$). The total-C and N contents were higher in the Qa soil than in the Pk soil throughout the soil profile, and decreased with increasing soil depth for both soils ($p < 0.001$). Differences in the average total-C and N contents of the upper layer of the soil (0–20 cm) between the Qa and Pk soils were 16.8 g kg^{-1} and 1.32 g kg^{-1}, while those of the lower layer (30–50 cm) were 3.81 g kg^{-1} and 0.27 g kg^{-1}, respectively. (Figures 2c and 2d). The $\delta^{13}C$ of total-C was less negative in the Pk soil than in the Qa soil throughout the soil profile, and increased to a depth of 20–30 cm in both soils ($p < 0.001$) (Figures 2c and 2d). On the other hand, the $\delta^{15}N$ of total-N increased from near 0‰ (surface layer) to near +7‰ at a depth of 20–30 cm beyond ($p < 0.05$) (Figures 2c and 2d).

3.2 Soil pH and mineral N contents

Soil pH decreased during the experimental year and soil depth regardless of tree species, and the decrease was greater in the Pk soil than in the Qa soil ($p < 0.05$) (Figure 3). In the first-year of the experiment, soil pH at 0–20 cm decreased sharply by 0.29 in the Qa soil and 0.41 in the Pk soil, which were lower than that of control soil throughout the experiment ($p < 0.001$). At the end, compared with the control

Figure 2. Some characteristic chemical properties of two different oak and pine forest floor soils prior to N treatment: (a) mineral N, (b) pH, (c) total-C and its $\delta^{13}C$, and (d) total-N and its $\delta^{15}N$. The error bars indicate ± one standard deviation (*n* = 3).

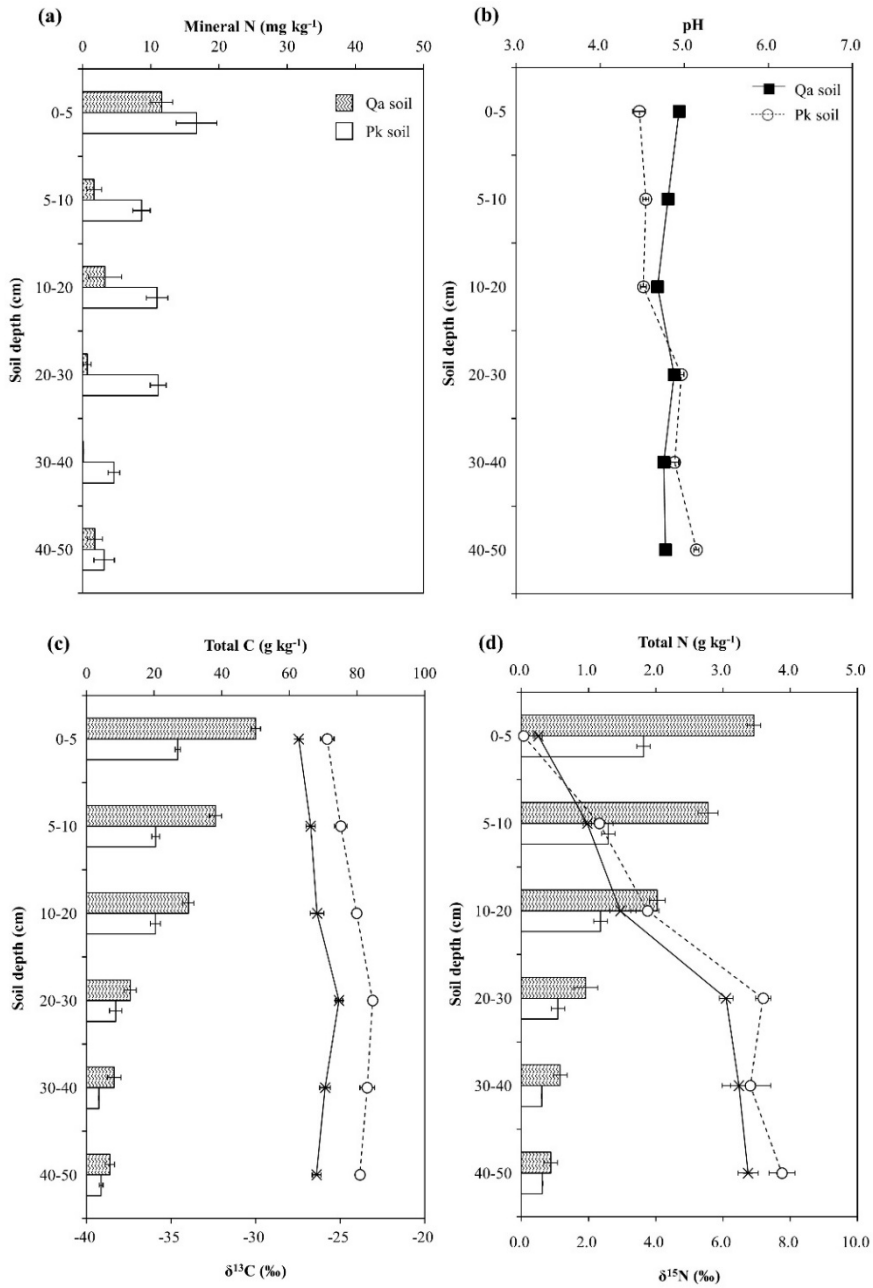

(a)
Mineral N (mg kg-1)
0
10
20
30
40
50
Qa soil
Pk soil
0-5
5-10
10-20
20-30
30-40
40-50
Soil depth (cm)
(b)
pH
3.0
4.0
5.0
6.0
7.0
Qa soil
Pk soil
Soil depth (cm)
(c)
Total C (g kg-1)
0
20
40
60
80
100
Soil depth (cm)
-40
-35
-30
-25
-20
δ13C (‰)
(d)
Total N (g kg-1)
0.0
1.0
2.0
3.0
4.0
5.0
Soil depth (cm)
0.0
2.0
4.0
6.0
8.0
10.0
δ15N (‰)

Figure 3. Annual variations in pH profiles after N deposition into two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

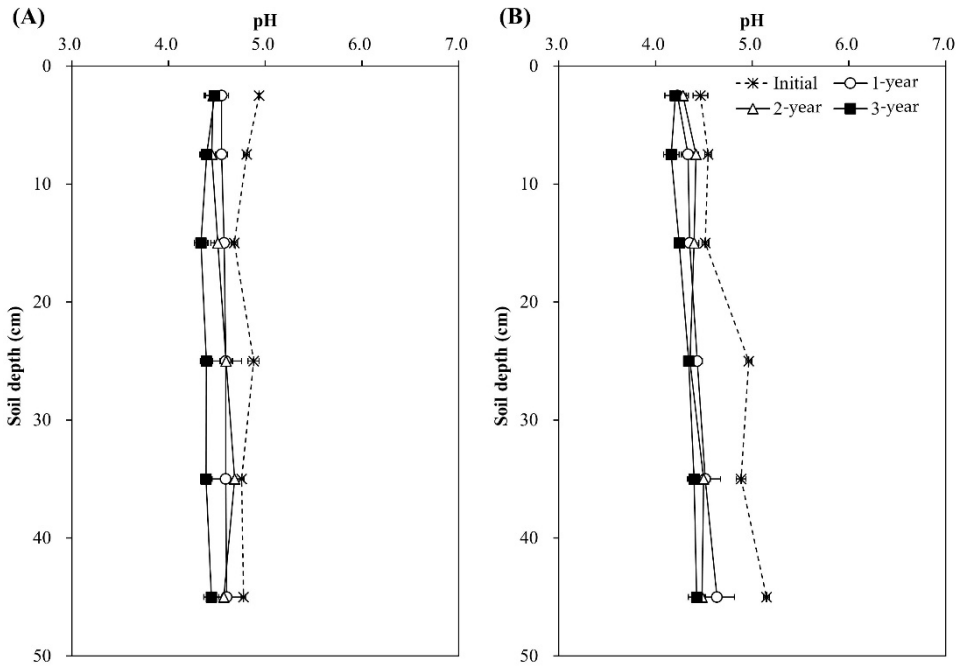

(A)
pH
3.0
4.0
5.0
6.0
7.0
0
10
20
30
40
50
Soil depth (cm)
(B)
pH
3.0
4.0
5.0
6.0
7.0
Initial
1-year
2-year
3-year
0
10
20
30
40
50
Soil depth (cm)

Table 2. Results of four-way analysis of variance (ANOVA) showing the significance of the effects of experimental parameters on pH, mineral N (NH_4^+–N and NO_3^-–N), total-N (TN), N derived from ^{15}N-labeled inputs (NDFI), total-C (TC), and $\delta^{13}C$.

Factors		Mineral N					
	pH	NH_4^+	NO_3^-	TN	NDFI	TC	$\delta^{13}C$
Time (T)	*	n.s.	n.s.	n.s.	*	n.s.	***
Treatment (N)	***	*	***	**	***	***	*
Soil depth (D)	***	***	***	***	***	***	**
Tree species (S)	***	n.s.	*	n.s.	n.s.	**	***
T × N	*	***	*	*	*	*	*
T × D	*	***	n.s.	*	*	**	*
T × S	*	*	*	n.s.	n.s.	n.s.	n.s.
N × D	***	***	***	***	***	***	**
N × S	***	**	***	*	*	***	n.s.
D × S	***	n.s.	*	n.s.	n.s.	**	**
T × N × D	n.s.	***	n.s.	n.s.	**	*	*
T × N × S	*	*	*	n.s.	*	*	*
T × D × S	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
N × D × S	**	*	*	*	n.s.	**	n.s.
T × N × D × S	*	n.s.	*	n.s.	*	n.s.	*

***: significant at the $p < 0.001$ level; **: significant at the $p < 0.01$ level; *: significant at the $p < 0.05$ level; n.s.: not significant ($p > 0.05$).

soil, the pH of N-treated soils decreased by 0.3 and 0.3 in the upper layers of the Qa and Pk soils, and by 0.3 and 0.6 in the lower layers (30–50 cm) of the Qa and Pk soils, respectively. Prior to N treatment, mineral N (NH_4^+–N and NO_3^-–N) contents were higher in the Pk soil than in the Qa soil, and this pattern was maintained throughout the experiment (data not shown). However, the levels of NH_4^+–N at soil depth of 0–5 cm in N-treated soils were not different during the first 2 year but increased during the third year ($p < 0.05$) (Figures 4A–a and 4B–a), while those of NO_3^-N increased in the Qa soil and decreased in the Pk soil throughout the experiment ($p < 0.05$) (Figures 4A–b and 4B–b).

3.3. Soil total-N content, NDFI, and ^{15}N recovery

Regardless of tree species, total-N content in N-treated soils increased with time, while those in the control soils remained virtually unchanged ($p < 0.05$) (Figure 5). The increasing effect due to N application was evident in the surface soil layer (0–5 cm), but not in the layer below ($p > 0.05$). The distribution patterns of the NDFI were uniform throughout the soil profile and similar in both N-treated soils during the first year, and the NDFI increased with time particularly in the surface soil layer ($p < 0.01$) (Figure 6). The increase in the NDFI in this layer was greater in the Pk soil than in the Qa soil. The percent ^{15}N recovery of soils was calculated based on the NDFI values (data not shown). At the end of experiment, the total recovery of ^{15}N from both N-treated soils calculated through the mass N-balance approach was 61.4 ± 4.8% for the Qa soil and 77.2 ± 10.6% for the Pk soil, with an average of approximately 30.7 % of N unrecovered portion.

Figure 4. Annual variations in (a) NH_4^+–N content and (b) NO_3^-–N content profiles after N deposition into two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. Each N content indicate the difference between N-treated and control soils: The error bars indicate ± one standard deviation (*n* = 3).

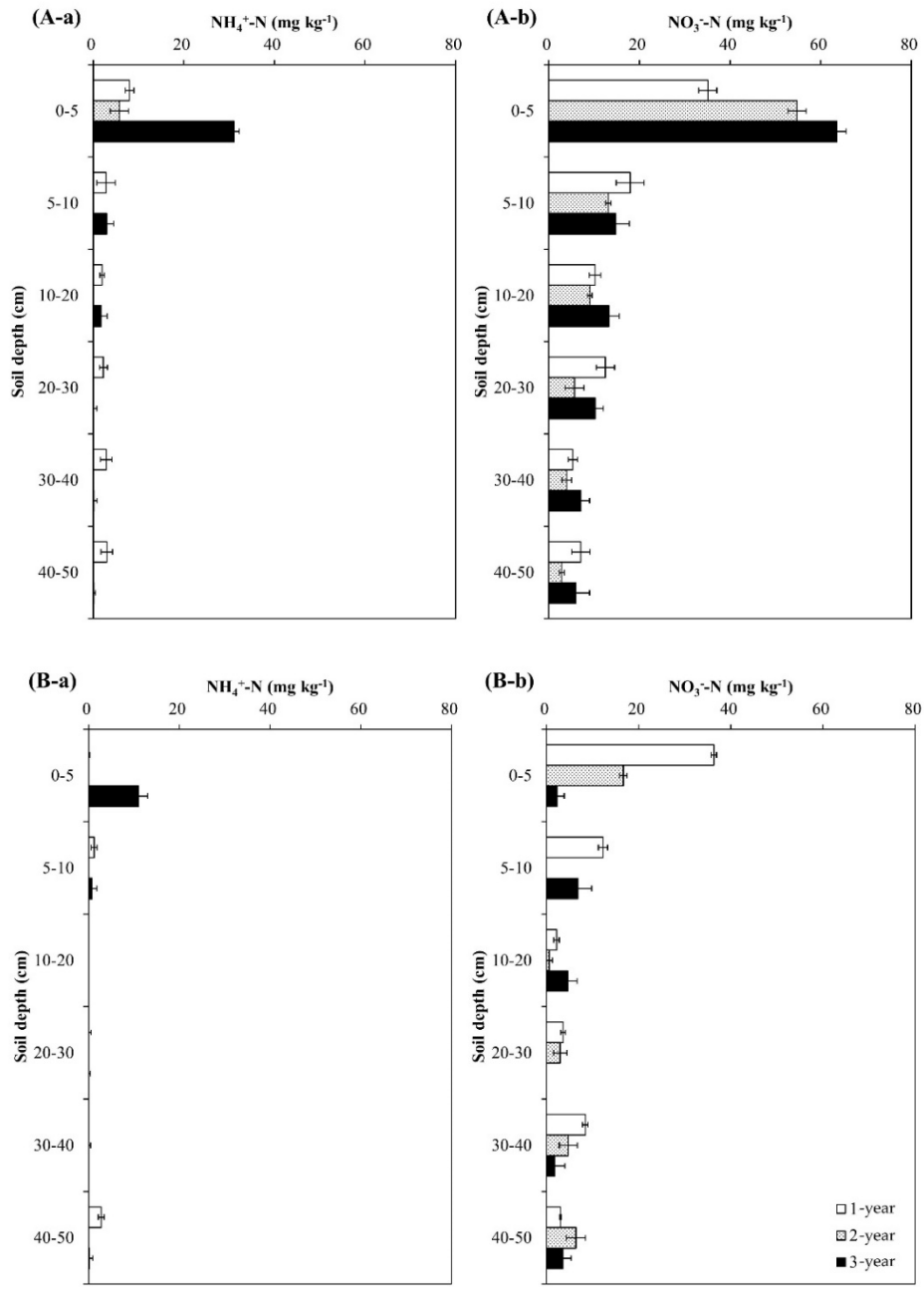

(A-a)
NH4+-N (mg kg-1)
0
20
40
60
80
0-5
5-10
10-20
20-30
30-40
40-50
Soil depth (cm)
(A-b)
NO3--N (mg kg-1)
(B-a)
(B-b)
1-year
2-year
3-year

Figure 5. Annual variations in total-N after (a) 1 year, (b) 2 years, and (c) 3 years of N deposition in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. For each N profile, different lowercase letters indicate significant difference ($p < 0.05$). The error bars indicate ± one standard deviation ($n = 3$).

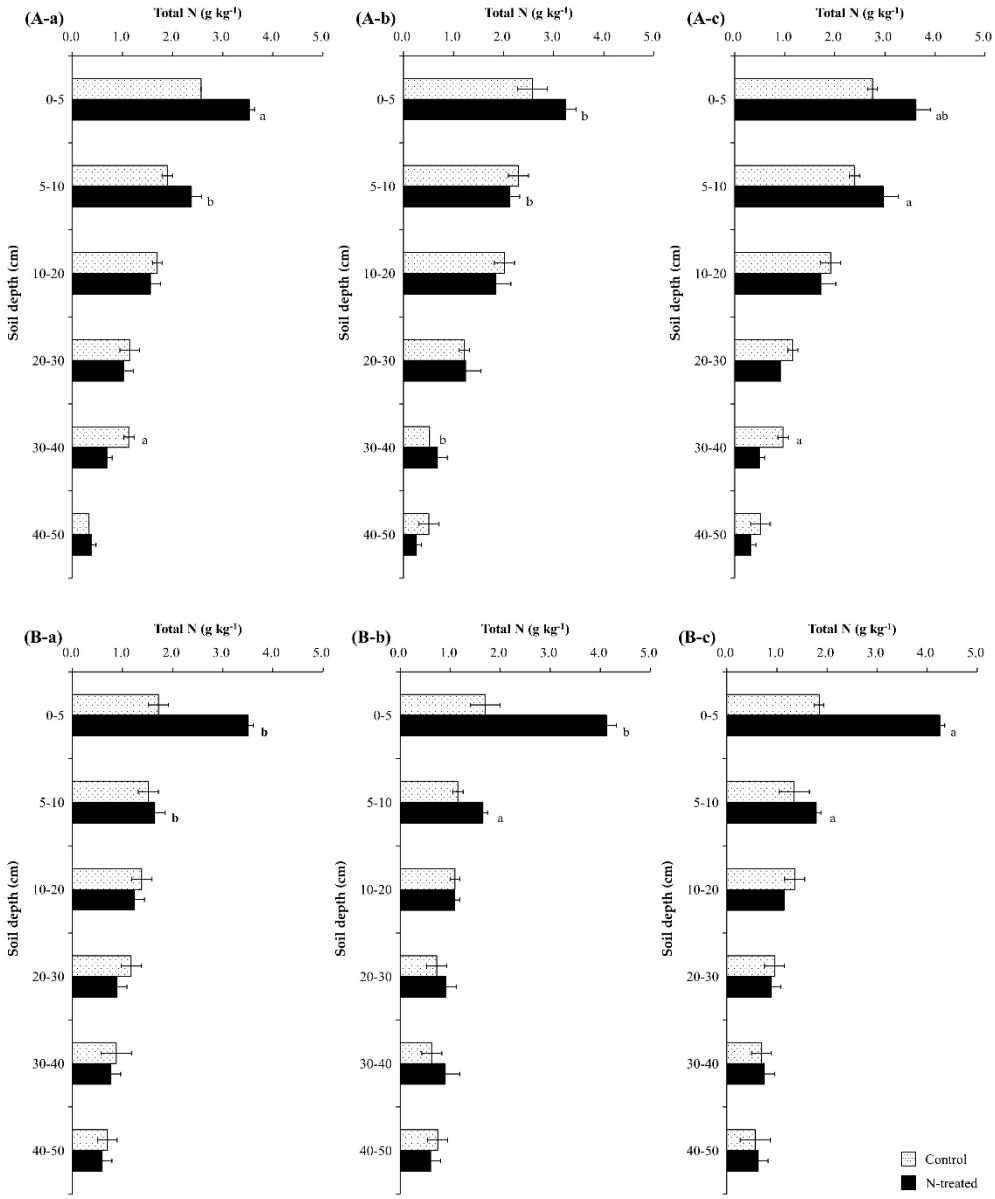

(A-a)
(A-b)
(A-c)
(B-a)
(B-b)
(B-c)
Total N (g kg-1)
0.0
1.0
2.0
3.0
4.0
5.0
Soil depth (cm)
0-5
5-10
10-20
20-30
30-40
40-50
a
b
ab
Control
N-treated

Figure 6. Annual variations in the amount of N derived from ^{15}N-labeled input sources (NDFI) along the soil profile in two different (A) oak (Qa) and (B) pine (Pk) tree forest soils. The error bars indicate ± one standard deviation (*n* = 3).

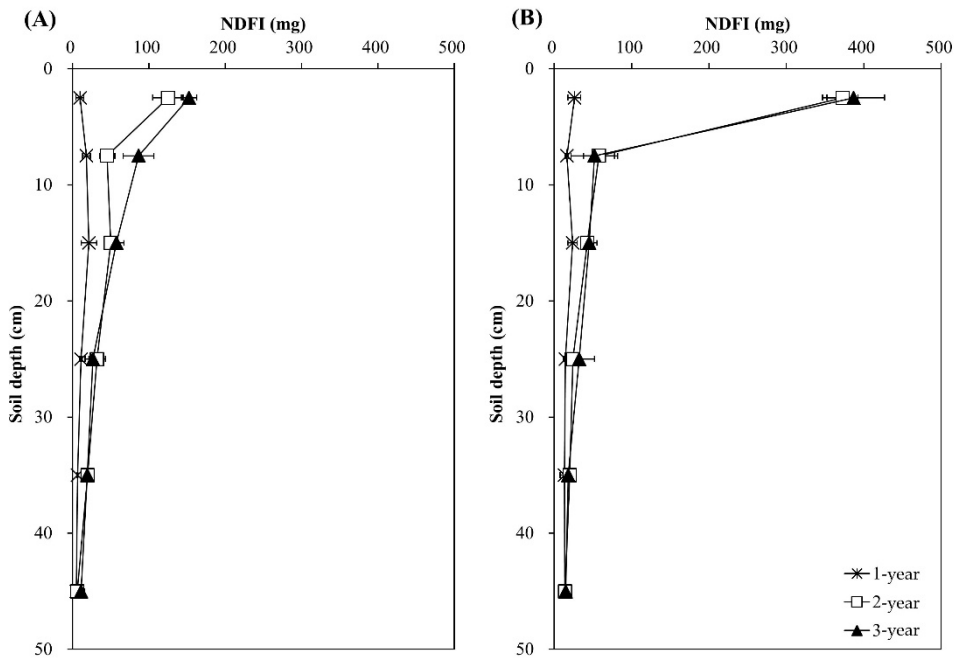
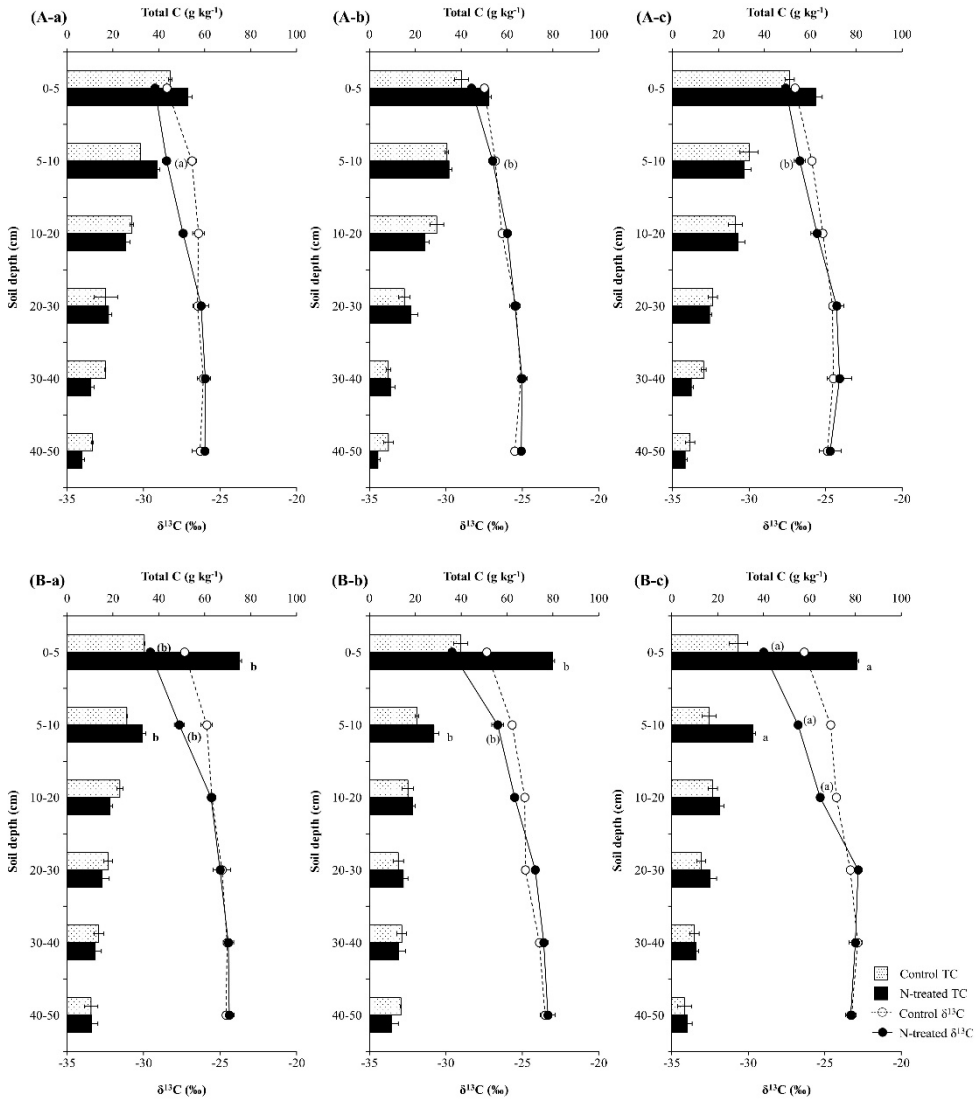

(A)
NDFI (mg)
0
100
200
300
400
500
Soil depth (cm)
0
10
20
30
40
50
(B)
NDFI (mg)
1-year
2-year
3-year

Figure 7. Annual variations in total-C (Top) and $\delta^{13}C$ (Bottom) after (a) 1 year, (b) 2 years, and (c) 3 years of N deposition in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. For each N profile, different lowercase letters indicate significant difference ($p < 0.05$). The error bars indicate ± one standard deviation ($n = 3$).


(A-a)
(A-b)
(A-c)
(B-a)
(B-b)
(B-c)
Total C (g kg-1)
Soil depth (cm)
0-5
5-10
10-20
20-30
30-40
40-50
δ13C (‰)
Control TC
N-treated TC
Control δ13C
N-treated δ13C

3.4. Soil total-C content and $\delta^{13}C$

Compared with the control soils, the application of N increased total-C content and decreased $\delta^{13}C$ values in the upper soil layer (0–10 cm) during the first year, and the effect was greater in the Pk soil than in the Qa soil ($p < 0.05$) (Figure 7). However, as time progressed, total-C content at soil depth of 5–10 cm increased in the Pk soil, while the increasing effect disappeared in the Qa soil (Figures 7A–a, 7A–b and 7A–c). During the same period, the decrease in $\delta^{13}C$ values in the upper soil layer due to N application disappeared in the Qa soil, while $\delta^{13}C$ values of total-C in deeper layer (10–20 cm) were suppressed more negatively, resulting in an increase in total-C content in the soil layer behind the advancing $\delta^{13}C$ values front ($p < 0.05$) (Figures 7B–a, 7B–b and 7B–c).

4. Discussion

4.1. Differences in initial chemical properties of two different forest soils

Since deciduous trees have higher nutrient levels and lower levels of lignin and polyphenols than coniferous trees, leaf-litter of the former decomposes faster than that of the latter (Prescott et al., 2000; Zhang et al., 2008; Chiti et al., 2012; Gurmesa et al., 2013), resulting in different soil chemical properties under these two contrasting forest stands (Figure 2). In this study, mineral N contents of the Pk soil were higher than those of the Qa soil (Figure 2a), and this difference can be explained by the differences in the N uptake patterns between tree species (Xing et al., 2010; Kristensen et al., 2014). In general, the amount of N uptake by coniferous trees is almost 56% of that absorbed by deciduous trees (Prescott et al., 2000; Zhang et al., 2008; Gurmesa et al., 2013). However, higher soil total-C and N contents in the Qa soil than in the Pk soil (Figures 2b and 2c) were due to lower levels of lignin and polyphenols of leaf-litter of Qa tree species (Prescott et al., 2000; Zhang et al., 2008; Chiti et al., 2012; Gurmesa et al., 2013) and its faster decomposition in the Qa soil (Turner et al., 1983; Fernandez et al., 2003; Boström et al., 2007). In particular, soil $\delta^{13}C$ and $\delta^{15}N$ values were higher in the lower layers than in the upper layers, and this difference in the isotopic C and N compositions of soil total-C and total-N pools between two soil zones could be explained by the mixing of fresh substrates having more negative $\delta^{13}C$ and lower $\delta^{15}N$ into the preexisting old SOC pools that had been enriched in ^{13}C and ^{15}N in the profiles for both soils (Turner et al., 1983; Boström et al., 2007).

Nevertheless, I could deduce that both $\delta^{13}C$ of soil total-C and $\delta^{15}N$ of total-N along the soil profile reflect isotopic fractionation associated with litter-fall decomposition and physical mixing processes. Since forest soils are characterized

by litter-fall and root exudates that are gradually mixed and decomposed down the soil profile (Acton et al., 2013), more decomposed SOM can exist lower in the soil profile as a result of decomposition and physical mixing while newer C and N inputs tend to exist shallower in the soil profile (Wang et al., 2018). It is well known that microbial enzymes discriminate against ^{13}C and ^{15}N during SOM decomposition, resulting in significant enrichment of ^{13}C and ^{15}N in SOM pools (Turner et al., 1983; Fernandez et al., 2003; Boström et al., 2007). Therefore, the experimental site well reflected the history of long-term enrichment of ^{13}C and ^{15}N in SOM pools and the current mixing of new SOM substrates into the soil profile (Figures 2b and 2c).

4.2. Effect of N treatment on soil pH and mineral N

With time, compared with the control soils, the pH in both N-treated soils decreased throughout the soil profile, with greater decreases in the Pk soil than in the Qa soil (Figure 3). In general, it is known that the decrease in soil pH in forest stands correlates well with the production of H^+ during nitrification (Davidson et al., 1992; Luo et al., 2015); however, NO_3^-–N contents were maintained at higher levels in the Qa soil than in the Pk soil (Figures 4A–b and 4B–b), unlike the previous investigations in which the rates of nitrification in coniferous tree stands are faster than in deciduous tree stands (Staelens et al., 2012). However, greater decreases in pH in the Pk soil than in the Qa soil could not be explained by nitrification alone. In addition to nitrification, the production of H^+ from leaf-litter decomposition may also affect soil acidification, since coniferous litter produces more organic acids than deciduous litter (Augusto et al., 2002).

Most previous studies have shown that greater and more frequent NO_3^- leaching occurred in soils under coniferous tree stands than under deciduous tree stands (Davidson et al., 1992; Luo et al., 2015) and the leaching of nitrate down the soil

profile occurred more rapidly when the C/N ratio of a SOM pool was below a threshold of 25 (Kristensen et al., 2014). Particularly, even at the same C/N ratio, leaching of nitrate was greater in soils under coniferous tree stands than under deciduous tree stands (Augusto et al., 2002; Staelens et al., 2012; Kristensen et al., 2014; Dawud et al., 2016). In this experimental sites, intense rainfall occurs from July to August during summer season (approximately 50% or more of the mean annual precipitation), and rainfall exceeds evapotranspiration.

This results of annual distribution patterns of NO_3^- (Figures 4A–b and 4B–b) were consistent with above-mentioned results of previous studies of NO_3^- leaching through the soil profile, and this pattern of NO_3^- transported by leaching was well corroborated by the NDFI profiles (Figure 6) in which N treated on the soil surface of the forest floor was transported down to the bottom of the profile column during the first year. However, during the following two years, greater portion of N was recovered in the upper surface layer in both soils, indicating the incorporation of urea-N into the formation of SOM and/or microbial mass.

4.3. Effect of N treatment on soil total-N contents

The application of N increased total-N content in the topmost soil layer (0–5 cm) (Figure 5) due to enhanced decomposition of leaf-litter containing about 5% N (Fog, 1988; Nowinski et al., 2008), and the increasing effect was greater in the Pk soils than in the Qa soils due to greater decomposition (Augusto et al., 2002; Dawud et al., 2016). A greater increase in total-N observed in the topmost soil layer was well corroborated by the recovery and partitioning of applied-N in the soil profile (Figure 6). In particular, a greater soil total-N in the Pk soil than in the Qa soil indicated faster decomposition of the Pk leaves in the forest floor, as evidenced by a greater increase in total-C and a concurrent decrease in soil-$\delta^{13}C$ (Figure 7).

At the end, total amounts of urea-N recovered and the corresponding recovery in the system were 353.1 ± 3.8 mg and 61.4 ± 4.8% for the Qa soil and 455.8 ± 2.9 mg and 77.2 ± 10.6% for the Pk soil (data not shown), indicating a considerable N loss from the system. The unrecovered loss of ^{15}N could be ascribed to N leaching, denitrification and/or NH_3 volatilization (Ro et al., 2018). However, NH_3 volatilization or denitrification would have contributed much less to ^{15}N loss than N leaching based on the amounts of NDFI throughout the profile (Figure 6), since the soil pH remained acidic (below 5) and the soil remained mostly aerobic (data not shown). In addition, dissimilatory nitrate reduction to ammonia (DNRA) could result in decreasing NO_3^- concentrations (Gundersen et al., 1998), but I could disregard the contribution of DNRA to N loss, since denitrification is negligible and NH_4^+ concentrations remained virtually at low levels in both soils (Figure 4). Therefore, I deduced that NO_3^- leaching denitrification was mostly responsible for the unrecovered portion of ^{15}N (N loss) in both N-treated forest systems (Staelens et al., 2012; Luo et al., 2015; Ro et al., 2018;).

4.4. Effect of N treatment on soil total-C contents and $\delta^{13}C$ values

It is well known that N treatment to the forest floor increased the soil microbial activity and the soluble- and insoluble organic C pools in the forest soils (Tietema and Wessel, 1992), and the magnitude of the increase in the soil microbial activity and SOC pools varied with the litter composition of the plant species (Gundersen et al., 1998). I also observed that N treatment to the surface of the forest soils obviously increased soil total-C contents at 0–10 cm soil depth in both forest soils ($p < 0.001$) (Figure 7) due to stimulated decomposition of leaf-litter and SOC pools, and this phenomenon was corroborated by a greater increase in total-N content in this surface soil layer ($p < 0.01$) (Figure 5). In particular, the portion of N recovered in the surface

layer of the soil was greater under coniferous tree (Pk) stands than under deciduous tree (Qa) stands (Figure 6), and this phenomenon could explain greater increase in total-C contents in the Pk soil due to greater litter decomposition (Figure 7). Unlike most previous observations that showed an increase in soil total-C content was greater under deciduous tree stands than under coniferous tree stands as a result of faster decomposition of SOM pools in the former forest soils than in the latter forest soils (Booth et al., 2005; Tonitto et al., 2014), I observed that N treatment caused a faster tree litter decomposition in the Pk soil than in the Qa soil, resulting in a greater increase in total-C content in the Pk soil than in the Qa soil. Finn et al. (2015) reported that the amounts of soil C and N pools remained in coniferous forest floors after decomposition at a constant temperature were larger than those in deciduous forest floors. In particular, even under similar environmental conditions (climate region, temperature, rainfall, and soil moisture), it is well-known that soil CO_2 efflux was highest from deciduous forests, next from mixed forests and lowest from coniferous forests (Lettens et al., 2005; Kim et al., 2010; Finn et al., 2015), as a result of higher lignin content of their leaf-litter (Laganière et al., 2010; Qualls, 2016) and lower SOM degradation (Kime et al., 2010). Compared with the control soils, N deposition obviously increased soil total-C contents ($p < 0.001$) (Figure 7), and this increase in SOC pools was well supported by the increase in ^{15}N recovery (Figure 6) and the concurrent decrease in soil- $\delta^{13}C$ to 0–10 cm soil depth in both forest soils (Figure 7).

It is believed that $\delta^{13}C$ reflects the decomposition and physical mixing of leaf-litter (new substrate) and SOC (old substrate) pools in forest soils (Farquhar et al., 1989; Davidson et al., 1992; Boström et al., 2007). I obviously showed that compared with their respective control soils, $\delta^{13}C$ of total-C in the surface soil layer (0–10 cm) decreased due to N deposition while total-C content increased, indicating the mixing and transport of new C substrates into old C substrates down the profile

($p < 0.001$) (Figure 7). During the same time, the decrease in $\delta^{13}C$ of total-C was maintained in the upper surface layer under the Pk stands (Figure 7), and this could reveal sustained incorporation of leaf-litter and release of newer C substrates from decomposition, resulting in a greater increase in total-C content. In particular, the increase in total-C content and the concurrent decrease in its $\delta^{13}C$ became greater with time in the Pk soil than in the Qa soil (Figure 7), and the annual increase in total-C content was well corroborated by the increase in ^{15}N recovery in this soil layer (Figure 6).

However, soil total-C contents were not different between both N-treated soils and the control soils below a soil depth of 10 cm, and this pattern indicated that new C substrates from litter decomposition did not penetrate into the soil profile below this soil depth (10 cm) during three years, since NO_3^- ions migrate much faster than organic C particles through the soil profile (Edmoned, 1991). The increasing patterns of $\delta^{13}C$ from the surface to this soil depth obviously reflected the physical mixing of new C substrates with old C substrates while migrating down the profile (Figure 7), even though the decomposition of SOC pools leaves behind the heavier ^{13}C substrates in the soil. Since forest soils are characterized by litterfall and SOM pools that are gradually mixed and degraded down the soil profile, more recalcitrant substrates (such as lignin, fat, and wax) are in deeper soil layers (Tietema and Wessel, 1992; Laganière et al., 2010). It is the common notion that N treatment causes an increase in SOC contents through increased soil microbial activity (Edmoned, 1991). However, this results invariably indicated that the decomposition of old SOC pools is very limited in deeper soil layers where the chemical and biological activities are limited.

5. Conclusions

I challenged a commonly-held belief that an increase in soil C pools due to N deposition is greater in deciduous forest soils than in coniferous forest soils. I hypothesized that N deposition to the surface of the forest floor would stimulate the decomposition of litter and SOC pools and affect the mixing of new C substrates released from decomposition into preexisting old SOC pools down the profile, thus causing a difference in the response to N deposition between two oak and pine forest soils. I interpreted the causal relations for the differences in SOC dynamics in a temperate natural oak and pine forest by analyzing natural ^{13}C abundances ($\delta^{13}C$) and by using the ^{15}N-isotope dilution technique. Compared with their respective control soils, the lowering of $\delta^{13}C$ with increasing total-C contents in the surface soil layers (0–20 cm) for both forest soils after N application well reflected the relative contribution of the production of fresh C substrates from litter decomposition and the subsequent physical mixing into old SOC pools down the soil profile. In addition, I found that the incorporation of new C substrates into old SOC pools was greater under coniferous pine tree stands than under deciduous oak tree stands at least in this temperate region. Particularly, an increasing pattern of $\delta^{13}C$ of the soil to a depth of 20 cm indicated deeper penetration of new C substrates into the soil profile as a result of ^{13}C isotope mixing with old C pools, and this phenomenon was well evidenced by an increase in ^{15}N recovery in this upper region with time. However, I could not fully explain how differences in nutrient composition of leaf-litter between two contrasting tree species affect litter decomposition and the formation of SOC pools, since no direct measurements were made on the kinetics of litter decomposition and microbial activity in both forest floor soils. Despite this lack of direct information supporting the contribution of leaf-litter decomposition to the increase in SOC pools, I revealed that an increase in total-C and N contents due to N deposition was greater

under coniferous forest stands than under deciduous forest stands as a result of greater mixing of new C substrates into the soil profile in this temperate forest. Therefore, the kinetics of litter decomposition of leaf-litter types, soil microbial activity, separation of the mixing of new substrates from the decomposition of old C substrates, and the formation and stabilization of SOC pools should be considered in advance.

CHAPTER II

Effects of nitrogen addition on soil carbon aggregate-size and fractions in soils under temperate oak and pine forest stands

Abstract

To investigate the effects of nitrogen (N) treatment on the soils of two different forest floors (deciduous trees, Qa; coniferous trees, Pk), specifically the effects of N on different sizes of soil aggregates and soil organic carbon (SOC) decomposition patterns, this study examined the temporal variation during 365 days of incubation. Soil samples were taken from two different forest stands and were divided into two treatment groups: a control treatment group and an N treatment group (^{15}N-urea at 100 kg N ha^{-1} yr^{-1}, 5 atom% excess). The soil samples were incubated at 25 ± 2°C for 365 days, after which they were analyzed for soil mineral N (NH_4^+–N + NO_3^-–N) content, dissolved organic carbon (DOC) content, total carbon (TC) content, $\delta^{13}C$, soil aggregate distribution, total nitrogen (TN) content and ^{15}N recovery. N treatment decreased both the mineral N content and DOC content in the soils regardless of tree species. Interestingly, different patterns of TC content between the Qa and Pk soils were observed as a result of differences in the decomposition characteristics associated with their chemical compositions. However, regardless of tree species, there was no significant difference in $\delta^{13}C$ values among the sizes of the aggregates, unlike in whole soil. At the end of incubation, the N treatment of the soils of both tree species reduced the proportion of aggregates that were 2000–1000 μm in size, whereas the proportion of aggregates that were 1000–250 μm, 250–53 μm, and <53 μm in size increased. Therefore, I conclude that N treatment affects both the decomposition of organic matter (OM) and soil aggregate distribution and that these differences depend on tree species.

Keywords: *Tree species; Forest soil; Nitrogen input; Soil aggregate; Deciduous tree*

1. Introduction

In general, soil organic matter (SOM) is the major reservoir of carbon (C) in terrestrial ecosystems, and SOM exists in various complex forms in the soil. Furthermore, SOM is an important determinant of soil quality, and qualitative and quantitative changes in SOM are determined by terrestrial ecosystem characteristics, such as physical (soil crust, moisture retention, aggregate stability, etc.), chemical (pH, cation exchange capacity, nutrient cycling, etc.), and biological (vegetation, soil enzymes, microorganisms, etc.) properties of the soil (Doran and parkin, 1996). In forest ecosystems, SOM is obtained from plant litterfall and woody debris on the soil surface, whereas subsoils obtain organic matter (OM) from the death and decay of tree roots. However, since the OM composition of leaf and root litter varies with the type (deciduous or coniferous) of tree species, the composition of tree communities predominantly determines OM decomposition rates as well as the size and quality of soil organic carbon (SOC) pools (Hobbie, 2015). For these reasons, it is important to understand how different tree species might affect SOC dynamics.

Soil aggregates are formed by the interaction and binding of SOM, microorganisms, and soil mineral particles; these particles are arranged in and formed of numerous sizes, varying the volume and size of soil pores (Horn et al., 1994; Brady and Weil, 2008). Soil aggregates are influenced by the physical, chemical and microbiological characteristics of soils, including OM, clay minerals, types of cations, plant roots, and various biological factors (Dorioz et al.,1993; Chenu et al., 2000). Among these characteristics, OM strongly influences the formation of the soil surface. In general, when the OM content of a soil increases, microbial propagation becomes active, and as a result, microbial secretions infiltrate the soil to form soil aggregates; consequently, the secretions promote OM humification (humin, humic acid and fulvic acid) and aggregate formation (Chaney

and Swift, 1984). Previous studies has shown that, in macro-aggregates (>250 μm), OM acts as a binding agent via plant roots and hyphae (Tisdall and Oades, 1982), whereas in micro-aggregates (<250 μm), OM acts on the charge of particles (Goldberg et al., 1990). The formation of these soil aggregates indicates that different mechanisms operate depending on the different size classes of soil aggregates (Amézketa, 1999). For example, macro-aggregates contain more SOM than do micro-aggregates because the former are composed of micro-aggregates held together by the organic material (Jastrow et al., 1996). Therefore, it is important to study SOC dynamics because SOM is considered an important factor in both the formation of soil aggregates and the improvement of the habitat environment for microorganisms.

In terrestrial ecosystems, nitrogen (N) is a critical constraint that determines the size and composition of SOC pools; N addition often increases productivity in agricultural environments, while N promotes the decomposition of SOM. Many researchers have reported that N addition to the soil increases soil microbial activity and soluble- and insoluble organic C pools in the soil, and the differences depend on the litter composition of the plant species (Tonitto et al., 2014; Finn et al., 2015). Although recent data from several studies suggest that N addition exerts an effect by increasing microbial and SOM decomposition during the initial stage, decomposition was suppressed toward the late stage, during which mainly lignin was decomposed (Zak et al., 2008; Song et al., 2013). Riggs et al. (2015) reported that N addition decreased the decomposition rate of SOM by alleviating microbial nutrient limitations and, as a result, increased SOC storage at grassland sites through increased C occlusion in macro-aggregates. Despite the long history of N input to the soil, the effects of N addition on SOC dynamics differ across studies (Riggs et al., 2015). Therefore, understanding the interactive effects between N addition to the soil and soil aggregates on SOC decomposition dynamics is essential for determining

the residence time and the size of an ecosystem's SOC pools.

The natural isotope abundance ($\delta^{15}N$ and $\delta^{13}C$) is useful as a reliable tool for studying N and C dynamics in the soil and reflects the interaction between the source isotopic composition and the isotope fraction. For example, applications of N enriched in stable isotopes (^{15}N tracers) have been artificially added to terrestrial ecosystems and used to identify the fate and retention of N according to a timescale. Farquhar et al. (1989) reported that the process of discrimination of stable C isotopes differs by the C transformation involved with the organic material constituents and the physical, chemical and metabolic processes, which then indicates a difference in the stable C isotope values. Therefore, since the decomposition of SOC directly affects its stable C isotope composition, it can be used to explain the C dynamics in the soil.

Therefore, I hypothesized that N addition to the soils of two different forest floors (deciduous trees and coniferous trees) would result in different sizes of soil aggregates through different SOC decomposition patterns. I tested this hypothesis with soil profile samples taken from two different forest floors on Mt. Taewha. I conducted batch incubation experiments and determined the time-course patterns of total carbon (TC) and soil aggregate distributions as well as the corresponding natural abundance of stable isotopes along the soil aggregate distribution.

2. Materials and Methods

2.1. Sampling sites and soil preparation

The study site was chosen in a relatively mild hilly area (200 m above sea level) in a mixed coniferous-broadleaf forest located within the Seoul National University Forest on Mt. Taewha, Geonggi-do (37°18'N, 127°17'E), Korea. The annual mean air temperature and precipitation at the site are 11°C and 1389 mm, respectively. Since a mix of deciduous oak (*Quercus acutissima* Carruth., Qa) and coniferous pine (*Pinus koraiensis* Siebold & Zucc., Pk) tree species are predominant in the canopy of this forest, one similarly aged (approximately 35–40 years old) forest stand of each tree species was chosen for comparison. This forest has not received any nitrogen, phosphorus, and potassium (NPK) fertilizer for 35–40 years since its establishment. Samples (0–30 cm in depth) were taken from each forest stand using a soil auger in April 2016. Immediately after sampling, the soil samples were air dried at room temperature, passed through a 2 mm sieve, and mixed homogeneously. The physical and chemical properties of the soil are shown in Table 1.

2.2. Batch incubation experiments

To investigate the effects of N on the distributions of soil C and aggregates in the soil under different tree species, 220 g of each soil sample was transferred to 250 mL plastic bottles; a total of 72 bottles were prepared for all soil treatments in triplicate, necessitating 6 destructive samplings. Each bottle was covered with a perforated cap to ensure gas exchange and was preincubated at 25 ± 2°C in the dark for 7 days, after which the soil water content was adjusted to 0.31 m^3 m^{-3} [field capacity (–33 kPa)].

Table 1. Chemical and physical properties of soil under *Q. acutissima* and *P. koraiensis* stands.

Soil properties	*Q. acutissima*	*P. koraiensis*
Texture[a]	Clay loam	Clay loam
Bulk density (Mg m^{-3})	1.1 ± 0.0	1.1 ± 0.0
Field capacity (m^3 m^{-3})	0.34 ± 0.1	0.33 ± 0.1
pH (soil:water = 1:5[b])	5.0 ± 0.0	4.9 ± 0.0
Inorganic N		
NH_4^+–N (mg kg^{-1})	44.0 ± 1.5	45.4 ± 1.0
NO_3^-–N (mg kg^{-1})	2.6 ± 0.9	2.3 ± 0.3
Total-C (g kg^{-1})	23.5 ± 0.3	20.6 ± 0.2
$\delta^{13}C$ (‰)	–25.3 ± 0.0	–23.5 ± 0.0
Total-N (g kg^{-1})	1.7 ± 0.1	1.8 ± 0.1
$\delta^{15}N$ (‰)	3.5 ± 0.0	4.6 ± 0.0
C/N ratio	13.8 ± 0.1	11.4 ± 0.2

[a] USDA classification scheme

[b] Soil-to-suspension ratio of 1:5

Following their preincubation period, the soil samples were divided into two (treating the soils under each species separately): control (untreated) soils and N-treated soils (^{15}N-urea at 100 kg N ha^{-1} yr^{-1}, 5 atom% excess). Thereafter, the bottles were incubated at 25 ± 2°C, after which the soil water content was adjusted to maintain the field moisture capacity during the 365 day batch experiment by adding deionized water to the bottles as necessary to maintain their initial conditions. Soil samples were taken at 5, 15, 80, 150, 250, and 365 days of incubation, and those samples were thoroughly homogenized for soil chemical analyses.

2.3. Soil aggregate separation and soils analyses

The separation of aggregates based on size was performed in accordance with the dry sieving method (Kemper and Rosenau, 1986) using a vibratory sieve shaker (Analysette 3, Fritsch, Germany) with a stack of stainless steel sieves. A 50 g air-dried soil sample was placed within the top sieve and submerged for 10 min (oscillation amplitude of 20 mm and a frequency of approximately 50 Hz). The soil particles that were retained on the 2000–1000 μm (large and median macro-aggregates), 1000–250 μm (small macro-aggregates), and 250–53 μm (micro-aggregates) screens and that passed through the 53 μm (silt plus clay-sized particles) screen were weighed. The soil pH was measured potentiometrically in a 1:5 (w/v) soil:water suspension using a pH meter (Orion 3 Star, Thermo Scientific, USA). For the determination of mineral N, approximately 15 g of fresh soil (8 g on an oven-dried basis) was extracted with 60 mL of 2 M KCl, after which the extract was filtered through a Whatman No. 42 filter paper and then a 0.45 μm nylon membrane. The filtrate was analyzed for NH_4^+–N and NO_3^-–N by steam distillation. A 30 mL aliquot of each filtrate was added to a distillation flask and steam-distilled with MgO for NH_4^+–N determination; thereafter, the sample in the flask was steam-distilled

again after the addition of Devarda's alloy for NO_3^-–N determination (Mulvaney, 1996). During each distillation, the liberated NH_3 was collected into a 0.005 M H_2SO_4 solution and then titrated with a 0.01 M NaOH solution using an automatic titrator (702 SM Titrino, Metrohm, Switzerland) for the determination of mineral N content. The concentrations of dissolved organic carbon (DOC) were analyzed with a total organic C analyzer (Sievers 5310 C, GE Analytical Instruments, USA). Approximately 8 g of fresh soil was extracted with distilled water at a soil:solution ratio of 1:5 (w/v), after which the extract was filtered through a Whatman No. 42 filter paper and then through a 0.45 μm nylon membrane. The dried soil samples were subsequently ground to very fine powder using a ball mill (MM400, Retsch, Germany), after which the total-C (TC,) and N (TN), and $\delta^{13}C$ and $\delta^{15}N$ were analyzed with a continuous-flow stable isotope ratio mass spectrometer linked to a CN elemental analyzer (IRMS, IsoPirme-EA, Micromass, UK).

2.4. Calculations and Statistical analysis

Natural abundances (δ) of the stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were calculated as:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} is either the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for the samples and $R_{standard}$ is the ratio for a standard [Pee Dee Belemnite for C and atmospheric N_2 (= 0.0036765) for N]. For ^{15}N-isotope dilutions, the ^{15}N recovery (%) in each soil sample that received ^{15}N inputs can be obtained using the following equations (Ro et al., 2018):

$$NDFI = T \times (AS / AF)$$

^{15}N Recovery (%) = NDFI / Nf

where NDFI is the percentage of N derived from ^{15}N-labeled inputs, Nf is the amount of N inputs, T is the total amount of N in the N-treated soil, AS is the atom% excess of ^{15}N in the soil sample, and AF is the atom% excess of ^{15}N in the N inputs.

All statistical analyses were performed with general linear model (GLM) procedures in SAS software (SAS Institute, Version 9.3, Cary, NC, USA). The effects of three factors (tree species, N treatment, and incubation time) and their interactions on soil NH_4^+–N, NO_3^-–N, DOC, TN, the percentage of N derived from ^{15}N-labeled inputs (NDFI), TC, $\delta^{13}C$ and aggregate distribution were evaluated. A three-way analysis of variance (ANOVA) for a completely randomized design with three replications was performed to test for significant differences among the treatment means within each factor and for interactions between factors. The least significant difference (LSD) test at the significance level of $p < 0.05$ was used to separate the means. The calculated *p*-values for the three main factors and the interactions are shown in Table 2.

3. Results

3.1. Soil mineral N and total-N contents, and N recovery

The soil mineral N content in both the Qa and Pk soils increased in response to N treatment, but there was no significant difference between the types of tree species (Table 2). Compared with that in the control soils, the mineral N content in the N-treated soils increased to 164.3 mg kg^{-1} for the Qa soil and to 151.7 mg kg^{-1} for the Pk soil during the initial incubation. Regardless of the N treatment, the relative proportions between the two components (NH_4^+–N and NO_3^-–N) of the soil mineral N content were similar depending on the day of incubation in all treatments (Figure 1). The initial NH_4^+–N content in soils within 5 days of incubation abruptly decreased at 80 days of incubation and gradually decreased thereafter (Figure 1A–a, 1B–a); however, the NO_3^-–N content increased during incubation, and this increasing pattern was reflected by the decrease in the NH_4^+–N content (Figure 1A–b, 1B–b). The NDFI also increased significantly for both of the N-treated soils (Table 3), and the ^{15}N contents exhibited similar patterns during incubation. In this study, the ^{15}N recovery was calculated using the NDFI (Table 3). During the incubation study, the ^{15}N recovery was determined at each sampling date. Similar to the NDFI, the ^{15}N recovery, regardless of tree species, did not change during the incubation period; the average ^{15}N recovery was 93%. The temporal variation in the TN content in the control soils did not change during the incubation period regardless of the tree species but slightly increased in the N-treated soils (Table 2). The TN content in the N-treated Qa soil increased to 1.80 g kg^{-1}, whereas that in the Pk soil increased to 1.76 g kg^{-1} after 365 days of incubation (data not shown)

Table 2. Results of three-way analysis of variance (ANOVA) showing the significance of the effects of experimental parameters on mineral N (NH_4^+–N, NO_3^-–N), dissolved organic carbon (DOC), total-C (TC), $\delta^{13}C$, total-N (TN), $\delta^{15}N$, and Aggregates.

Factors	Mineral N							
	NH_4^+	NO_3^-	DOC	TC	$\delta^{13}C$	TN	NDFI	Aggregate
Treatment (N)	**	**	***	*	*	*	***	**
Incubation time (T)	**	**	**	*	n.s.	n.s.	*	*
Tree species (S)	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
N × T	*	*	*	*	n.s.	n.s.	*	**
N × S	*	*	*	*	*	n.s.	*	*
T × S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N × T × S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

***:significant at the $p < 0.001$ level; **: significant at the $p < 0.01$ level; *: significant at the $p < 0.05$ level; n.s.: not significant.

Figure 1. Temporal variations in (a) NH_4^+–N content and (b) NO_3^-–N content after nitrogen (N) treatment of two different soils of (A) oak (Qa) and (B) pine (Pk) forest floors. The error bars indicate ± one standard deviation (*n* = 3).

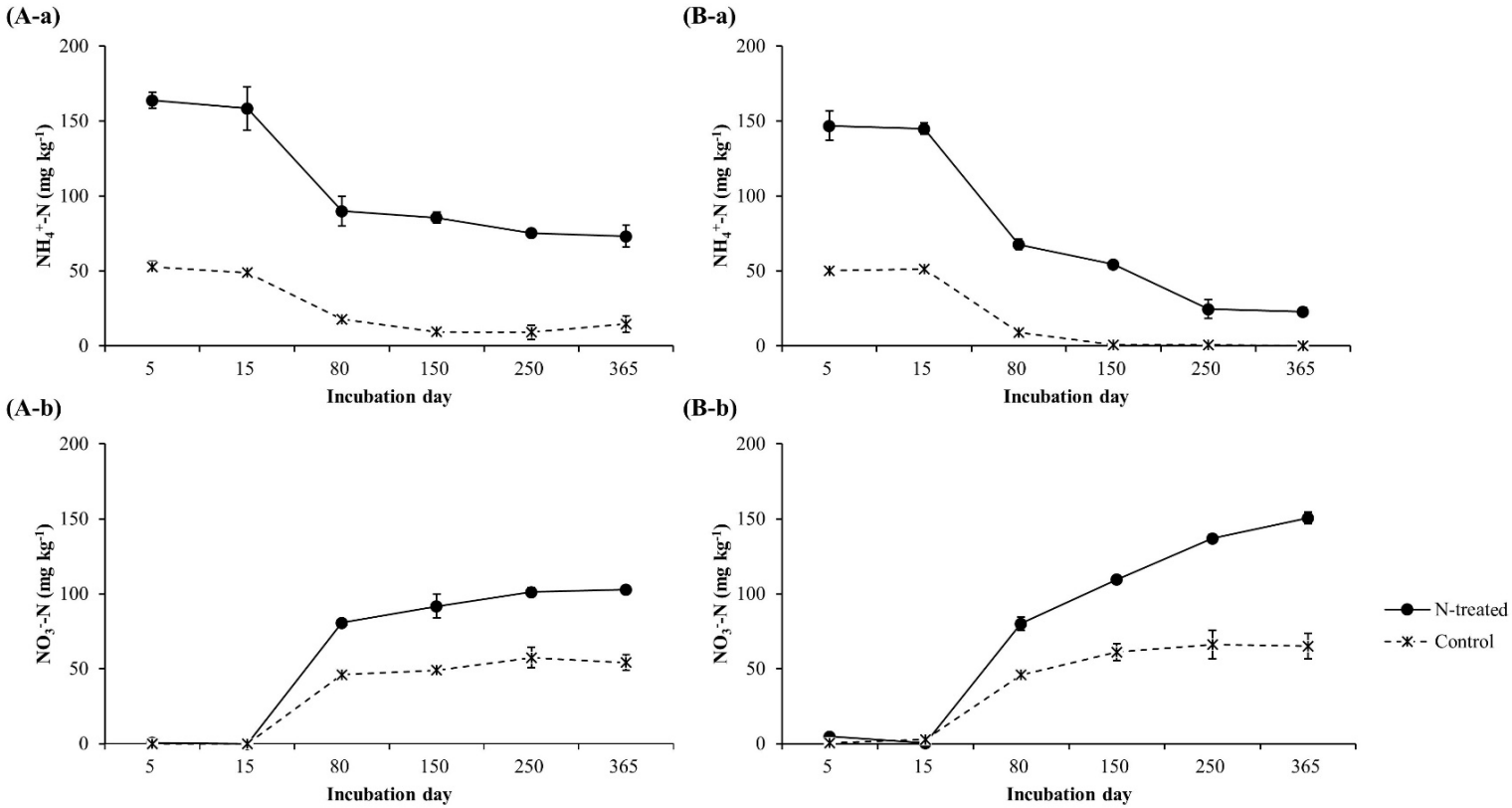

(A-a)
(B-a)
(A-b)
(B-b)
NH_4^+-N (mg kg^{-1})
NO_3^--N (mg kg^{-1})
0
50
100
150
200
5
15
80
150
250
365
Incubation day
N-treated
Control

Table 3. Amount of N derived from ^{15}N-treated soil calculated using the isotope method and total % recovery of ^{15}N isotope.

Aggregate Size (μm)	*Q. acutissima*		*P. koraiensis*	
	NDFI (mg)	Recovery (%)	NDFI (mg)	Recovery (%)
Whole	54.8 ± 2.4	91.4 ± 3.3	57.3 ± 2.4	95.4 ± 1.9
2000–1000	44.1 ± 1.4	73.6 ± 1.3	45.5 ± 2.2	75.8 ± 1.2
1000–250	51.7 ± 2.0	86.2 ± 3.5	51.4 ± 1.2	85.7 ± 5.6
250–53	58.0 ± 5.5	96.7 ± 5.3	60.8 ± 5.1	101.3 ± 6.3
<53	64.9 ± 9.4	108.2 ± 10.3	68.9 ± 7.1	114.8 ± 12.3

3.2. Soil dissolved organic carbon (DOC)

The DOC content varied temporally during the incubation period in both types of N-treated soils, but there was no significant difference in response to tree species (Table 2). Moreover, the temporal variation in the DOC content in the control soils did not change during the incubation period regardless of tree species. The DOC content in the N-treated Qa soil and the Pk soil, which ranged from 203.1 mg kg^{-1} to 219.7 mg kg^{-1} during the initial days of incubation after N treatment, decreased to a range of 45.2 mg kg^{-1} to 57.8 mg kg^{-1} at the end of incubation (Figure 2). Compared with control soils, the N-treated soils presented increased DOC contents during the initial days of incubation (5 days of incubation), but at the end of incubation, the DOC contents were lower than those in the control soils (132.7 mg kg^{-1} and 138.5 mg kg^{-1} for the Qa and Pk soils, respectively)

3.3. Soil aggregates

Before the start of incubation, the soil aggregate proportions of the Qa and Pk soils were 41.6% and 29.9% for 2000–1000 μm, 33.9% and 46.1% for 1000–250 μm, 15.9% and 15.8% for 250–53 μm, and 8.3% and 7.5% for <53 μm, respectively. However, regardless of tree species and N treatment, there were various changes in soil aggregates in response to all treatments during incubation: the 2000–1000 μm soil aggregate proportion decreased, whereas the 250–53 μm proportion increased. Despite these changes, compared with the control treatment, the N treatment resulted in different soil aggregate proportions (Figure 3). At the end of the incubation period, the N treatment of the soil of both tree species reduced the proportion of the 2000–1000 μm aggregates, while that of the aggregates of other sizes (1000–250, 250–53 and <53 μm) increased. However, there was no significant difference in soil

Figure 2. Temporal variations in dissolved organic carbon (DOC) after nitrogen (N) treatment of two different soils of (A) oak (Qa) and (B) pine (Pk) forest floors. The error bars indicate ± one standard deviation (*n* = 3).

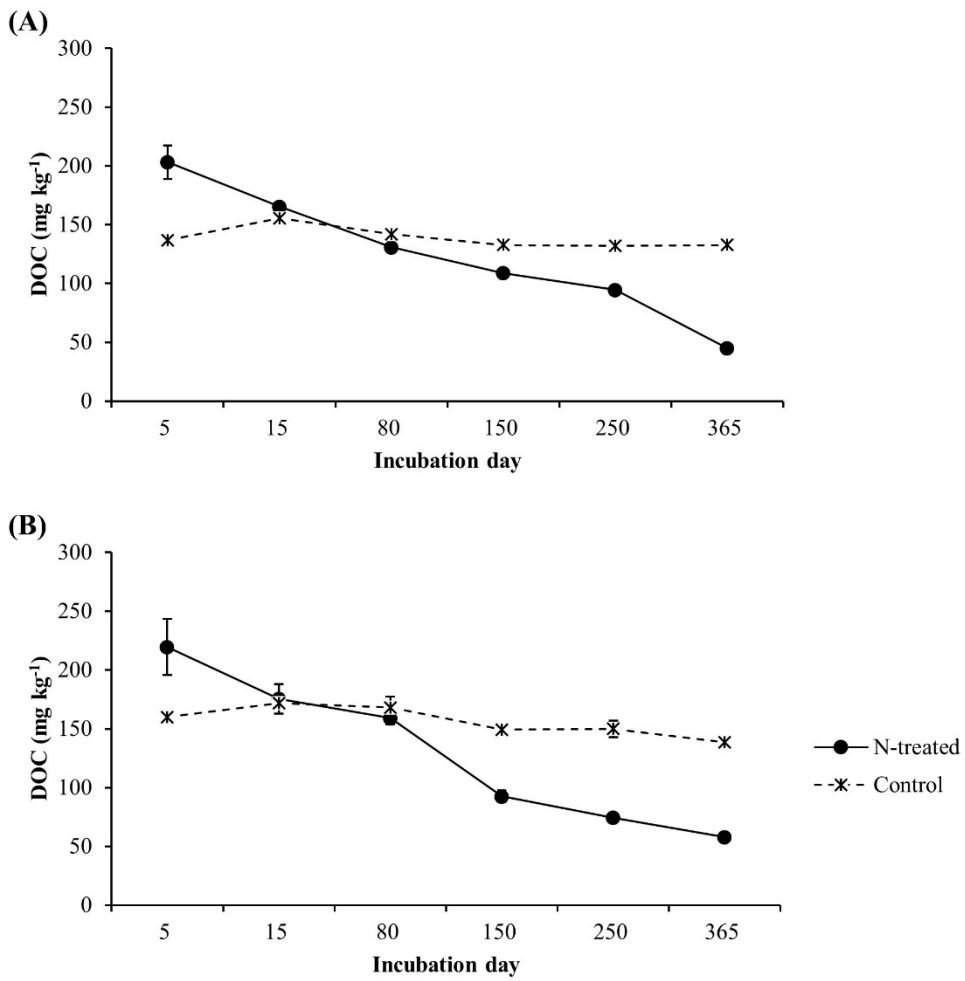

(A)
300
250
200
150
100
50
0
DOC (mg kg-1)
5
15
80
150
250
365
Incubation day
(B)
300
250
200
150
100
50
0
DOC (mg kg-1)
5
15
80
150
250
365
Incubation day
N-treated
Control

Figure 3. Particle-size distribution at 365 days after nitrogen (N) treatment of soils of two different forest floors.

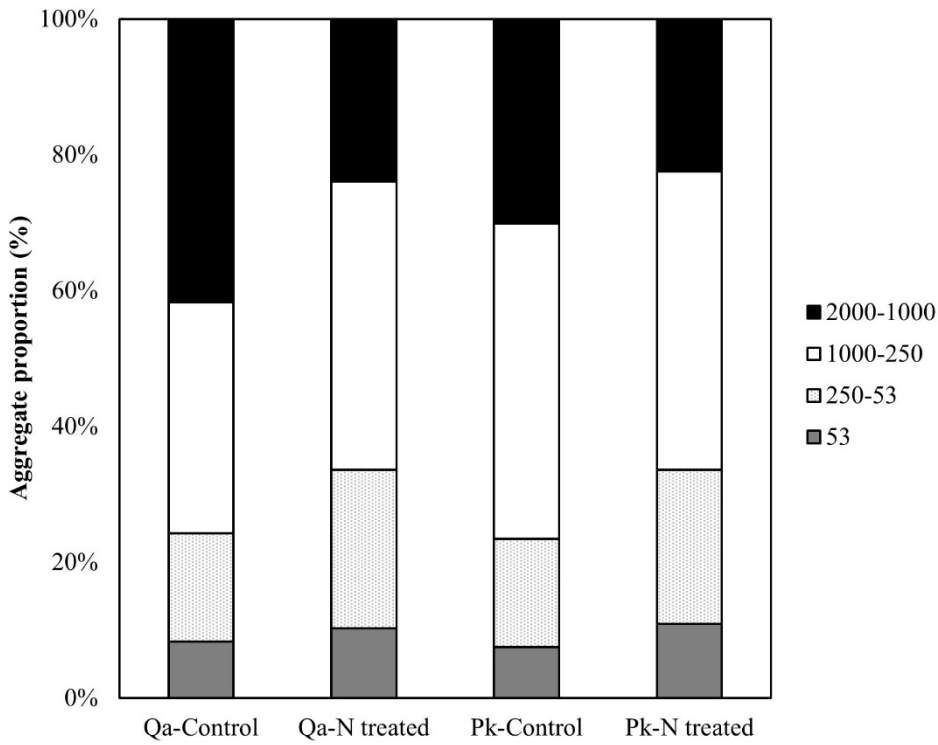

100%
80%
60%
40%
20%
0%
Aggregate proportion (%)
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
2000-1000
1000-250
250-53
53

Figure 4. Temporal variations in total carbon (TC) content (left axis) and $\delta^{13}C$ (right axis) after nitrogen (N) treatment in soils of (A) oak (Qa) and (B) pine (Pk) forest floors. The error bars indicate ± one standard deviation (*n* = 3).

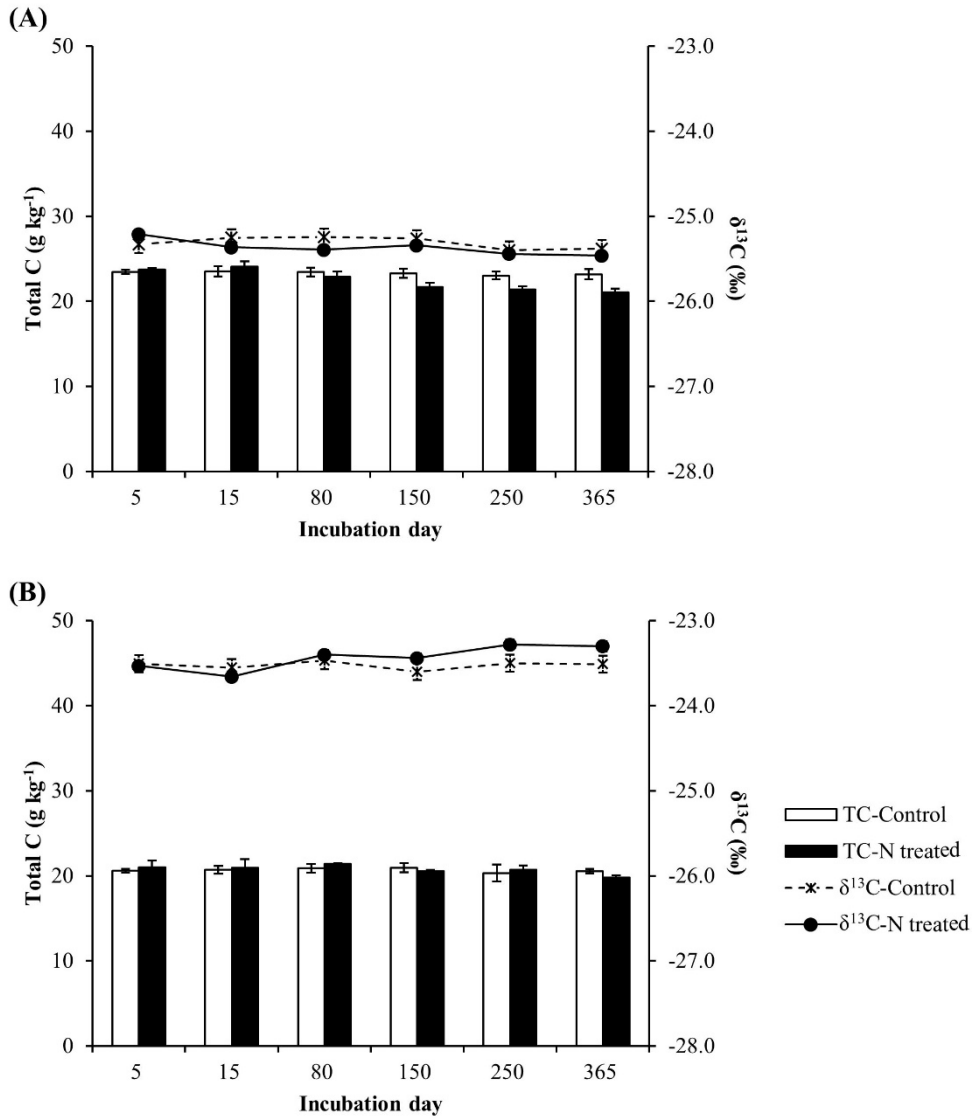

(A)
Total C (g kg-1)
δ13C (‰)
Incubation day
(B)
Total C (g kg-1)
δ13C (‰)
Incubation day
TC-Control
TC-N treated
δ13C-Control
δ13C-N treated

Figure 5. Temporal variations in total carbon (TC) content (left axis) and $\delta^{13}C$ values (right axis) after nitrogen (N) treatment in 2000–1000 μm aggregates in soils of (A) oak (Qa) and (B) pine (Pk) forest floors. The error bars indicate ± one standard deviation (*n* = 3).

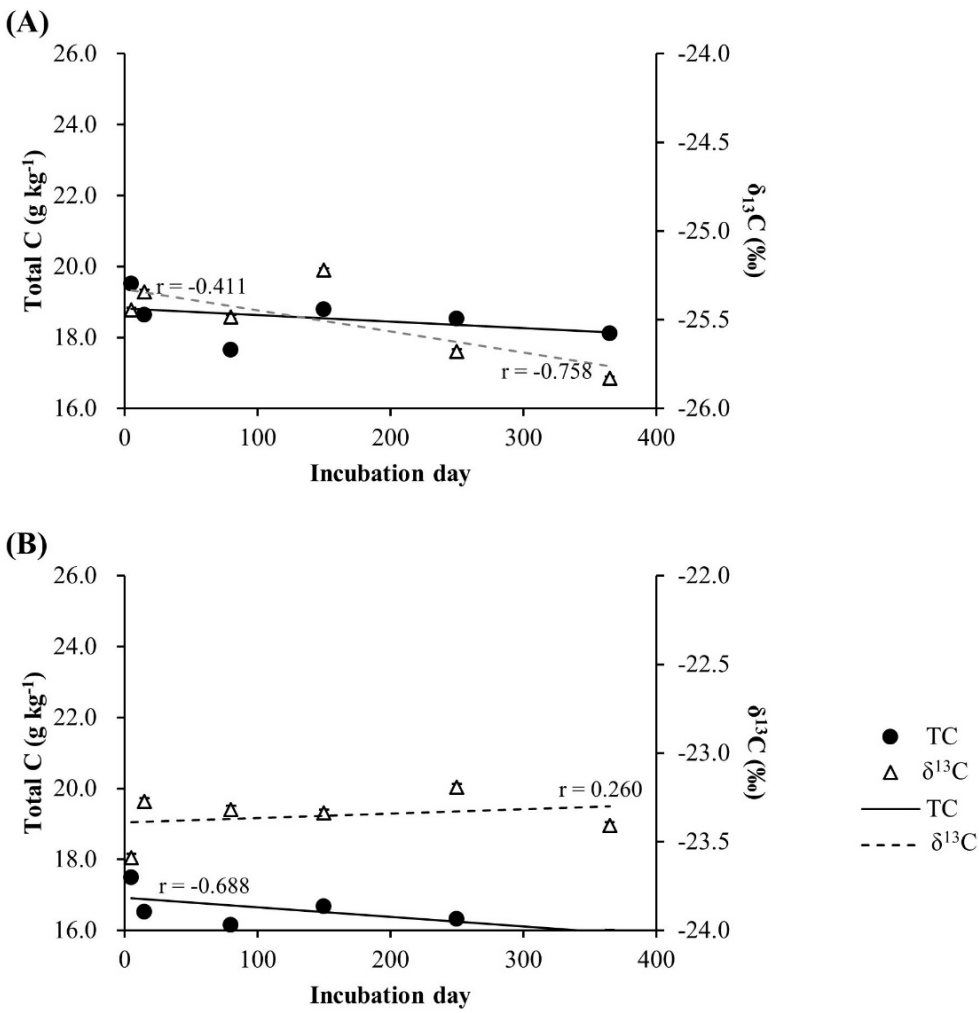

(A)
Total C (g kg-1)
δ13C (‰)
Incubation day
r = -0.411
r = -0.758
(B)
Total C (g kg-1)
δ13C (‰)
Incubation day
r = 0.260
r = -0.688
TC
δ13C
TC
δ13C

Figure 6. Temporal variations in total carbon (TC) content (left axis) and $\delta^{13}C$ values (right axis) after nitgen (N) treatment in 1000–250 μm aggregates in soils of (A) oak (Qa) and (B) pine (Pk) forest floors. The error bars indicate ± one standard deviation (*n* = 3).

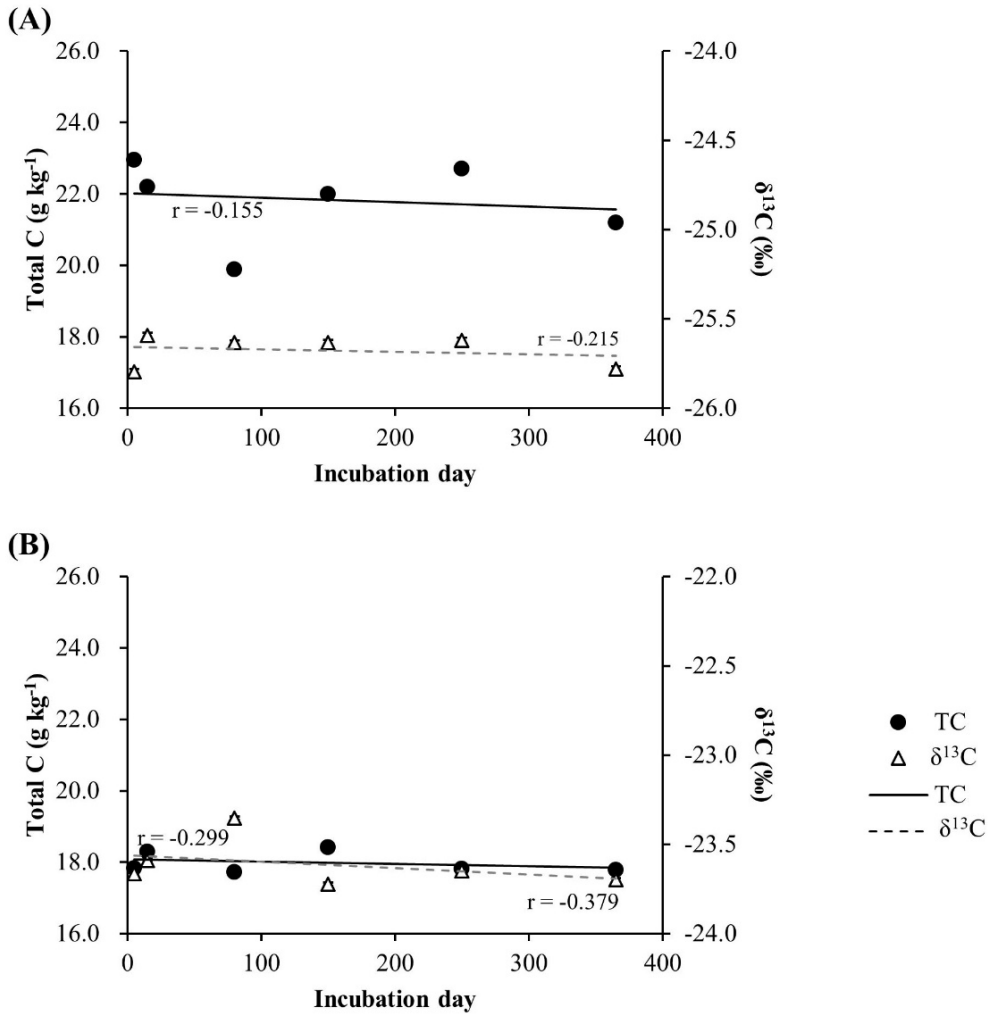

(A)
Total C (g kg-1)
δ13C (‰)
Incubation day
r = -0.155
r = -0.215
(B)
Total C (g kg-1)
δ13C (‰)
Incubation day
r = -0.299
r = -0.379
TC
δ13C
TC
δ13C

Figure 7. Temporal variations in total carbon (TC) content (left axis) and $\delta^{13}C$ values (right axis) after nitrogen (N) treatment in 250–53 μm aggregates in soils of (A) oak (Qa) and (B) pine (Pk) forests. The error bars indicate ± one standard deviation (*n* = 3).

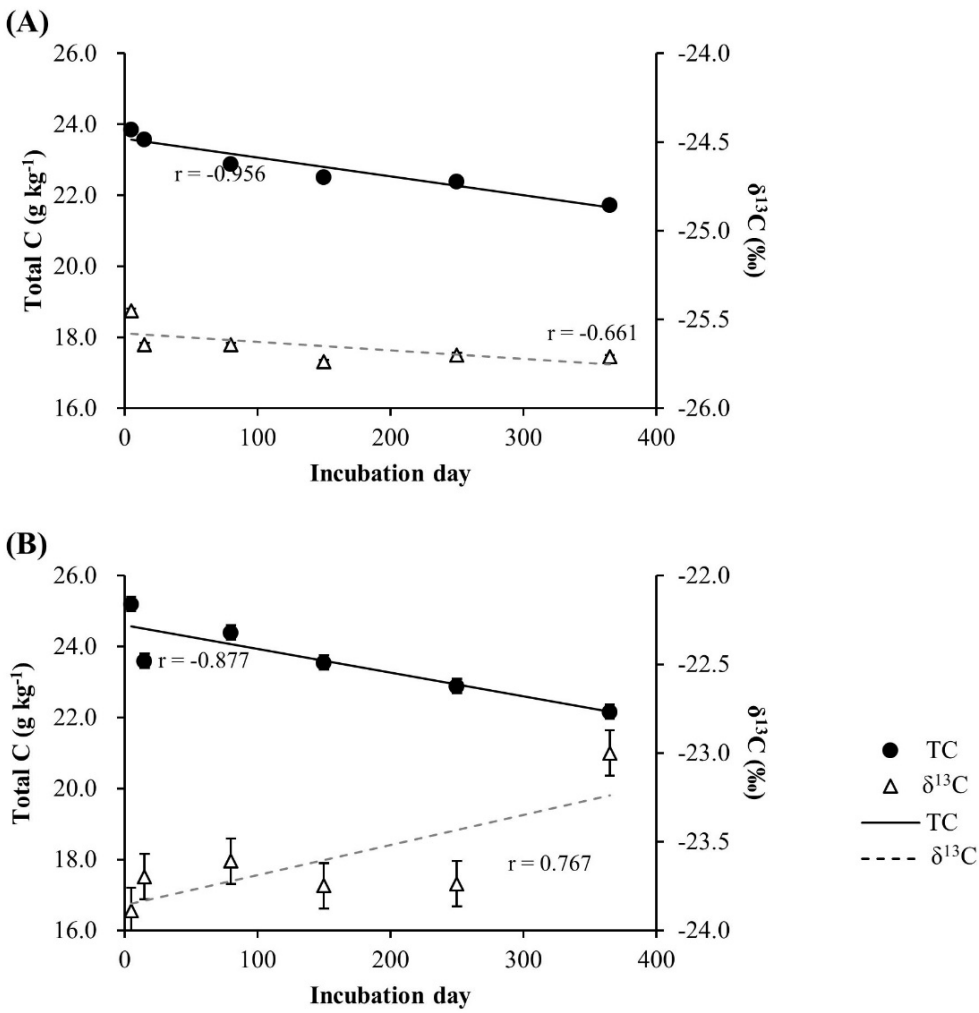
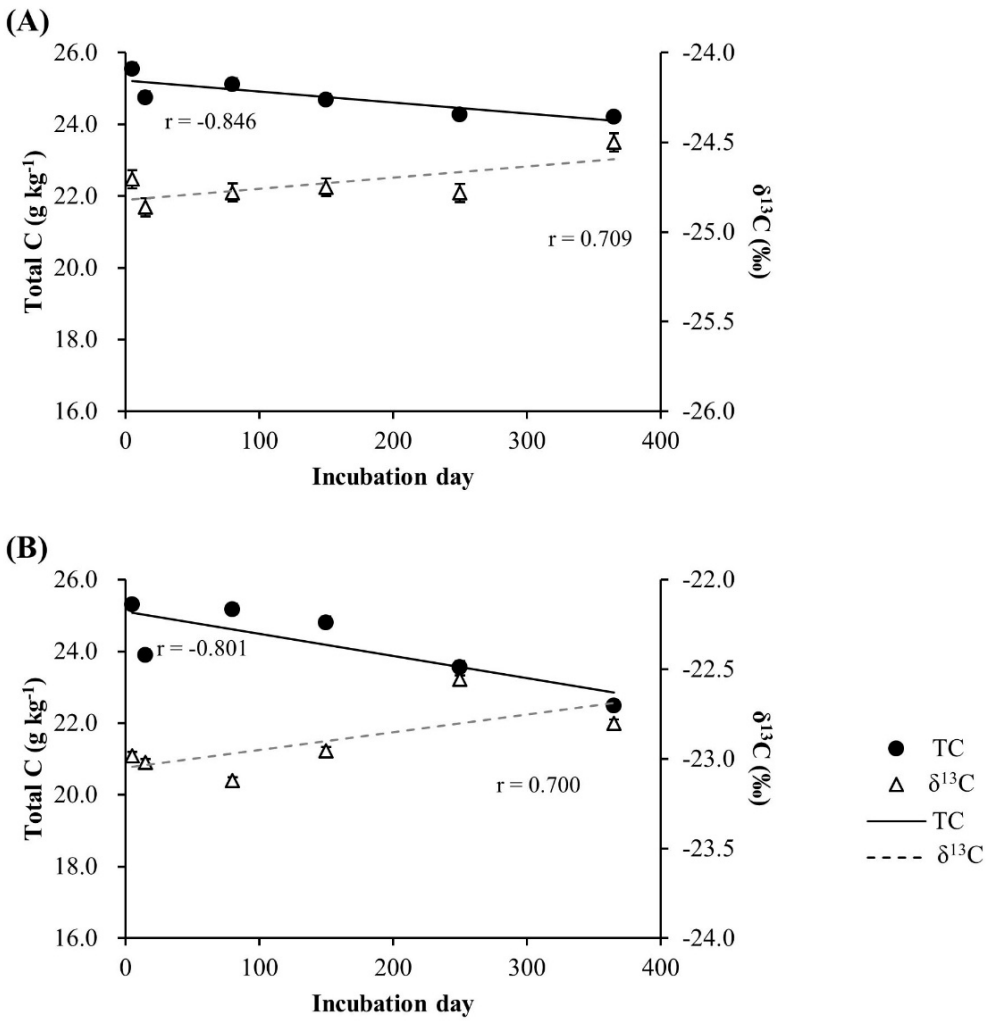

(A)
Total C (g kg-1)
δ13C (‰)
r = -0.956
r = -0.661
Incubation day
(B)
Total C (g kg-1)
δ13C (‰)
r = -0.877
r = 0.767
Incubation day
TC
δ13C
TC
δ13C

Figure 8. Temporal variations in total carbon (TC) content (left axis) and $\delta^{13}C$ values (right axis) after nitrogen (N) treatment in <53 µm aggregates in soils of (A) oak (Qa) and (B) pine (Pk) forest floor. The error bars indicate ± one standard deviation (*n* = 3).


(A)
Total C (g kg-1)
δ13C (‰)
r = -0.846
r = 0.709
Incubation day
(B)
Total C (g kg-1)
δ13C (‰)
r = -0.801
r = 0.700
Incubation day
TC
δ13C
TC
δ13C

.

aggregate proportions in response to tree species (Table 2).

3.4. Soil total-C contents and $\delta^{13}C$

The total-C (TC) contents and $\delta^{13}C$ values of the N-treated Qa and Pk soils are shown in Figure 4. The temporal variation in the TC contents and $\delta^{13}C$ values in the control soils did not change during the incubation period regardless of tree species. In response to N treatment, the TC content in the Qa soil decreased during the incubation period, and that in the Pk soil changed slightly; however, the $\delta^{13}C$ values in the Pk soil increased (Figure 4). The soil TC content and $\delta^{13}C$ values of each soil aggregate differed among the four areas (Figures 5–8). In the majority of the N-treated soils, the TC contents in the soil aggregates under both tree species slightly decreased, especially for aggregates whose sizes were 250–53 μm and <53 μm (Figures 7 and 8). However, regardless of tree species, the $\delta^{13}C$ values did not change significantly with aggregate size during the incubation period in any soil treatment.

4. Discussion

In all treatments, the constant of mineral N (NH_4^+–N + NO_3^-–N) content (data not shown) indicated that there was no apparent gain or loss during the incubation experiments, and as time progressed, decreases in the NH_4^+–N content and increases in the NO_3^-–N content were observed. Regardless of tree species, the mineral N content in the N-treated soils was higher than that in the control soils from the start until the end of incubation; therefore, I considered these mineral N content increases in the soils to have been caused by the N treatment (Figure 1). In this study, I confirmed that much of the NH_4^+–N content was converted to NO_3^-–N content after 80 days of incubation. In general, when urea is introduced into the soil, hydrolysis, ammonification, and nitrification occur, which ultimately produce nitrate under aerobic incubation conditions (Davidson et al., 1992). Therefore, unlike in the control soils, in the N-treated soils, the NH_4^+–N tended to decrease gradually, which is considered a result of the continuous nitrification process (Chen et al., 2013). During the incubation period, the ^{15}N recovery did not change in either N-treated soil, which was because the soils under both tree species were weakly acidic; as such, NH_3 volatilization did not occur, and NO_3^- ions did not leach due to the closed experimental conditions of plastic bottles (Table 3). However, regardless of tree species, the aggregates of N-treated soils had slightly different ^{15}N recovery rates: the lowest were in the 2000–1000 μm aggregates; the highest, in the <53 μm aggregates. In a study investigating soil aggregate sizes by N treatment, Wang et al. (2015) reported that the mineral N content was higher in microaggregates than in macro-aggregates; specifically, NO_3^-–N was more than 2-fold greater in micro-aggregates than in macro-aggregates, and the ^{15}N values affected by the mineral N content increased as the aggregate size decreased. In general, the smaller the size of soil aggregates is, the larger the affinity for ions; macro-aggregates are formed by

the combination of organic residues and clay bonds (Brady and Weil, 2008). Therefore, in the present study, the ^{15}N recovery rate was higher in micro-aggregates than in macro-aggregates because the N treatment was present in the form of NO_3^-–N through nitrification (Figure 1 and Table 3). Regardless of tree species, the TN content was slightly higher in the N-treated soils than in the control soils, but there was no significant change in the N-treated soils during the incubation period. These increases in the TN content were attributed to the N treatment, and the lack of change in the TN contents in all treatments was due to the lack of NH_3 volatilization and NO_3^- leaching, as mentioned above.

Dissolved organic carbon is the major form involved in C dynamics within the soil and can represent a significant factor in the C budget; DOC contents are affected by various processes such as root exudation, microbial decomposition and consumption, leaching and ecosystem CO_2 exchange (Kalbitz et al., 2000; Neff and Asner, 2001). In turn, these processes are affected by various environmental factors (temperature increases, soil water, soil pH, OM content, N and sulfur (S) effects, atmospheric CO_2 concentration, etc.) (Evans et al., 2008), and N treatment of the soil increases both microbial activity and the soluble and insoluble organic C pools in the soil (Tonitto et al., 2014); the magnitude of these differences depends on the litter composition of the plant species (Finn et al., 2015). However, in the present study, the DOC content did not significantly differ based on the tree species regardless of N treatment during the incubation period (Table 2). In terrestrial ecosystems, N treatment of the soil leads to DOC leaching due to the resulting increase in net productivity (Vitousek and Howarth, 1991) and leads to an increase in available simple C forms resulting from increased OM decomposition caused by the increase in enzyme activity (Homyak et al., 2017). As previously reported, I observed an initial sharp increase in DOC contents in both the Qa and Pk soils in response to N treatment. However, despite the initial increase in DOC contents, both soils treated

with N presented lower levels than did the control soils at 80 days of incubation; however, at the end of incubation, these levels decreased to approximately 25% of the initial DOC contents (Figure 2). Previous studies have shown that N addition to the soil induces a variety of reactions involved in soil CO_2 flux (Geng et al., 2017) and increases the enzyme activity of bacteria, fungi, and actinomycetes (Lv et al., 2017); in turn, the soil the microbial activity consumes DOC (Lei et al., 2017). Therefore, I infer that the OM decomposition was promoted by the N treatment, which resulted in an increase in the DOC content during the initial days of incubation, and as the incubation period progressed, the DOC content in the soil of both tree species decreased due to the consumption by microorganisms.

In this study, there were various changes in soil aggregates in all treatments during the incubation period: the 2000–1000 μm soil aggregate proportion decreased, whereas the 250–53 μm proportion increased (Figure 3). Possible reasons may explain the changes in the soil aggregate distributions in all treatments regardless of N treatment. Soil aggregate stability influences a wide range of physical and biogeochemical processes in both natural and agricultural environments (Chenu et al., 2000). In general, soil aggregates are destroyed by conditions such as repeated drying-wetting or repeated freezing-thawing. Previous incubation studies have shown that, because the mucilage component of plant root exudates decreases as the microbial activity increases within environments where water and temperature conditions are optimized, the soil aggregates are destroyed as time progresses (Morel et al., 1991; Lehrsch and Brown, 1995; Erktan et al., 2016). Therefore, I could infer that the changes in the soil aggregates in all treatments during the incubation period were based on the results of previous studies. Soil aggregation is the process by which soil particles of different sizes are joined and held together by different materials (Dorioz et al., 1993). According to the aggregate hierarchy model, macro-aggregates form with temporary binding agents such as OM particles, microbial

exudates, fungal hyphae and micro-aggregates, whereas micro-aggregates form with primary mineral particles bound together by various cementing agents such as persistent organic materials, crystalline oxides and aluminosilicates (Tisdall and Oades, 1982; Six et al., 2000). Among these agents, SOM is a nucleus for aggregate formation, and upon OM decomposition, macro-aggregates form micro-aggregates and eventually stable organo-mineral complexes, that is, physical SOC stabilizes as micro-aggregates are formed (Gale et al., 2000, Six et al., 2000). Therefore, the rate of SOC decomposition is affected by its spatial distribution within soil aggregates, and in this study, regardless of species, N treatment resulted in a decrease in soil TC contents and changes in soil aggregate distributions (Figures 3 and 4). In general, macro-aggregates are unstable and have higher turnover rates than do micro-aggregates because the former are composed of fresh and easily degradable organic materials (Gale et al., 2000). The results of the present study showed that, in the N-treated soils, the 2000–1000 μm aggregates increased at the end of the incubation period, whereas the 1000–250 μm aggregates decreased (Figure 3). Therefore, as mentioned above, because the decomposition of OM causes temporary bonds within aggregates to be broken, the smaller-sized aggregates presumably increase in size (Gale et al., 2000; Six et al., 2000). Moreover, the results concerning the soil TC content and the $\delta^{13}C$ values differed in this study (Figure 4). Many researchers have reported that N treatment of the soil increases soil microbial activity and the soluble and insoluble organic C pools in the soil and that the magnitude of these differences depends on the litter composition of the plant species (Tonitto et al., 2014; Finn et al., 2015); the soil CO_2 efflux, which was greater for deciduous forests than for coniferous forests (Laganière et al., 2010); and the increase in heterotrophic decomposition of SOC with N addition, which resulted in declining SOC concentrations (Khan et al. 2007; Mulvaney et al., 2009). Therefore, in the present study, I concluded that the reason why the TC content in the Qa soil decreased more

than that in the Pk soil was probably due to the differences in the OM composition and decomposition rate of each tree species (Figure 4). These differences also affected the $\delta^{13}C$ values. Farquhar et al. (1989) reported that the process of discrimination of stable C isotopes differs based on the C transformation involved with the specific organic material constituents and the physical, chemical, and metabolic processes involved, which then results in differences in the stable C isotope values in the products of these processes. Balesdent and Mariotti (1996) reported that the total $\delta^{13}C$ values of the soil are usually different, and because of isotopic discrimination during OM decomposition, there are differences in the decomposition rate with respect to the ^{13}C signature and the ^{13}C:^{12}C isotopic ratio. Boström et al. (2007) reported that C isotope fractionation in soils is caused by kinetic discrimination during microbial respiration processes and the preferential decomposition of specific materials. Thus, I inferred that the increase in the $\delta^{13}C$ values in the N-treated Pk soil was caused by both the ^{13}C:^{12}C isotope ratio and kinetic discrimination due to the decomposition of OM in the experimental conditions without new OM (Figure 4).

In contrast, unlike my hypothesis in which changes in TC contents in the 2000–1000 μm or 1000–250 μm aggregates would be larger than those in the other aggregates (Figures 5 and 6), the TC contents changed mostly in the 250–53 μm and <53 μm aggregates (Figures 7 and 8). Since the SOM within the existing large aggregates (2000–1000 μm and 1000–250 μm) was broken down and was more prevalent in the small aggregates, the TC content in the broken large aggregates affected the TC content in the new small aggregate distribution (250–53 μm or <53 μm), not that in the existing large aggregates. As a result, a decrease in the soil TC content was observed in the 250–53 μm and <53 μm aggregates, but the aggregate distribution did not change (Gale et al., 2000, Six et al., 2000). These results are consistent with those of Liu et al. (2018), who reported that the micro-aggregates

appeared to be the final products. In the case of $\delta^{13}C$ values, there was no significant difference in the size of each aggregate regardless of tree species, unlike in whole soils. Previous studies have shown that ^{13}C isotopic fractionation cannot be explained by aggregate formation with SOM input or decomposition, and no definitive evidence that micro-aggregates were more enriched in ^{13}C than were macro-aggregates was found (Helfrich et al., 2006; Gunina et al., 2014). However, I cannot explain the relative contribution of the $\delta^{13}C$ values to the SOC decomposition dynamics or changes in soil aggregate distribution because no direct measurements affected the response of the $\delta^{13}C$ values for the soil aggregate distribution in relation to the N treatment of the soil. Therefore, this situation warrants further study of the effects of N treatments on SOM decomposition, soil organic functional groups, and humic substances as well as the interactive effects between N treatments and microbial dynamics on C dynamics.

5. Conclusions

I hypothesized that N addition to the soils of two different forest floors (deciduous trees, Qa; coniferous trees, Pk) would result in different sizes of soil aggregates via different SOC decomposition patterns. During the incubation period, regardless of tree species, no significant changes were observed in most of the experimental parameters in the control soil. However, after N treatment, the mineral N content, DOC content and TC content substantially decreased, while there was a change in aggregate distribution, indicating that N treatment led to an increased distribution of 1000–250 μm aggregates in the soil. In particular, unlike my hypothesis in which changes in TC contents in the 2000–1000 μm and 1000–250 μm aggregates would be greater than those of the other aggregates, the TC contents changed mostly in the 250–53 μm and <53 μm aggregates. However, for the $\delta^{13}C$ values, regardless of tree species, there was no significant difference in the size of any aggregate, unlike in whole soils. On the basis of my results, I cannot explain the relative contribution of $\delta^{13}C$ values to the SOC decomposition and changes in soil aggregate distributions. Therefore, I conclude that N treatment affects the decomposition of OM and soil aggregate distribution and that these differences depend on tree species. To investigate this phenomenon more closely, it will be necessary to investigate the effects of N application on soil organic functional groups and humic substances further, and a long-term analysis and interpretation will also be needed to understand soil C dynamics fully in relation to the effects of tree species.

CHAPTER III

Effect of N addition on some functional groups in humic substances of soils under temperature oak and pine forest soil

Abstract

To investigate the effect of nitrogen (N) treatment on the soil of two different forest floors (deciduous trees, Qa, and coniferous trees, Pk) and the effects of humic substances and soil organic functional group patterns following the soil aggregate-size fraction, this study examined the temporal variation in incubation periods over 365 days. Soil samples were taken at two different forest stands and divided into two treatment groups: a control group and a N treatment group (urea at 100 kg N ha^{-1} yr^{-1}). Each soil was incubated at 25±2°C for 365 days and analyzed for total-N, humic substances, and soil organic functional groups. The nitrogen treatment caused a change in the humic substances and soil organic functional groups according to the soil aggregate-size fraction. Interestingly, different patterns of the soil organic functional groups between the Qa and Pk soils were observed because of the differences in the decomposition characteristics associated with their chemical compositions. Therefore, I conclude that the N treatment affects the humic substances and soil organic functional groups and that these differences depend on the tree species.

Keywords: *Tree species; Forest soil; Nitrogen input; Humic substance; Organic matter functional groups*

1. Introduction

The increase in atmospheric nitrogen (N) deposition has been a major global environmental concern recently. Since carbon (C) and N are major elements in ecosystems and their cycling processes are strongly coupled (Asner et al., 1997), it is well known that N treatment to the forest floor increases the soil microbial activity and the soluble and insoluble organic C pools in forest soils (Tietema and Wssel, 1992), and the magnitude of the increase in the soil microbial activity and soil organic carbon (SOC) pools varies with the litter composition of the plant species (Gundersen et al., 1998). However, despite the long history of N input into the soil, the response of soil C dynamics to external N deposition remains poorly understood (Cotrufo et al., 2013).

Soil organic matter (SOM) consists of partially decayed animal and plant residues, soil microorganisms and the by-products of decomposition that lead to the production of humic substances (HSs). Humic substances are relatively stable in soils and are very important in SOM dynamics. In general, HSs are divided into three fractions based on their solubility, and each has many molecular components and chemical characteristics (Stevenson, 1994). Humins are insoluble in water at any pH condition, and their molecular weights range from approximately 100,000 to 10,000,000 (Pettie, 2006). Humins are the most resistant to decomposition within the soil and play an important role in soil structure, stability, and fertility. Humic acids (HAs) consist of compounds of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids that are soluble in alkaline conditions but are insoluble under acid conditions, and their molecular weights range from approximately 10,000 to 100,000. Fulvic acids (FAs) also consist of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids that are soluble in water under all pH conditions. Moreover, fulvic acids are more chemically reactive due to an oxygen

(O) content 2-fold greater than the O content of HA, and because of their relatively small size (approximately 1,000 to 10,000), they are the key ingredients of high-quality foliar fertilizers in the soil. In soil structure, HSs are key components of soil aggregates, which are complex carbohydrates synthesized by bacteria together with clay and silt (Stevenson, 1994; Ghabbour, 2014). Therefore, the HSs within soil aggregates are present in a very stable form for a long time because of this complex with soil minerals (Pettie, 2006). However, most recent studies have reported that additional sources of C and N stimulated microbial degradation of HSs (Fog, 1988; Ghabbour, 2014). Although many soil studies have been conducted on the degradation or inactivation of HSs, most of them have focused solely on the mean residence times, particularly on soil microbial activity and litter decomposition.

Since the quality and decomposition of SOM is affected by the type of tree species and environmental conditions, these two major factors will, in turn, affect the SOC stocks in the forest (Cotrufo et al., 2013; Meng et al., 2017). However, since SOM decomposes over a long period of time, it is insufficient to fully evaluate its variation within a short time frame; thus, specific studies are needed. In general, the decomposition and mineralization of SOM entail changes in functional group chemistry, such as the corroborative increase in aromatic groups to the aliphatic (carbon chains) group during decomposition (Hsu and Lo, 1999). Fourier-transform infrared (FTIR) spectroscopy can be used to help explain SOM transformations and stabilization and enables the rapid characterization of relative changes in the SOM functional groups (e.g., carboxylic, phenolic) (Martin-Neto et al., 2009) because many infrared bands are characteristic of the molecular structure and functional groups (Ellerbrock and Kaiser, 2005). Particularly, many previous studies have reported that FTIR spectroscopy was used to describe the decomposition processes through a reduction of carbohydrate markers and as quantitative indicators of the SOM in different soil horizons (Haberhauer et al., 2000; Artz et al., 2008). Therefore,

FTIR is a very useful tool to observe the structural variations in soil due to the changes in the chemical and microbial activity that result from N addition.

Therefore, I hypothesized that the addition of N to the soil of two different forest floors (deciduous trees and coniferous trees) would result in different distribution patterns of HSs and a decomposition of the SOC functional groups in the soil systems. I tested this hypothesis with soil profile samples that were taken from two different forest floors in Mt. Taewha, Korea. I conducted batch incubation experiments and determined the time-course patterns of HSs and the corresponding soil organic functional groups along the soil aggregate-size fractions.

2. Materials and Methods

2.1. Sampling sites and soil preparation

The study site was chosen in a relatively mild hilly area (200 m above sea level) in a mixed coniferous-broadleaf forest located within the Seoul National University Forest on Mt. Taewha, Geonggi-do (37°18'N, 127°17'E), Korea. The annual mean air temperature and precipitation at the site are 11°C and 1389 mm, respectively. Since a mix of deciduous oak (*Quercus acutissima* Carruth., Qa) and coniferous pine (*Pinus koraiensis* Siebold & Zucc., Pk) tree species are predominant in the canopy of this forest, one similarly aged (approximately 35–40 years old) forest stand of each tree species was chosen for comparison. This forest has not received any nitrogen, phosphorus, and potassium (NPK) fertilizer for 35–40 years since its establishment. Samples (0–30 cm in depth) were taken from each forest stand using a soil auger in April 2016. Immediately after sampling, the soil samples were air dried at room temperature, passed through a 2 mm sieve, and mixed homogeneously. The physical and chemical properties of the soil are shown in Table 1.

2.2. Batch incubation experiments

To investigate the N effect on the soil organic matter functional groups and humic substance distributions on the different type of tree species soil, 220 g of a soil sample were transferred into 250-mL plastic bottles, and a total of 72 bottles were prepared for the soil treatments in triplicate, with 6 destructive samplings. Each bottle was covered with a perforated cap to ensure gas exchanges and was pre-incubated at 25±2°C in the dark for 7 days, and the soil water content was adjusted to 0.31 m^3 m^{-3} (filed capacity –33kPa). After pre-incubation, each of the two tree

species soil samples was divided into two treatments: a control (untreated) soil and a N-treated soil (urea at 100 kg N ha^{-1} yr^{-1}). Thereafter, the bottles were incubated at 25±2°C, and the soil water content was adjusted to maintain the field moisture capacity during the 365-day batch experiment by adding deionized water to the bottles as necessary to maintain their initial condition. Soil samples were taken at 5, 15, 80, 150, 250, and 365 days of incubation, and these samples were thoroughly homogenized for soil chemical analyses.

2.3. Soil aggregate-size fractions and analyses of the soils

Soil aggregate-size fractions were determined by the dry sieving method (Kemper and Rosenau, 1986) using a vibratory sieve shaker (Analysette 3, Fritsch, Germany) with a stack of stainless-steel sieves. The 50-g air-dried soil sample was placed on the top sieve and submerged for 10 min (oscillation amplitude 20 mm and a frequency of approximately 50 Hz). Soil was retained on the 2,000-1,000 μm (large and median macro-size), 1,000-250 μm (small macro-size), and 250-53 μm (micro-size) screens and was passed through the 53 μm (silt-plus clay-size particles) screen. The dried soil samples were ground into very fine powders with a ball mill (MM400, Retsch, Germany) and then analyzed for the total-N content, extract of HSs and FTIR spectra. The total-N content was measured using an elemental analyzer (Flash EA 2000, Thermo Scientific, UK).

2.4. Humic substances (HSs)

The humic substances were partitioned into three following humic products (humin, humic acid (HA), fulvic acid (FA)) according to the extract procedure (Lamar et al., 2014). Humin is the insoluble material obtained from extracts with 0.1 M NaOH.

Table 1. Chemical and physical properties of soil under the *Q. acutissima* and *P. koraiensis* stands.

Soil properties	*Q. acutissima*	*P. koraiensis*
Texture[a]	Clay loam	Clay loam
Bulk density (Mg m^{-3})	1.1 ± 0.0	1.1 ± 0.0
Field capacity (m^3 m^{-3})	0.34 ± 0.1	0.33 ± 0.1
Total-C (g kg^{-1})	23.5 ± 0.3	20.6 ± 0.2
Total-N (g kg^{-1})	1.7 ± 0.1	1.8 ± 0.1
C/N ratio	13.8 ± 0.1	11.4 ± 0.2

[a] USDA classification scheme

[b] Soil-to-suspension ratio of 1:5

After 6 h of shaking, the solutions remained for 1 to 6 h until complete dissolution and were then centrifuged at 3,900 × g for 10 min to separate any insoluble material (humin) from the dissolved extract solution (HA and FA). Humic acid is the alkaline extraction obtained from an insoluble product with an acidic condition. To flocculate the HA, concentrated HCl (1:1) was added dropwise to the alkaline extract until the pH of 1.0 ± 0.05 was reached and was then subjected to shaking for 1 h. After mixing, the solutions stayed overnight until the HA was completely precipitated and was then separated from the dissolved solution (FA is an acid-soluble compound) supernatant by centrifuging at 3,900 × g for 0.5 h.

2.5. Fourier-transform infrared (FTIR) spectroscopy

The soil functional groups of whole soils and aggregate-size fractions were examined by FTIR. The FTIR spectra were obtained with an IR Tracer-100 FT-IR spectrometer (Shimadzu, Japan) with the KBr-technique according to Celi et al. (1997). Approximately 1 mg of air-dried whole soils and aggregate-size fraction samples from each treatment was mixed with 100 mg of KBr, which was finely ground and compressed to form KBr pellets. The spectra were recorded in the range of 4,000-500 cm^{-1}, with a resolution of 1 cm^{-1}. Sixty-four scans were collected for each sample, and they were averaged and corrected against ambient air (H_2O and CO_2) as the background.

The experimental calculation procedures basically followed Margenot et al. (2015) and Veum et al. (2014). The soil functional groups were calculated into two band ratios based on their wavenumber assignments (Table 2). These two band ratios represent functional group types (e.g., aliphatic, O functional group), which in previous studies have been established as indexes of the relative decomposition and recalcitrance of SOM (Margenot et al., 2015).

$$Index\ \ I\ \ = \frac{1650 + 920 + 840}{2924 + 2850 + 1470 + 1405}$$

$$Index\ \ II\ \ = \frac{2924 + 2850 + 1650 + 1470 + 1405 + 920 + 840}{3400 + 1270 + 1110 + 1080}$$

Index I is an index of decomposition as a ratio of the aromatic and aliphatic functional groups, and the ratio of the bands that show these two functional groups have shown increases with an increasing degree of decomposition. Index II is a ratio of the C to O functional groups, which is thought to be related to the recalcitrance of SOM.

2.6. Calculation and statistical analysis

All statistical analyses were performed with the General Linear Model (GLM) procedures in SAS software (SAS Institute, Version 9.3, Cary, NC, USA). The effects of four factors (the tree species, N treatment, incubation time and aggregate-size fraction) and their interactions on the soil total N content (TN), HSs, humification, and Indexes I and II were evaluated. A four-way analysis of variance (ANOVA) for a completely randomized design with three replications was performed to test for the significant differences among the treatment means within a factor and for the interactions between factors. The least significant difference (LSD) test at the confidence level of $P < 0.05$ was used to separate the means. The calculated *P*-values for the three main factors and the interactions are shown in Table 3.

Table 2. Functional group assignments used to evaluate the FT-IR spectra of soil based on Parikh et al. (2014).

Band, cm^{-1}	Assignment[a]
3400	ν(N–H), ν(O–H)
2924	aliphatic ν_{as}(C–H)
2850	aliphatic ν_{s}(C–H)
1650	aromatic ν (C = C)
1575	amide δ(N–H) and ν(C = N)
1470	aliphatic δ(C–H)
1405	aliphatic δ(C–H)
1390	aliphatic δ(C–H), potential contributions from carboxylate ν_{s}(C–O)
1270	phenol νas(C–O), carboxylic acid ν(C–O)
1110	polysaccharide ν_{s}(C–O)
1080	polysaccharide ν_{s}(C–O)
920	aromatic δ(C–H)
840	aromatic δ(C–H), less substituted

[a] ν, stretching vibration; ν_{as}, asymmetric stretching vibration; ν_{s}, symmetric stretching vibration; δ, bending vibration

3. Results

3.1. Soil total-N content

The total N content of the Qa and Pk soils are shown in Table 4. The temporal variation in the total N content in the control soil did not change during the incubation period regardless of the tree species ($p > 0.01$). Meanwhile, compared with the control soils, the total N content for both the Qa and Pk soils increased with the N treatment throughout the experiment, but there was no significant difference between the types of tree species. With a N treatment, the total N contents in different aggregate-size fractions were seemingly increased with decreased soil aggregate sizes ($p < 0.01$).

3.2. Humic substances fraction

Compared with the control soils, the proportion of HSs during the incubation periods was different depending on the soil aggregate-size fractions ($p < 0.01$). Regardless of the tree species, the proportion of HSs in the whole soils of all treatments did not change with the incubation time ($p > 0.01$) (Figure 1). Meanwhile, the proportion of the FA fraction in the 2000–1000 and 1000–250 μm aggregate-size fractions showed, no change in the control soils, but there was a decrease in both of the N-treated soils (Figures 2 and 3). However, the proportion of the FA fraction in the 250–53 and <53 μm aggregate-sizes fractions increased more in both of the N-treated soils than the control soils during the incubation periods ($p < 0.01$) (Figures 4 and 5). At the end of incubation, compared with the initial soils, the HA/FA ratio, which indicates the degree of humification, showed the most significant decrease in the 250–53 and <53 μm aggregate-sizes in the N-treated soil regardless of the tree species ($p < 0.01$)

(Table 4). The HA/FA ratio in both the N-treated soils decreased at whole soil ranging between 0.21 and 0.53 in the whole soil, while this ratio decreased between 0.36 and 0.88 in the 250–53 μm aggregate-size fraction and between 0.32 and 0.39 in the <53 μm aggregate-size fraction ($p < 0.01$) (Table 5).

3.3. Soil FTIR

The FTIR spectra of each treatment soil aggregate-size fractions were different among the five-figures (Figures 6–10). Regardless of the tree species and N treatment, the soil organic functional groups of the FA fractions did not change significantly during the entire incubation period ($p > 0.01$). Changes in most soil organic functional groups, except the FA fractions, were observed at 2924, 2850, 1100, 1080, and 920 cm^{-1}, and there were differences according to the tree species and N treatment ($p < 0.01$). In both of the N-treated soils, the changes in the soil organic functional groups were more significant at the 2000–1000, 250–53 and <53 μm aggregate-sizes ($p < 0.01$) and showed different trends for humin and the HA fractions (Figures 9 and 10). Indexes I and II, which are shown by using the calculated by characteristic organic functional groups of the FTIR bands, were changed by the N treatment compared with the initial soils in most soil aggregate-sizes and HSs after 365 days ($p < 0.01$) (Figures 11–14). Compared with the initial soils, index I of the N-treated soils at the end of incubation increased in the Qa soil and decreased in the Pk soil. These changes in index I were the largest in the humin fractions and the smallest in the FA fractions, regardless of tree species (Figures 12 and 14). However, index II increased only in the whole soil of the N-treated Qa soil, while the other N-treated soils did not change (Figure 11).

Table 3. Results of a four-way analysis of variance (ANOVA) that showis the significance of the effects of the experimental parameters on the soil total-N content (TN), humic substances (HSs), humification (HI), and indexes I and II.

Factors	TN	HSs	HI	Index I	Index II
Treatment (N)	*	*	*	**	*
Incubation time (T)	n.s.	*	*	*	n.s.
Tree species (S)	n.s.	n.s.	*	*	*
Aggregate-size (A)	n.s.	**	**	**	*
N × T	*	*	*	*	n.s.
N × S	n.s.	n.s.	*	*	*
N × A	*	*	*	*	*
T × S	n.s.	n.s.	n.s.	n.s.	n.s.
T × A	*	*	*	n.s.	n.s.
S × A	n.s.	n.s.	n.s.	n.s.	n.s.
N × T × S	n.s.	n.s.	n.s.	*	n.s.
N × T × A	n.s.	*	*	*	*
N × S × A	n.s.	n.s.	n.s.	*	*
T × S × A	n.s.	n.s.	n.s.	n.s.	n.s.
N × T × S × A	n.s.	n.s.	n.s.	n.s.	n.s.

***: significant at the $p < 0.001$ level; **: significant at the $p < 0.01$ level; *: significant at the $p < 0.05$ level; n.s.: not significant.

Figure 1. Temporal variations in the humic substances of whole soils after N treatment in (a) humin, (b) humic acid, and (c) fulvic acid in (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

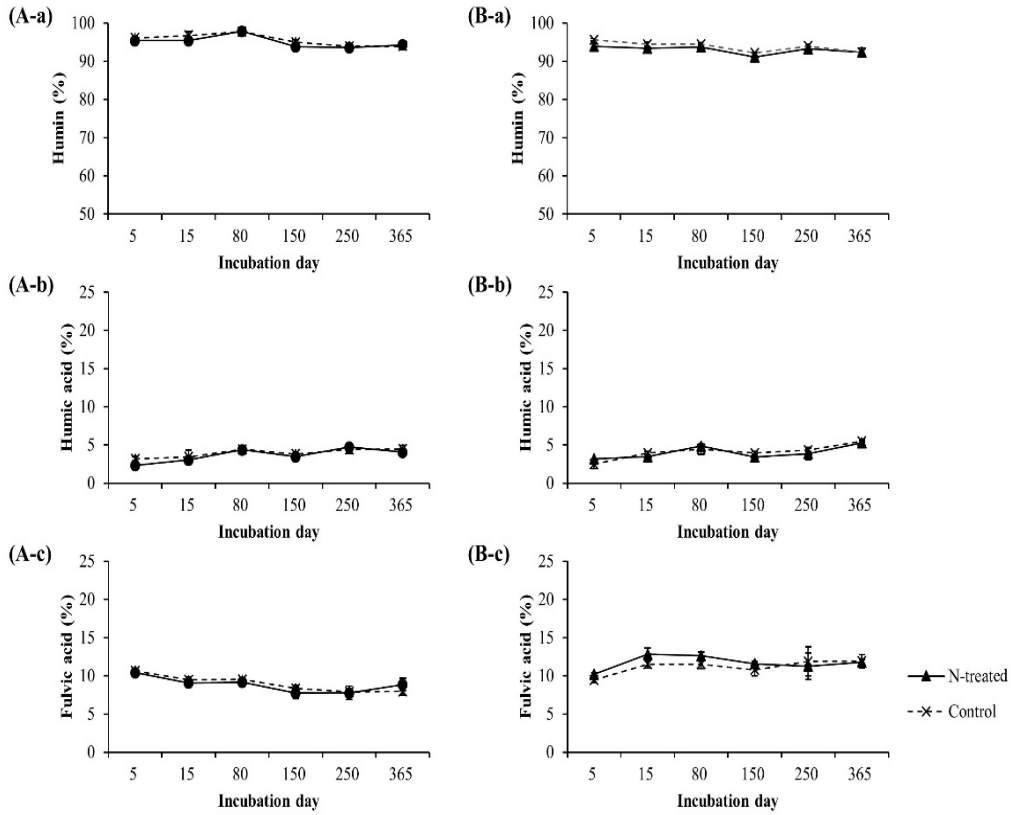

(A-a)
(B-a)
Humin (%)
(A-b)
(B-b)
Humic acid (%)
(A-c)
(B-c)
Fulvic acid (%)
Incubation day
5
15
80
150
250
365
N-treated
Control

Figure 2. Temporal variations in the humic substances of 2000–1000 μm size after N treatment in (a) humin, (b) humic acid, and (c) fulvic acid in (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

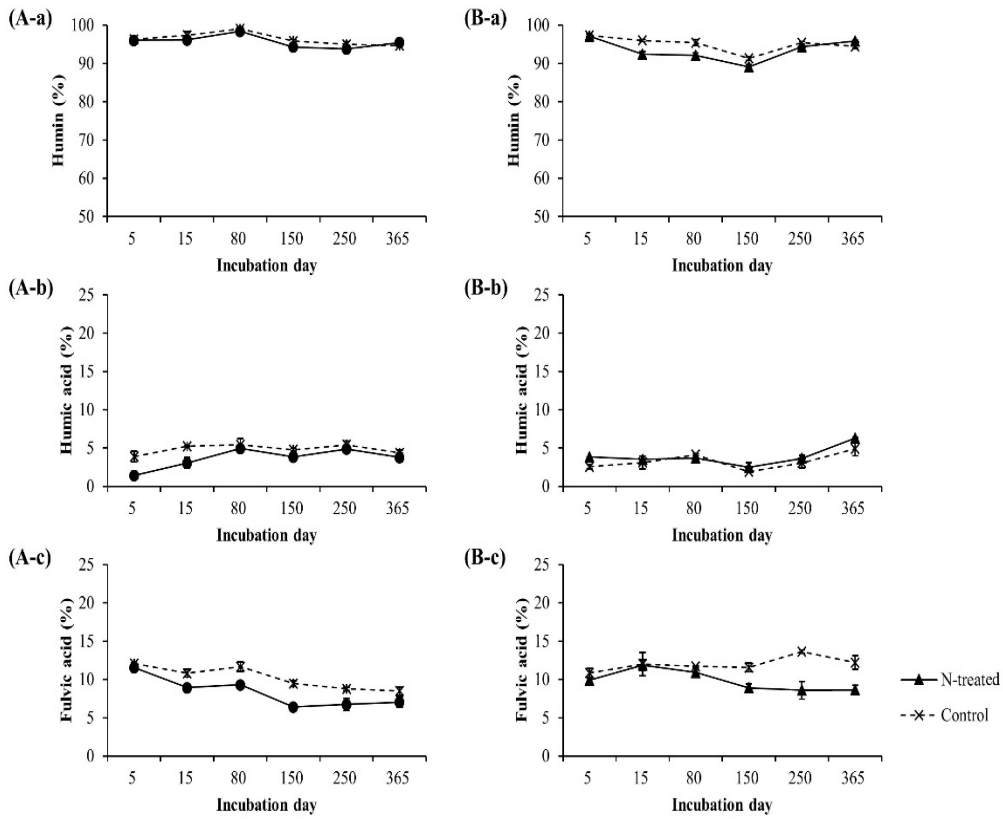

(A-a)
(B-a)
Humin (%)
(A-b)
(B-b)
Humic acid (%)
(A-c)
(B-c)
Fulvic acid (%)
Incubation day
5
15
80
150
250
365
N-treated
Control

Figure 3. Temporal variations in the humic substances of 1000–250 μm size after N treatment in (a) humin, (b) humic acid, and (c) fulvic acid in (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

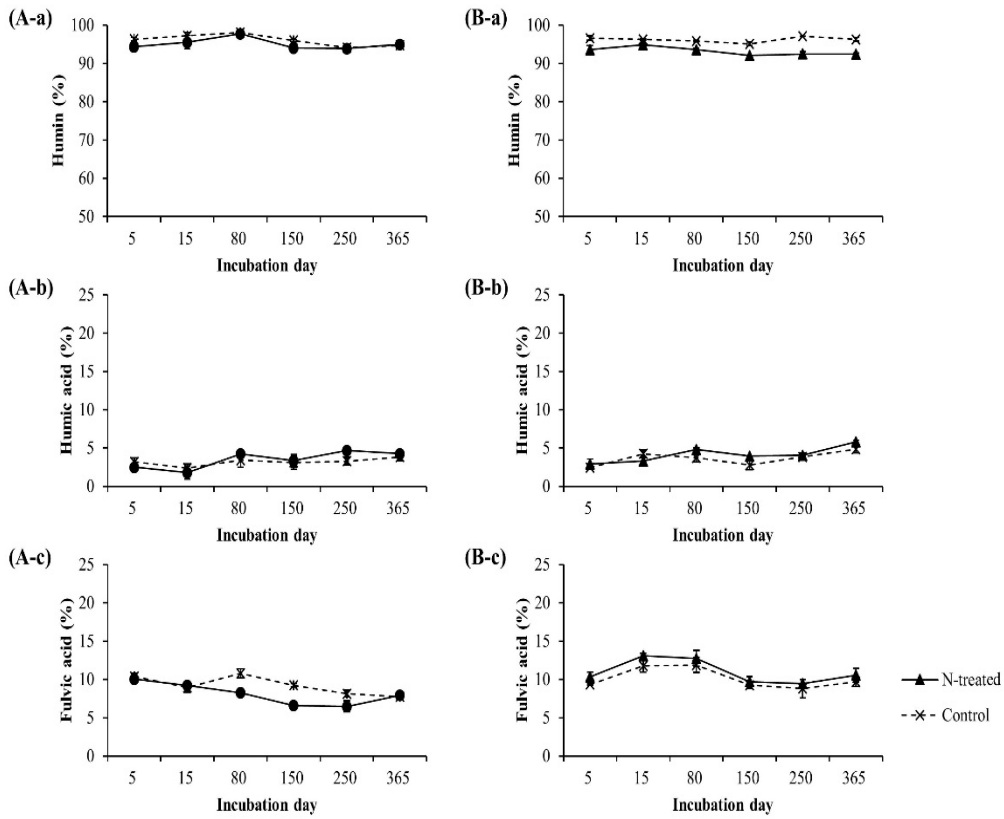

(A-a)
(B-a)
(A-b)
(B-b)
(A-c)
(B-c)
Humin (%)
Humic acid (%)
Fulvic acid (%)
Incubation day
5
15
80
150
250
365
N-treated
Control

Figure 4. Temporal variations in the humic substances of 250–53 μm size after N treatment in (a) humin, (b) humic acid, and (c) fulvic acid in (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

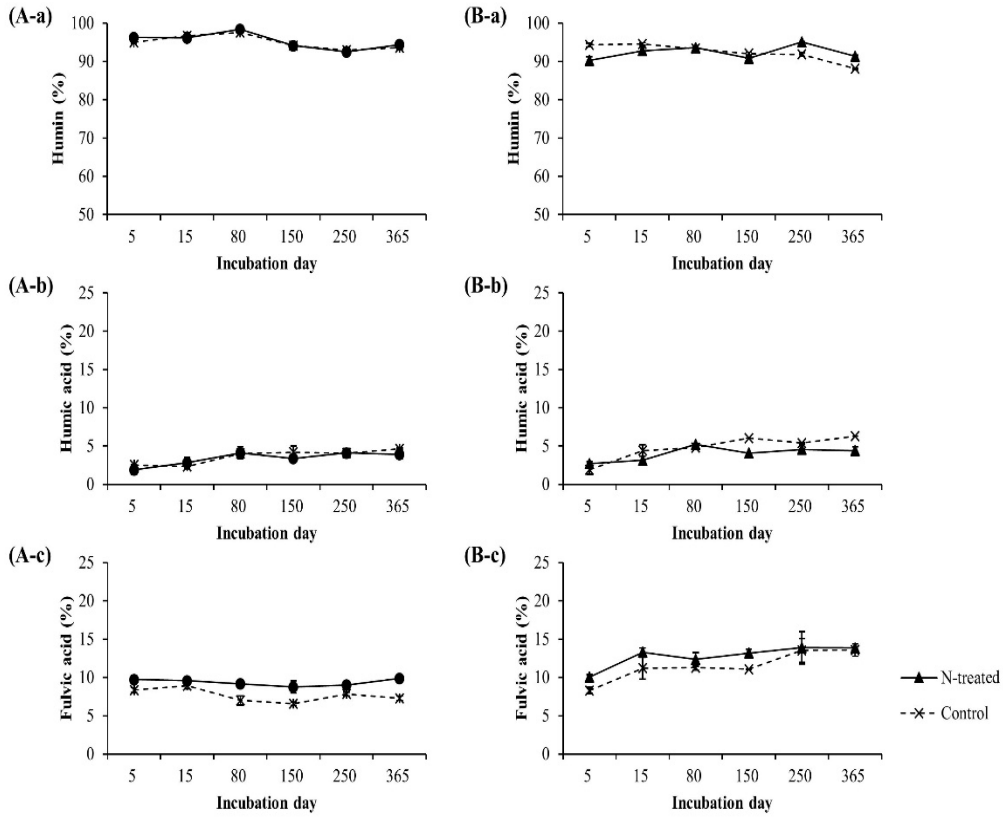

(A-a)
(B-a)
Humin (%)
(A-b)
(B-b)
Humic acid (%)
(A-c)
(B-c)
Fulvic acid (%)
Incubation day
5
15
80
150
250
365
N-treated
Control

Figure 5. Temporal variations in the humic substances of <53 μm size after N treatment in (a) humin, (b) humic acid, and (c) fulvic acid in (A) oak (Qa) and (B) pine (Pk) forest floor soils. The error bars indicate ± one standard deviation (*n* = 3).

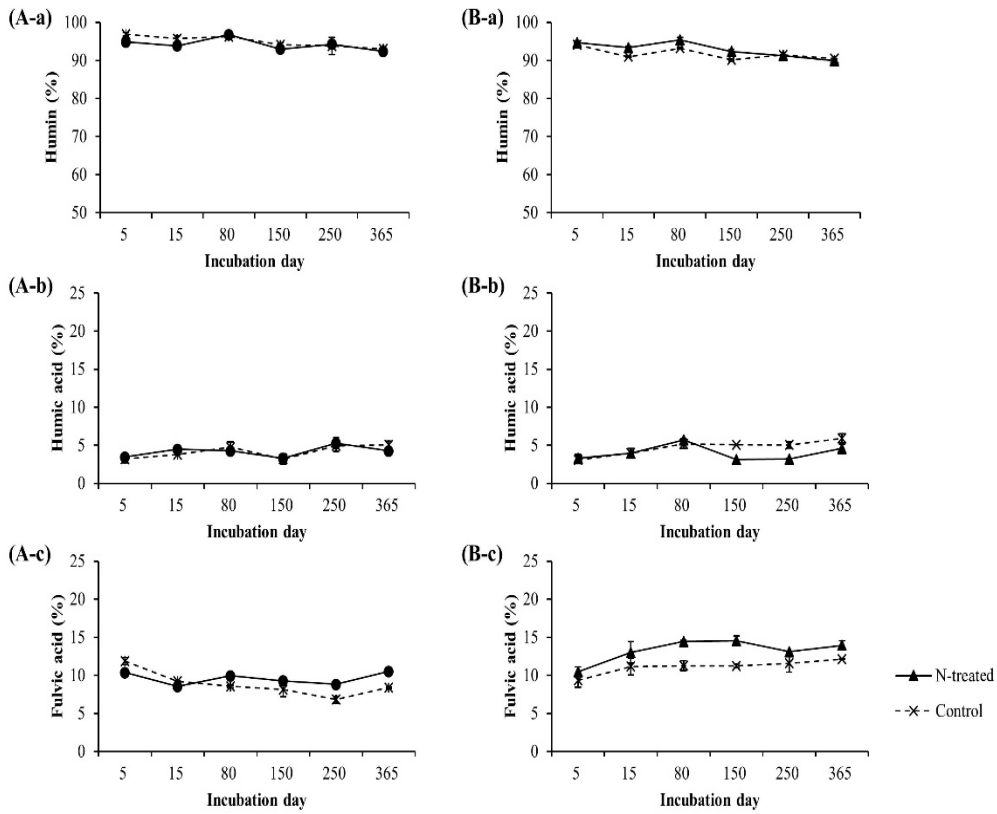

(A-a)
Humin (%)
100
90
80
70
60
50
5 15 80 150 250 365
Incubation day
(B-a)
Humin (%)
100
90
80
70
60
50
5 15 80 150 250 365
Incubation day
(A-b)
Humic acid (%)
25
20
15
10
5
0
5 15 80 150 250 365
Incubation day
(B-b)
Humic acid (%)
25
20
15
10
5
0
5 15 80 150 250 365
Incubation day
(A-c)
Fulvic acid (%)
25
20
15
10
5
0
5 15 80 150 250 365
Incubation day
(B-c)
Fulvic acid (%)
25
20
15
10
5
0
5 15 80 150 250 365
Incubation day
N-treated
Control

Table 4. Soil total- N contents at365 days after N deposition in two different forest floor soils.

Aggregate size (μm)	*Q. acutissima*		*P. koraiensis*	
	Control	N-treated	Control	N-treated
	g kg^{-1}			
Whole soil	1.7 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	2.0 ± 0.5
2000–1000	1.5 ± 0.0	1.6 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
1000–250	1.6 ± 0.1	1.7 ± 0.0	1.5 ± 0.1	1.5 ± 0.1
250–53	1.7 ± 0.1	1.8 ± 0.0	2.0 ± 0.1	2.2 ± 0.1
<53	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.2	2.4 ± 0.1

Table 5. Soil HA/FA ratios at 365 days after N deposition in two different forest floor soils.

Aggregate size (μm)	HA/FA ratio			
	Q. acutissima		*P. koraiensis*	
	Initial	After 365 days	Initial	After 365 days
Whole soil	0.99	0.46	0.66	0.45
2000–1000	1.03	0.54	0.72	0.73
1000–250	1.08	0.54	0.50	0.55
250–53	1.28	0.40	0.68	0.32
<53	0.73	0.41	0.72	0.33

Figure 6. Stacked FT-IR spectra in the whole soil of two different forest floors after 365 days of the control and N treatment: (a) soil, (b) Humin, (c) Humic acid, and (d) Fulvic acid. The bands (13 total) indicate the organic functional groups.

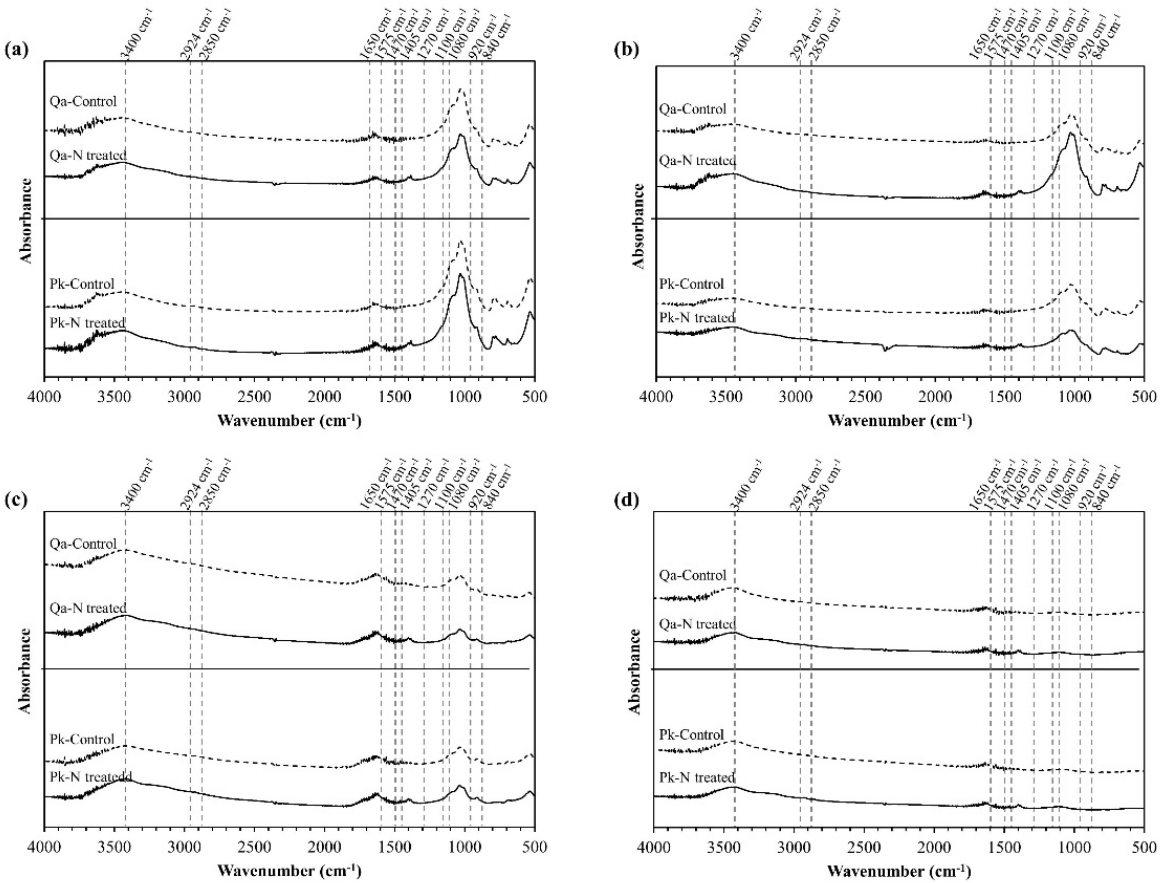

(a)
(b)
(c)
(d)
3400 cm-1
2924 cm-1
2850 cm-1
1650 cm-1
1575 cm-1
1470 cm-1
1405 cm-1
1270 cm-1
1100 cm-1
1080 cm-1
920 cm-1
840 cm-1
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
Absorbance
Wavenumber (cm-1)
4000
3500
3000
2500
2000
1500
1000
500

Figure 7. Stacked FT-IR spectra in the 2000–1000 μm size soil of two different forest floors after 365 days of the control and N treatment: (a) soil, (b) Humin, (c) Humic acid, and (d) Fulvic acid. The bands (13 total) indicate the organic functional groups.

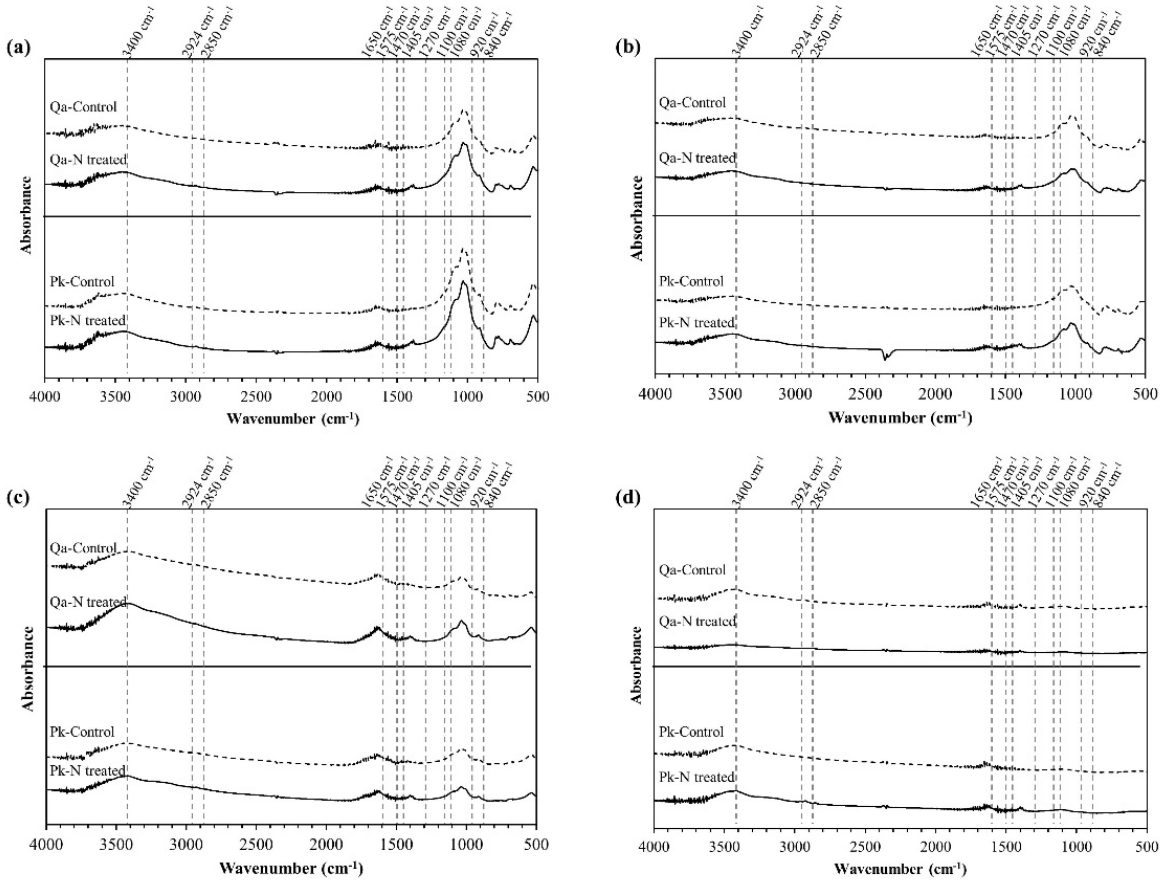

(a)
(b)
(c)
(d)
3400 cm-1
2924 cm-1
2850 cm-1
1650 cm-1
1575 cm-1
1470 cm-1
1405 cm-1
1270 cm-1
1100 cm-1
1080 cm-1
920 cm-1
840 cm-1
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
Absorbance
Wavenumber (cm-1)
4000
3500
3000
2500
2000
1500
1000
500

Figure 8. Stacked FT-IR spectra in the 1000–250 μm size soil of two different forest floors after 365 days of the control and N treatment: (a) soil, (b) Humin, (c) Humic acid, and (d) Fulvic acid. The bands (13 total) indicate the organic functional groups.

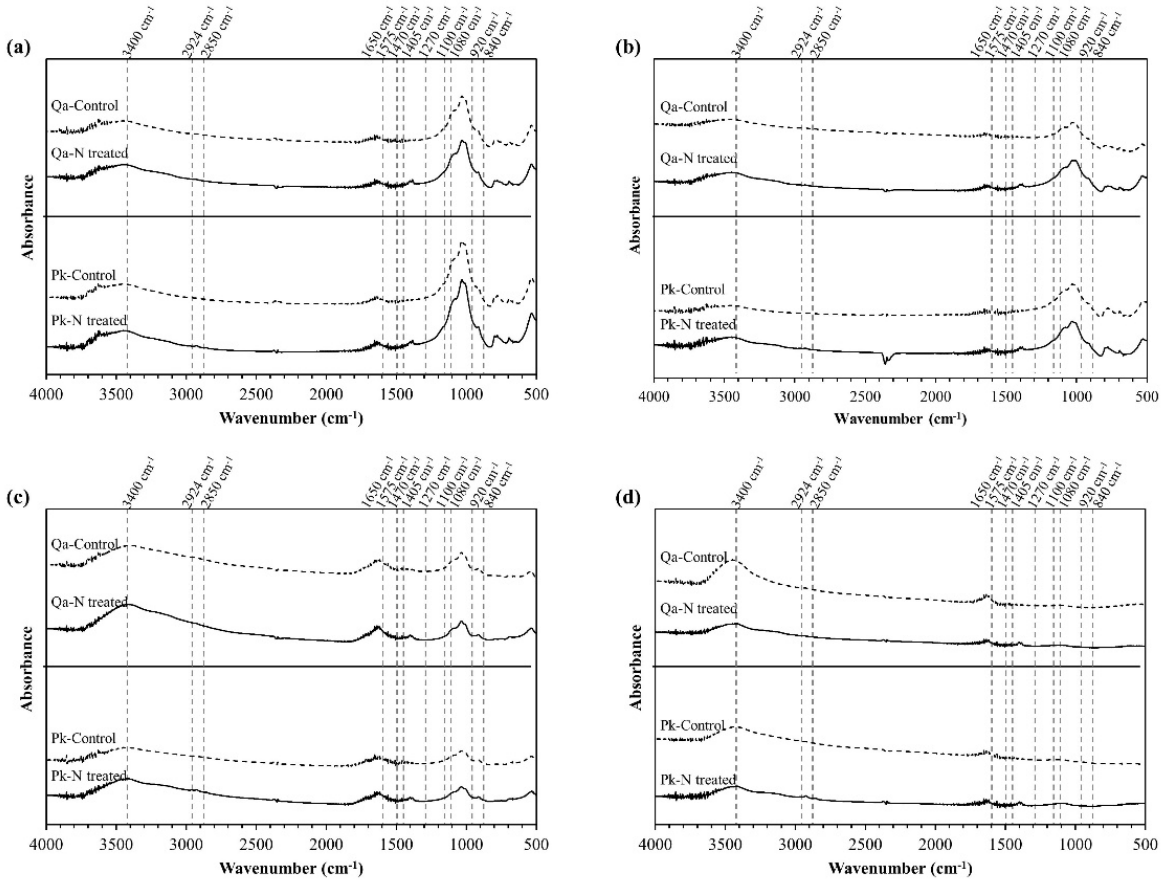

(a)
(b)
(c)
(d)
3400 cm-1
2924 cm-1
2850 cm-1
1650 cm-1
1575 cm-1
1470 cm-1
1405 cm-1
1270 cm-1
1100 cm-1
1060 cm-1
1000 cm-1
920 cm-1
840 cm-1
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
Absorbance
Wavenumber (cm-1)
4000
3500
3000
2500
2000
1500
1000
500

Figure 9. Stacked FT-IR spectra in the 250–53 μm size soil of two different forest floors after 365 days of the control and N treatment: (a) soil, (b) Humin, (c) Humic acid, and (d) Fulvic acid. The bands (13 total) indicate the organic functional groups.

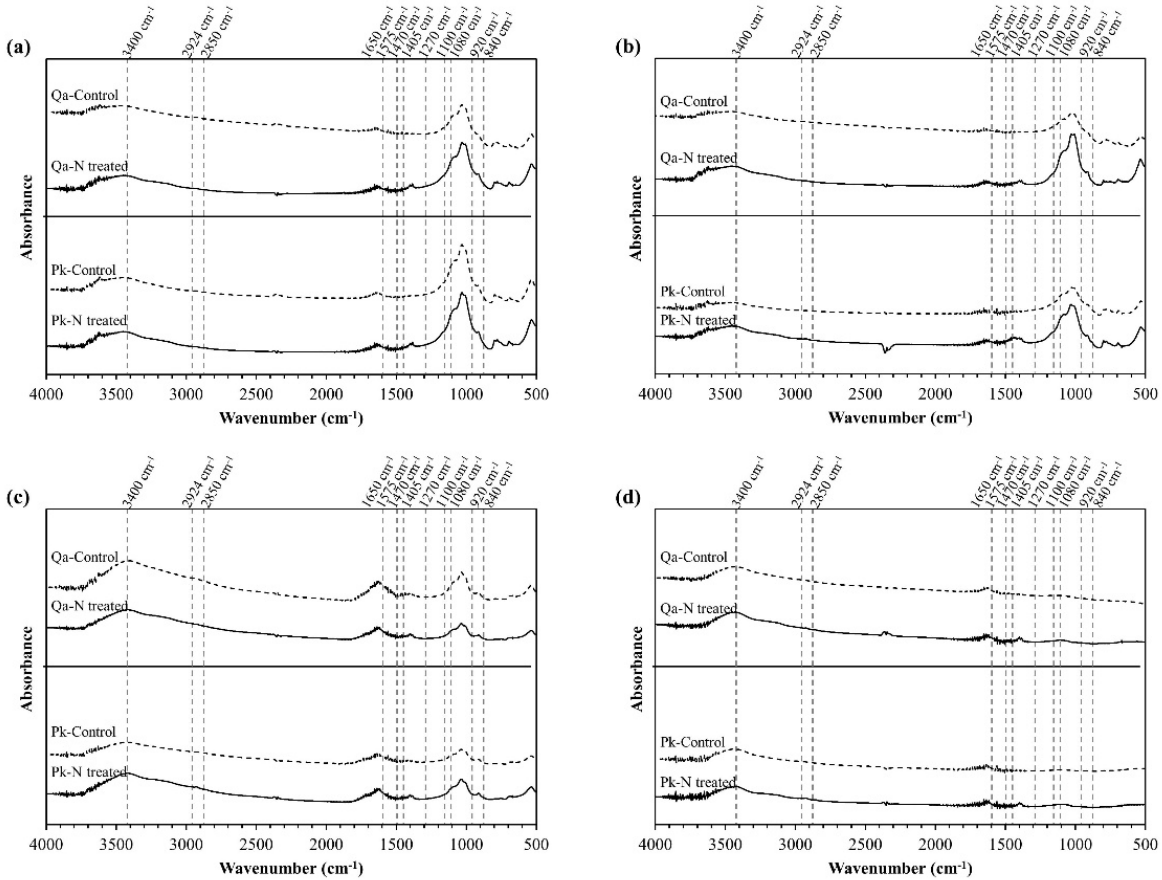

(a)
(b)
(c)
(d)
3400 cm-1
2924 cm-1
2850 cm-1
1650 cm-1
1575 cm-1
1470 cm-1
1405 cm-1
1270 cm-1
1100 cm-1
1080 cm-1
920 cm-1
840 cm-1
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
Absorbance
Wavenumber (cm-1)
4000
3500
3000
2500
2000
1500
1000
500

Figure 10. Stacked FT-IR spectra in the <53 μm size soil of two different forest floors after 365 days of the control and N treatment: (a) soil, (b) Humin, (c) Humic acid, and (d) Fulvic acid. The bands (13 total) indicate the organic functional groups.

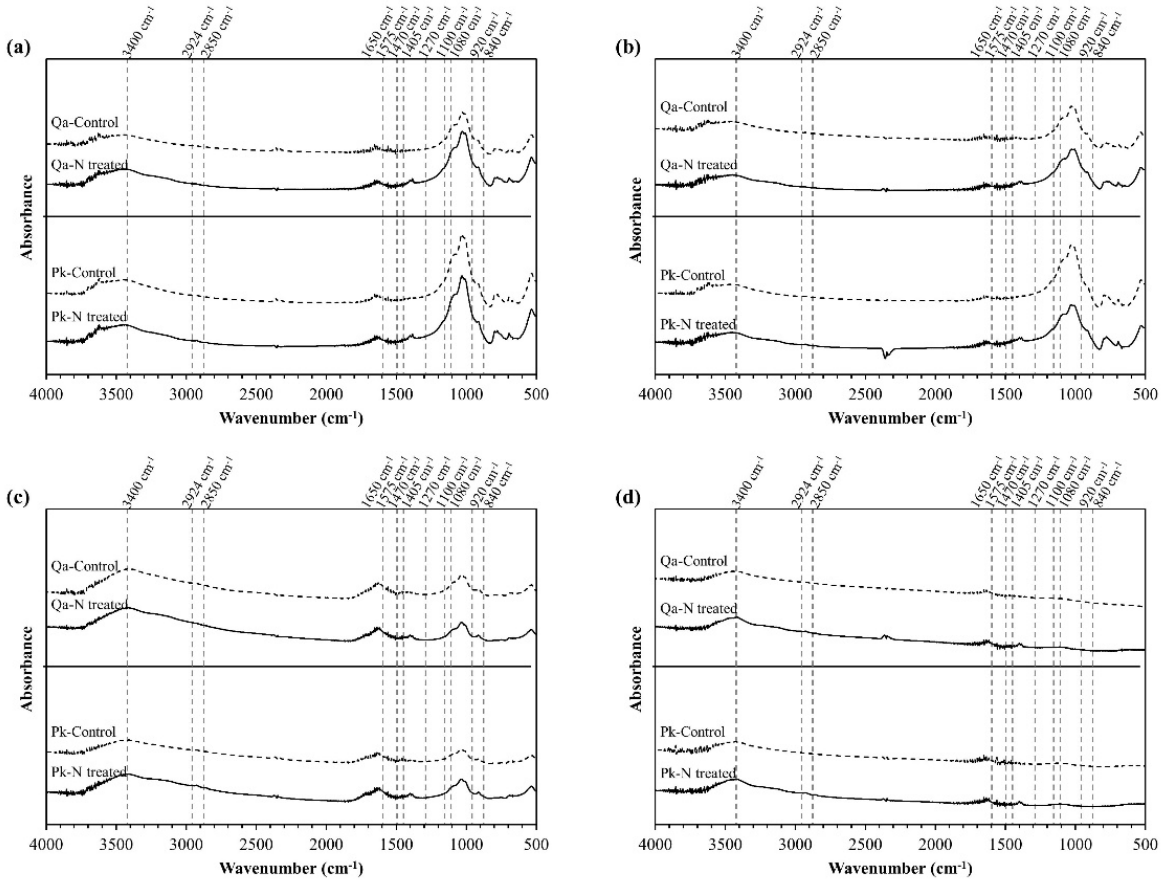

(a)
(b)
(c)
(d)
3400 cm⁻¹
2924 cm⁻¹
2850 cm⁻¹
1650 cm⁻¹
1575 cm⁻¹
1470 cm⁻¹
1405 cm⁻¹
1270 cm⁻¹
1100 cm⁻¹
1080 cm⁻¹
1000 cm⁻¹
920 cm⁻¹
840 cm⁻¹
Qa-Control
Qa-N treated
Pk-Control
Pk-N treated
Absorbance
Wavenumber (cm⁻¹)
4000
3500
3000
2500
2000
1500
1000
500

Figure 11. Ratio of the whole soil after N treatment to (a) index I and (b) index II in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. Indexes I and II indicate the relative degree of decomposition and the recalcitrance of soil organic matter.

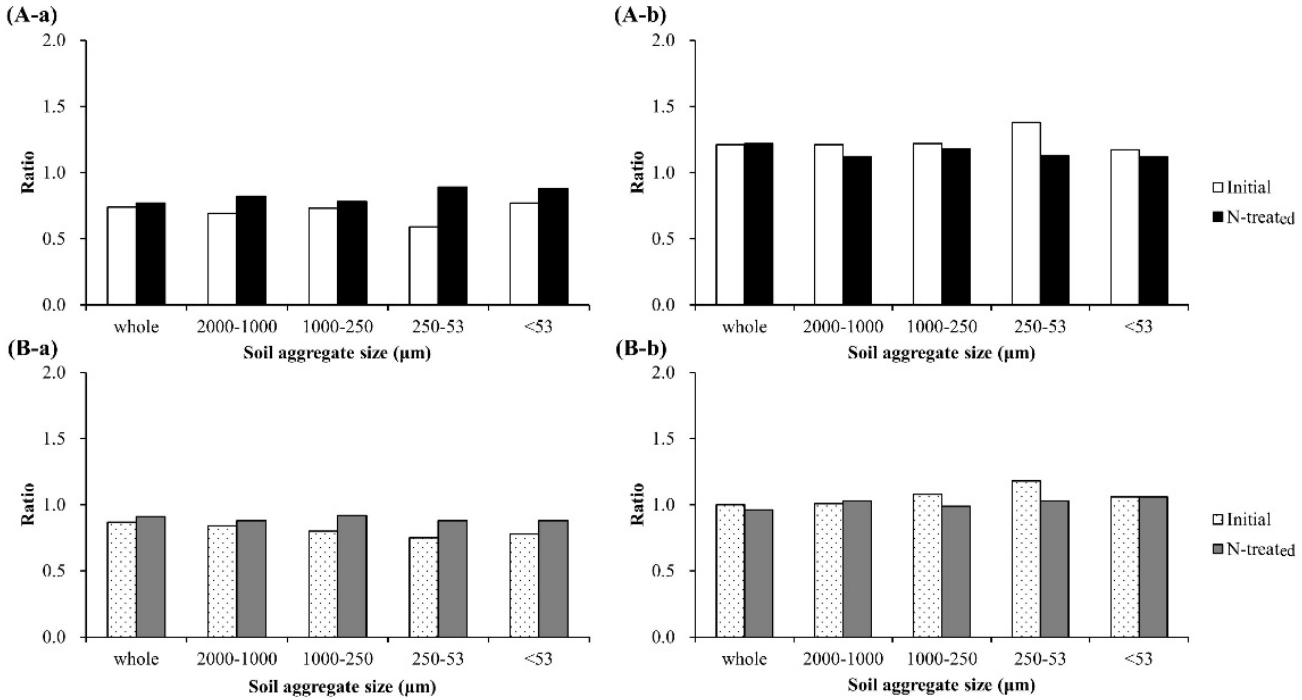

(A-a)
(A-b)
(B-a)
(B-b)
Ratio
2.0
1.5
1.0
0.5
0.0
whole
2000-1000
1000-250
250-53
<53
Soil aggregate size (μm)
Initial
N-treated

Figure 12. Ratio of humin after N treatment to (a) index I and (b) index II in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. Indexes I and II indicate the relative degree of decomposition and the recalcitrance of soil organic matter

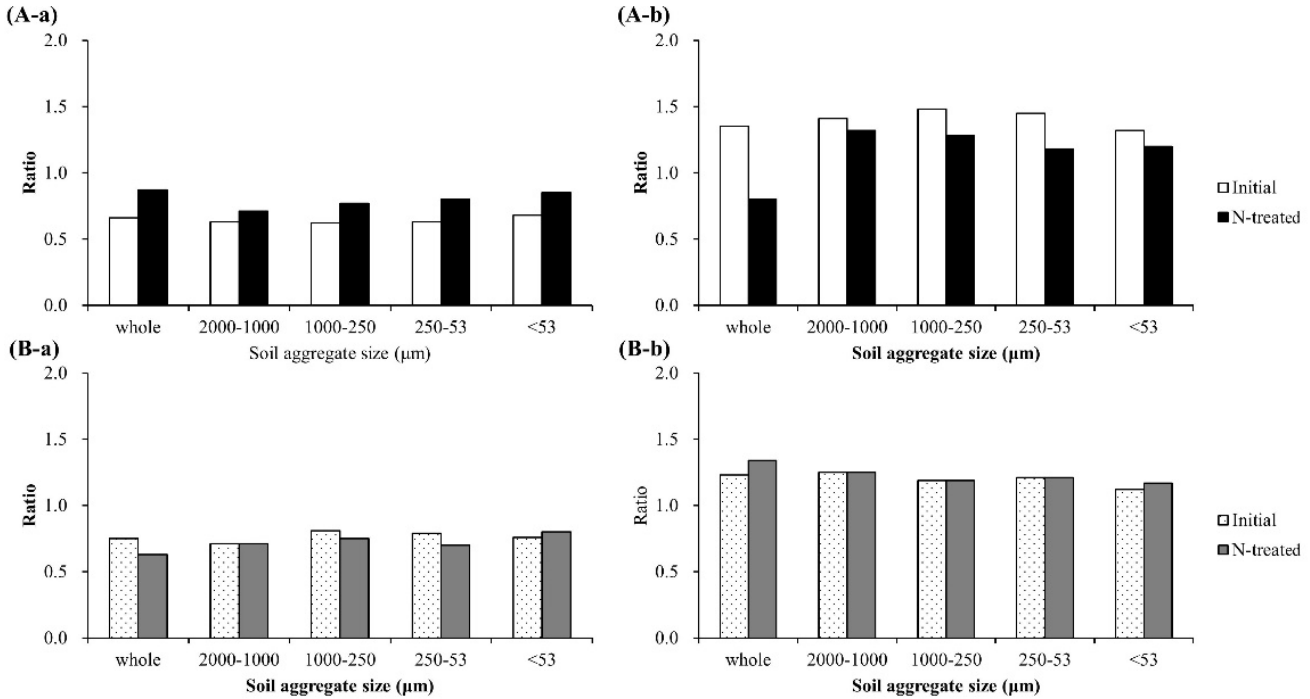

(A-a)
(A-b)
(B-a)
(B-b)
Ratio
2.0
1.5
1.0
0.5
0.0
whole
2000-1000
1000-250
250-53
<53
Soil aggregate size (μm)
Initial
N-treated

Figure 13. Ratio of humic acid after N treatment to (a) index I and (b) index II in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. Indexes I and II indicate the relative degree of decomposition and the recalcitrance of soil organic matter.

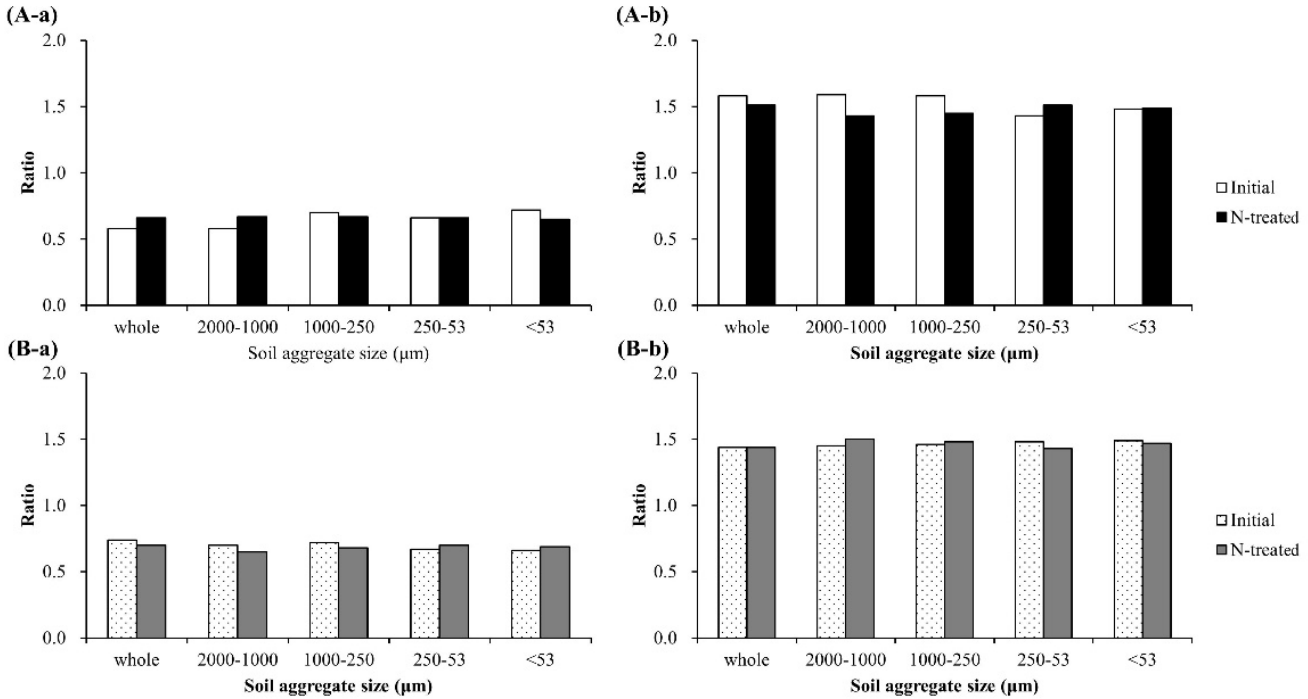
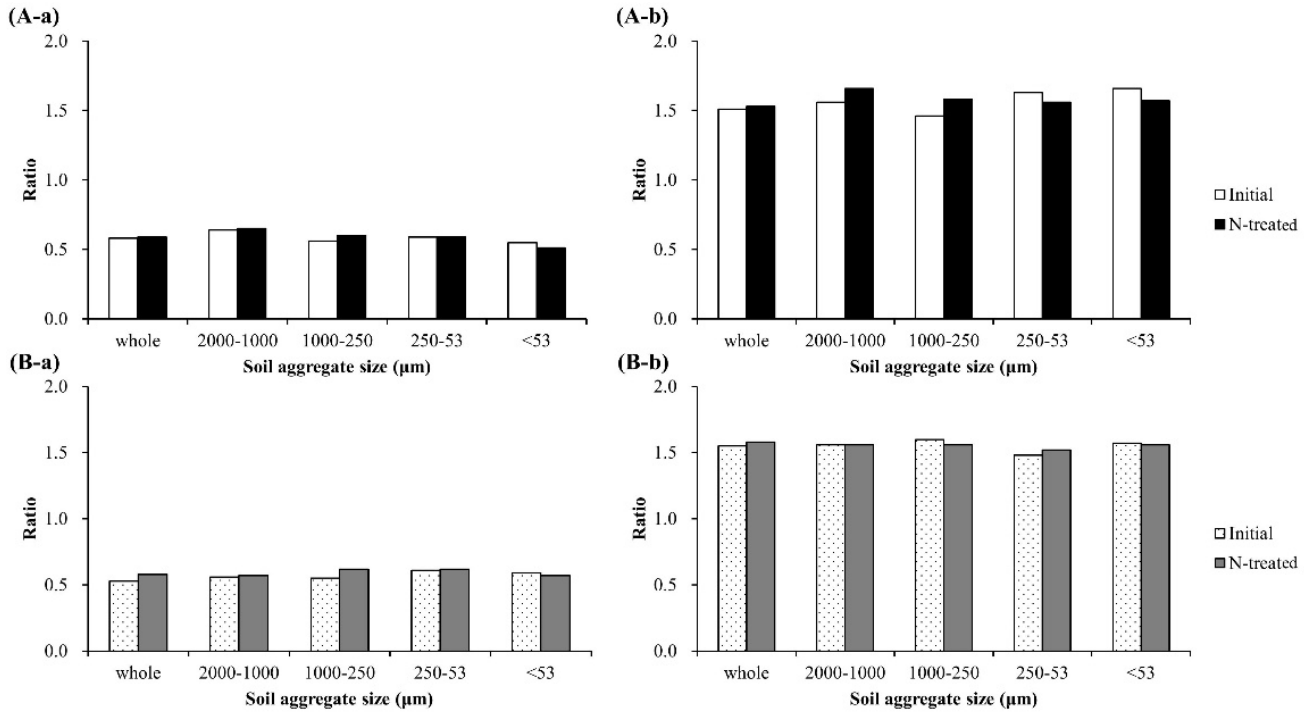

(A-a)
(A-b)
(B-a)
(B-b)
Ratio
2.0
1.5
1.0
0.5
0.0
whole
2000-1000
1000-250
250-53
<53
Soil aggregate size (μm)
Initial
N-treated

Figure 14. Ratio of fulvic acid after N treatment to (a) index I and (b) index II in two different (A) oak (Qa) and (B) pine (Pk) forest floor soils. Indexes I and II indicate the relative degree of decomposition and the recalcitrance of soil organic matter.


(A-a)
(A-b)
(B-a)
(B-b)
Ratio
2.0
1.5
1.0
0.5
0.0
whole
2000-1000
1000-250
250-53
<53
Soil aggregate size (μm)
Initial
N-treated

4. Discussion

The N treatment caused an increase of the total N content in the soil of both tree species (Table 3), but the total N content did not change during the incubation periods regardless of the N treatment (the data are not shown). In general, when urea is added to the soil under aerobic and weak acid conditions, the nitrate ions are produced through the hydrolysis, ammonification and nitrification processes (Davidson et al., 1992; Chen et al., 2013); consequently, the concentration of nitrate ion in the soil increases with incubation time (the data are from Chapter 2 Figure 2). Therefore, it is concluded that there was no change in the total N content in all treatments regardless of the N treatment and tree species since the nitrate ion leaching did not occur under limited experimental conditions, as in this study.

During the incubation periods, the proportion of HSs was different according to the tree species and the soil aggregate-size fractions, and the proportion of FA was higher than the proportion of HA (Figures 4–5). In the soil aggregate-size fractions, the whole soil showed no change in the proportion of HSs, regardless of the N treatment (Figure 1). However, in both N-treated soils, FA decreased in the 2000–1000 and 1000–250 μm aggregate sizes and increased in the 250–53 and <53 μm aggregate sizes.

There are two possible reasons to explain the changes in the HSs proportion of the N-treated soil depending on the soil aggregate size. The first reason is the change of the proportion of HSs due to the change in the soil aggregate-size fraction. In general, soil aggregate size is formed by the combination of various aggregate-size soils and other organic material, and large aggregate sizes are less stable than small aggregate sizes because they are bound with relatively less degraded organic materials (Kemper and Rosenau, 1986; Six et al., 2000). In addition, the N treatment is known to promote soil microbial activity (Tietema and Wessel, 1992), and

insoluble and soluble C pools and microbial activity are closely related to the proportion of HSs in the soil. Previous studies have reported that the proportion of micro-aggregates increased because of the temporary bond break between the macro-aggregates due to the repeated drying-wetting or the decomposition of SOM by the N treatment during the incubation period (Gale et al., 2000; Six et al., 2000; Erktan et al., 2016), which is a trend also shown in my previous study (the data from Chapter 2). Therefore, it is considered that the change in the HSs proportion in this study is due to the decomposition of soil C, considering my previous research in which the decomposition of the SOC was greater in the soil with large aggregate sizes than in the soil with small aggregate sizes (the data from Chapter 2). The second reason is a change in the proportions of HA and FA. In general, HA is a bioactive substance because of its high chemical reactivity and chelating ability in the soil (Mayhwe, 2004), and it will degrade to become FA (Pettie, 2006). Therefore, in this study, the decreases in HA and FA of the 2000–1000 and 1000–250 μm aggregate sizes and the increases in FA of the 250–53 and <53 μm aggregate sizes are considered to be the results of the degradation of HA or the temporary bond breaks due to the decomposition of organic matter (Pettie, 2006; Guimarães et al., 2013).

Meanwhile, the HA/FA ratio that indicates the degree of humification of the SOC showed a tendency to decrease with the N treatment at the end of incubation, but it varied depending on the tree species. Since deciduous trees have lower levels of lignin and polyphenols than coniferous trees, the leaf litter of deciduous trees decomposes faster than the leaf litter of coniferous trees (Chiti et al., 2012; Kristensen et al., 2014), which results in different soil HA/FA ratios under these two contrasting forest stands (Table 5).

Compared with the control soils, the band that represents the soil organic functional groups showed significant differences among the tree species and soil aggregate-size fractions (Figures 6–10). At the same aggregate-size soils, the N

treatment resulted in differences in the soil organic functional groups, and these changes were higher in the Qa soil than in the Pk soil. The major components of the change in the soil organic functional groups were aliphatic C–H (2924 and 2850 cm^{-1}) and polysaccharide (1110 and 1080 cm^{-1}), which influence important properties of the organic matter, such as stability against microbial degradation, and are derived mainly from plants or microorganisms (Haberhauer et al., 1998). The second changes in the functional groups of the soil were aromatic (920 cm^{-1}). These changes in the spectral bands of the soil functional groups might reflect the decomposition of SOM and probably results from the different degradation of the tree species (Margenot et al., 2015; Shiau et al., 2017). In particular, most of the changes in the soil organic functional groups were more pronounced in the Qa soil than in the Pk soil. These changes were in the order of soil sample > humin > HA, which is also shown in the results of Indexes I and II, which indicates the decomposition of SOM. According to previous studies, as the composting process proceeds, in the band that represents the soil organic functional groups, the aromatics and polysaccharides groups increased; however, the aliphatic group decreased (Inbar et al., 1989; Hsu and Lo, 1999). Therefore, as I can see from the HA/FA ratio result for the tree species (Table 5), since the SOC of the Pk soil was less decomposed than the Qa soil, which has organic matter that is relatively easy to decompose (Kristensen et al., 2014; Margenot et al., 2015; Shiau et al., 2017), in this study, the change of the soil organic functional groups is considered to reflect these characteristics of the tree species.

Meanwhile, the change of the soil organic functional groups according to the soil aggregate-size was also found to be significant as the proportion of the HSs results. Regardless of the tree species, the aliphatic C–H (2924 and 2850 cm^{-1}) tended to decrease in all soil aggregate sizes, but polysaccharides (1110 and 1080 cm^{-1}) and aromatics (920 cm^{-1}) increased in the 250–53 and <53 μm aggregate sizes (Figures 9 and 10). Similar to the FTIR results, most of the changes in the results of Indexes

I and II occurred in the 250–53 and <53 μm aggregate sizes (Figures 11–14). These results imply that the undecomposed recalcitrant substances tended to accumulate in the small aggregate-size fractions of the soils (Kavdir et al., 2005). Therefore, this result indicates that the recalcitrant soil organic compounds, such as polysaccharides and aromatic compounds, were typically found in stable bindings with fine clay minerals (Calabi-Floody et al., 2012), which is consistent with my proportion of the HSs results.

5. Conclusions

I hypothesized whether the addition of N to the soil of two different forest floors (deciduous trees, Qa, and coniferous trees, Pk) would result in different soil aggregate-size fractions through different HSs and soil organic functional group patterns. During the incubation period, regardless of the tree species, no significant changes were observed in most of the experimental results in the control soil. However, after the N treatments, there was a change in the HSs and soil organic functional groups according to the soil aggregate-size fraction. In particular, unlike my hypothesis that the changes in the HSs for the 2000–1000 μm or 1000–250 μm aggregate sizes would be larger, the HSs mostly changed in the aggregate sizes of 250–53 μm and <53 μm, regardless of the tree species. However, the soil organic functional groups changed more significantly at the 2000–1000, 250–53 and <53 μm aggregate sizes and showed different trends for the humin and HA fractions. With my results, I cannot explain the relative contribution of the soil organic functional groups to the SOC dynamic or the change in the soil aggregate-size distribution. Therefore, I conclude that the N treatment affects the HSs and soil organic functional groups and that these differences depend on the tree species. To investigate this phenomenon more closely, it is necessary to further investigate the effects of the labelled C application on the soil organic functional groups and HSs, and a long-term analysis and interpretation is also required to fully understand the soil C dynamics in relation to the effect of the tree species.

CHAPTER IV

Effect of liming on chemical speciation and in bioavailability of phosphorus in acidic forest soils

Abstract

A hypothesis of whether an increase in Ca^{2+} due to liming acid soils causes an increase in Ca-phosphates with a concomitant decrease in Al- and Fe-phosphates was tested on a relatively short-term time frame, as observed in the investigations of chemical phosphorus (P) speciation. Soil samples were taken at a deforested site and split into three treatment groups: a control and two $CaCO_3$ treatments (0.1 and 0.4 g $CaCO_3$ in 100 g soil). Each soil was incubated at 25±2°C for 40 days. Inorganic-P was partitioned into three fractions: Fraction-A (Al- and occluded Fe-P), Fraction-B (non-occluded Fe- and adsorbed-P), and Fraction-C (Ca-P). The pH of $CaCO_3$-treated soils decreased right after liming, indicating a probable formation of solid phosphate compounds. Available P in $CaCO_3$-treated soils decreased from 0.67 mg kg^{-1} to control levels at the end of incubation. Total-P and organic-P of $CaCO_3$-treated soils were virtually the same as those of control soils, while inorganic-P varied. Fraction-A in both $CaCO_3$-treated soils peaked at 10 days of incubation, and then decreased below control levels, while Fraction-B decreased abruptly right after liming, and thereafter gradually decreased. Liming decreased Fraction-C, and caused an increase in Al- and Fe-P due to an increase in exchangeable Al^{3+} and Fe^{3+}. My results challenge the hypothesis by showing the unexpected opposite data for Al-, Fe- and Ca-P compounds at least in the early stage of equilibrium disturbances due to liming an acidic soil.

Keywords: *Calcium, deforestation, equilibrium, fractionation, interconversion*

1. Introduction

Deforestation is the removal of trees in a forest where the land is thereafter converted to a non-forested land, entailing a potential threat to the health of terrestrial and aquatic ecosystems. The removal of trees without sufficient reforestation has led to unfavorable consequences, including loss of habitat, loss of biodiversity, and poor soil quality. Changes in soil chemical properties due to deforestation considerably decreased the levels of soil nutrients, pH and available phosphorus, since the lowering of soil pH alters nutrient availability and thereby affects plant growth and productivity in highly acidic soils (Zaman et al., 2010).

Phosphorous (P) has been recognized as a major limiting nutrient for sustaining the productivity and resilience of terrestrial ecosystems (Poeplau et al., 2016), and the availability and chemical form is very sensitive to changes in soil pH. In acidic soils Al^{3+} and Fe^{3+} react extensively with phosphates ($H_2PO_4^-$ and HPO_4^{2-}) to form insoluble Al and Fe phosphate compounds (Ro and Cho, 2000; Verma et al., 2005), while in neutral and alkaline soils Ca^{2+} readily reacts with phosphates to form less insoluble Ca-P compounds (Lindsay, 1979). However, recent evidence suggests the possibility of conversion of Ca-P compounds into even more insoluble compounds, decreasing their solubilities (Mahdi et al., 2012).

In general, liming is known to increase the availability of P in acidic soils by stimulating mineralization of soil organic P pools (Haynes, 1982; Sharma et al., 2013). However, another line of investigation suggests that liming decreased soluble-P and labile-P until the pH reached about 6.5 (Delgado and Torrent, 2000), thus indicating the occurrence of chemical disturbances in the early stage of liming, contrary to the widely-held notion. This contrasting effect of liming obviously suggests that the solubility and interaction of each P compound predominantly governs its availability and chemical speciation in soils (Haynes, 1982; Mahdi et al.,

2012), and that the patterns of partitioning and chemical speciation of P would vary with time.

Liming with $CaCO_3$ or $CaSO_4$ is an effective way of increasing the soil pH of acidic soils, and has two major effects on the soil chemical status, since it adds a large amount of Ca ions (Fransson et al., 1999). However, liming can reduce the availability of P in soils due to extensive interaction of P with Ca^{2+}, Al^{3+} and Fe^{3+} ions (Machado and Silva, 2001), thus causing a shift in ionic composition. The changes in ionic composition due to changes in soil pH can in turn lead to a shift in chemical equilibria by increasing concentrations of dissolved Ca^{2+} and displaced hydrolytic Fe and Al species (hereafter, be referred to as Al^{3+} and Fe^{3+}, respectively) that can decrease the availability of P in the soil solution (Ro and Cho, 2000). Since these reactions involve large numbers of chemical forms of P in soils, which are bound to metals or free cations and differ in their mobility and availability, the different P species, such as labile P, reductant P, and metal-bound P, coexist and the distribution of P species can be separated by different sequential extraction schemes, the so-called P-fractionations (Kaiserli et al., 2002).

Nevertheless, the mechanism responsible for variations in P chemical speciation in soils due to liming is still not fully understood. Although a large majority of efforts has been so far directed to long-term effects of liming on the chemical P speciation (Patzold et al., 2013), too little attention has been paid to relatively short-term effects right after liming, although the time-dependent relative thermodynamic stability and the kinetics of interconversion from various P compounds are required. Moreover, a chemical disturbance caused by liming in turn triggers complex chemical reactions in which a large number of chemical interconversions among P compounds occurs. However, the series of sequential or parallel interconversion reactions that determines inorganic P speciation processes is unresolved because of insufficient data or information on the Gibbs free energy of formation or the equilibrium constant

for each binary combination of P compounds.

In addition, some recent evidence suggests an inconsistency in the effects of liming on the distribution of Ca-P species and the availability of P (Machado and Silva, 2001; Mahdi et al., 2012), and this situation necessitates a testing of the possibility of conversion of Ca-P compounds into less soluble compounds and/or concurrent formation of Al- and/or Fe-P compounds in order to bridge the knowledge gap for the effect of liming at least in the early stage of chemical disturbances. Therefore, I challenged the widely-held notion that P availability increases due to liming by hypothesizing that an increase in soil pH induced by liming would instantaneously disturb chemical equilibria among inorganic P species through extensive interactions with increased Ca^{2+} due to dissolution of $CaCO_3$ and with displaced Al^{3+} and Fe^{3+}. Here, I tested this hypothesis by conducting batch incubation experiments and determined the time-course distribution patterns of chemical P species by sequential P extraction and X-ray diffraction analysis. As evidenced by recent evidence of decreasing P availability and solubility (Machado and Silva, 2001; Mahdi et al., 2012), the response of the speciation and availability of P to liming practices should be reevaluated, particularly for forest tree nurseries for reforestation and forest management.

2. Materials and Methods

2.1. Sampling sites and soil preparation

The study site was located in the Seoul National University Forest, Mt. Backwoon, Jeollanam-do (35º2'N, 127º35'E). The deforestation site was clear-cut 5 years ago, mostly to allow cultivation of pitch pine (*Pinus rigida* Mill.). Soil samples (0–30 cm in depth) were taken in February 2012. Immediately after sampling, soil samples were air-dried at room temperature, passed through a 2–mm sieve, and mixed thoroughly. The physical and chemical properties of the soil were shown in Table 1.

2.2. Lime requirement

Lime requirement was determined by the incubation pH buffer curve method (Kuo, 1996). One hundred grams of an air-dried soil sample was placed into each of seven separate 250 mL Erlenmeyer flasks, and these samples were adjusted to field moisture capacity (0.31 m^3 m^{-3}) and mixed with calcium carbonate (0, 0.1, 0.2, 0.3, 0.5, 0.7, and 0.9 g). Each mixture was sealed with Parafilm®, and was shaken at 120 rpm at 25±2 ºC for 5 days. The soil was then dried at room temperature. The soil pH was measured potentiometrically in a 1:5 (w/v) soil:water suspension using a pH meter (Orion 3 star, Thermo Scientific, NH, USA). The pH data were used to draw a pH buffer curve to obtain the lime requirement values for the desired target pH.

2.3. Batch incubation experiments

In order to investigate the liming effect on the pH and P fractions on deforestation soil, 100 g of soil sample was transferred into 250 mL plastic bottles, and a total of 45 bottles were prepared for three treatments (control and adjustments to pH 6 and 7) in triplicate with 5 destructive samplings. The soil samples were split into three treatments: a control and two lime-treated soils (adjusted to pH 6 and 7, respectively), and each treatment group was pre-incubated at 25±2 ºC in the dark. The deforestation soil was limed to the target pH by adding laboratory grade, powdered $CaCO_3$. The soil water content was adjusted to 0.31 $m^3\ m^{-3}$ (field capacity). After pre-incubation, two lime-treated soils were mixed with either 0.1 g $CaCO_3$ (referred to as low $CaCO_3$ or LCC) or 0.4 g $CaCO_3$ (referred to as high $CaCO_3$ or HCC), and these two terms represent adjustments to pH 6 and 7, respectively. The bottles were incubated at 25±2 ºC for 40 days. The soil water content was adjusted to maintain the field moisture capacity during the 40–day batch experiment by adding deionized water to the bottles as necessary to maintain their initial condition.

2.4. Sampling and analyses of soils

Triplicate soil samples for each treatment were prepared for analysis of the chemical properties at 0 (5 hours), 1, 10, 25, and 40 days of incubation. Soil samples were air-dried at room temperature, passed through a 2–mm sieve, mixed thoroughly, and analyzed for soil pH, total-P, organic-P, available-P, inorganic-P and exchangeable Ca^{2+}, Al^{3+} and Fe^{3+}. The soil pH was determined in a 1:5 (w/v) soil:water suspension using a pH meter (Orion 3 star, Thermo Scientific, USA). Total-P was determined by the ammonium paramolybdate-vanadate colorimetric method after a perchloric acid digestion (Kuo, 1996) using a UV-visible spectrophotometer (UV-1601,

Shimadzu, Japan). Organic P was determined by the ignition method (Saunders and Williams, 1955), and available P was determined by the Bray-1 procedure (Bray and Kurtz, 1945) using a UV-visible spectrophotometer.

The inorganic P was partitioned into three chemical fractions (Fraction-A, Fraction-B, and Fraction-C) according to Fractionation procedure (Chang and Jackson, 1957). Fraction-A denotes the NaOH extractable P fraction (a mixture of Al-P and occluded Fe- P) that is obtained from soil extracts with 1 M NH_4Cl + 0.5 M NH_4F + 0.1 M NaOH. Fraction-B denotes the citrate-dithionite-bicarbonate extractable P fraction (a mixture of non-occluded Fe- P and adsorbed-P) that is obtained by adding 0.3 M $Na_3C_6H_5O_7$ + 1 M $NaHCO_3$ to the residue and heating the suspension in a water bath at 85 ºC and then adding 1 g $Na_2S_2O_4$. Fraction-C is referred to as the H_2SO_4 extractable P fraction (Ca-P) that is separated by adding 0.25 M H_2SO_4 and shaking for 1 h. Saturated NaCl was used to wash the residue twice after each extraction step. The P concentrations in each fraction were determined by the ascorbic acid method (Murphy and Riley, 1962). The unaccounted-for P was calculated from the difference in total-P and the sum of inorganic P fractions. Exchangeable Ca was obtained by the 1 M ammonium acetate (pH 7) method (Sumner and Miler, 1996), and exchangeable Al and Fe were obtained by the 1 M potassium chloride, nitric acid method (Bertsch and Bloom, 1996) and DTPA-TEA method (Loeppert and Inskeep, 1996), respectively. These exchangeable cations were measured using an ICP-OES (inductively coupled plasma-optical emission spectrometer, ICPS-1000IV, Shimadzu, Japan).

To predict the time-course interconversion processes from the thermodynamic data and the presence of the crystalline P compounds available in the literature, the mineralogical composition of soil samples collected at a given time was analyzed using a Bruker AXS D8 ADVANCE with a DAVINCI design X-ray diffractometer

Table 1. Some relevant physicochemical properties of the soil.

Soil parameters	Values
Texture[a]	Loam
Bulk density (Mg m^{-3})	1.1
pH (soil:water = 1:5[b])	5.4
Organic carbon (g kg^{-1})	44.5
Total-P (g kg^{-1})	0.26
Available P (mg kg^{-1})	0.78
CEC (cmol kg^{-1})	20.1

[a]USDA classification scheme

[b]Soil-to-suspension ratio of 1:5

(Cu Kα radiation with a λ of 15.418Å), equipped with a 3-circle goniometer. Samples were step scanned between 5 and 90º (2θ) with an increment of 0.02º and as counting time per step at room temperature. The mineralogical composition of a soil sample was identified by using the Bruker DIFFRAC plus software with the Joint Committee on Powder Diffraction Standards (JCPDS) PDF-1 and PDF-2 databases.

2.5. Statistical analysis

Data were analyzed using the General Linear Model procedures (SAS Institute, Version 9.3, Cary, NC, USA). The effects of two factors ($CaCO_3$ treatment and time) and interactions on pH, total-P, organic-P, available-P, inorganic P fractions, and exchangeable cations were evaluated. A two-way analysis of variance (ANOVA) for a completely randomized design with three replications was performed to test for significant differences among the treatment means within a factor and for interactions between factors. The least significant difference (LSD) test at a confidence level of $p < 0.05$ was used to separate means. The calculated p values for the two main factors and the interaction were shown in Table 2.

3. Results

3.1. Effect of liming on P measures and exchangeable cations

The soil pH initially increased up to 6.9 for the HCC treatments and 6.3 for the LCC treatments as compared with control soils (pH of 5.5), and then quickly decreased to 6.7 for the former and 6.0 for the latter within 1 day of incubation (Figure 1). Total-P and organic-P of incubated soils did not change significantly in control and $CaCO_3$-treated soils (Figures 2A and 2B). The concentration of available-P was lower in $CaCO_3$-treated soils than in control soils (Figure 2C), showing a negative relationship between liming and available-P in this study. However, the levels of available-P did not differ between the $CaCO_3$-treated soils.

During incubation, the addition of $CaCO_3$ caused an early increase in the levels of exchangeable Ca^{2+}, Al^{3+} and Fe^{3+} ion species, and the increase was greater at higher rate of $CaCO_3$ (Figure 3). Unlike the temporal variation patterns of Al^{3+} ions (Figure 3B), the initial increase in the concentrations of exchangeable Fe^{3+} ions decreased within 10 days of incubation and thereafter remained virtually unchanged (Figure 3C).

3.2. Effect of liming on inorganic P speciation

The levels of Fraction-A in $CaCO_3$-treated soils peaked at 10 days of incubation, and then decreased below control levels at 40 days (Figure 4A). The initial sharp increase in Fraction-B for $CaCO_3$-treated soils within a day of incubation abruptly decreased at 10 days of incubation, and gradually increased thereafter (Figure 4B). During incubation, the levels of Fraction-C were lower in $CaCO_3$-treated soils than in control soils (Figure 4C).

The mass-balance distribution of the unaccounted-for P, which was not recovered by the sequential P fractionation procedure adopted herein, was calculated by subtracting the sum of inorganic-P fractions from the initial amount of total-P at each sampling time (Figure 5). Compared with control soils, the addition of $CaCO_3$ decreased the portions of the unaccounted-for P during the early incubation (10 days of incubation) as consequences of the initial increase in Fraction-B and the subsequent increase in Fraction-A. At the end of incubation, however, the unaccounted -for P in $CaCO_3$-treated soils increased as a result of the concomitant decreases in Fraction-A and -C, although the portions of raction-B slightly increased. The addition of $CaCO_3$ also decreased the portions of available-P (Figures 2C and 5), but did not affect those of organic-P.

Compared with control soils, despite the relatively weak intensities of the peaks, liming obviously induced the conversion of Al-P compounds from crystalline to amorphous phase within a day of incubation and the subsequent formation of crystalline $AlPO_4$ (berlinite) and its conversion into another crystalline hydrated Al-P compounds (Figure 6).

Figure 1. Temporal variations in the soil pH during batch incubation. The error bars indicate ± standard deviation.

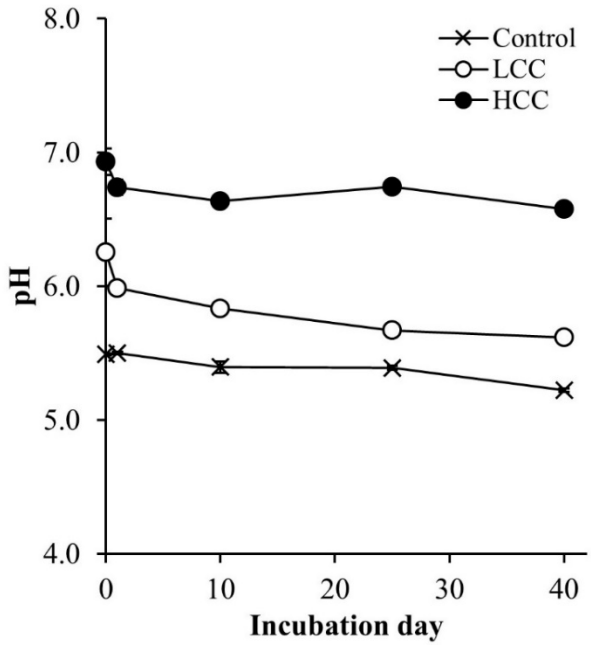

8.0
7.0
6.0
5.0
4.0
pH
0
10
20
30
40
Incubation day
Control
LCC
HCC

Figure 2. Temporal variations in the concentrations of (A) Total-P, (B) Organic-P and (C) Available-P during batch incubation. The error bars indicate ± standard deviation.

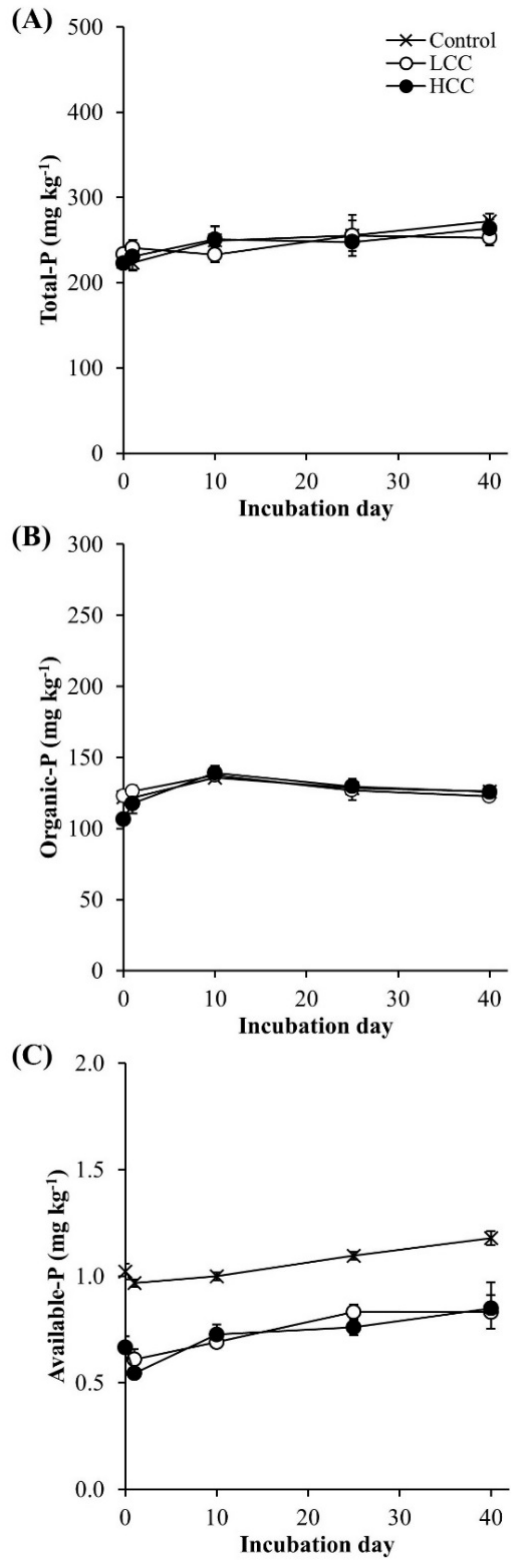

(A)
Control
LCC
HCC
Total-P (mg kg-1)
500
400
300
200
100
0
0
10
20
30
40
Incubation day
(B)
Organic-P (mg kg-1)
300
250
200
150
100
50
0
0
10
20
30
40
Incubation day
(C)
Available-P (mg kg-1)
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
Incubation day

Figure 3. Temporal variations in the concentrations of exchangeable (A) Ca^{2+}, (B) Al^{3+} and (C) Fe^{3+} during batch incubation. The error bars indicate ± standard deviation.

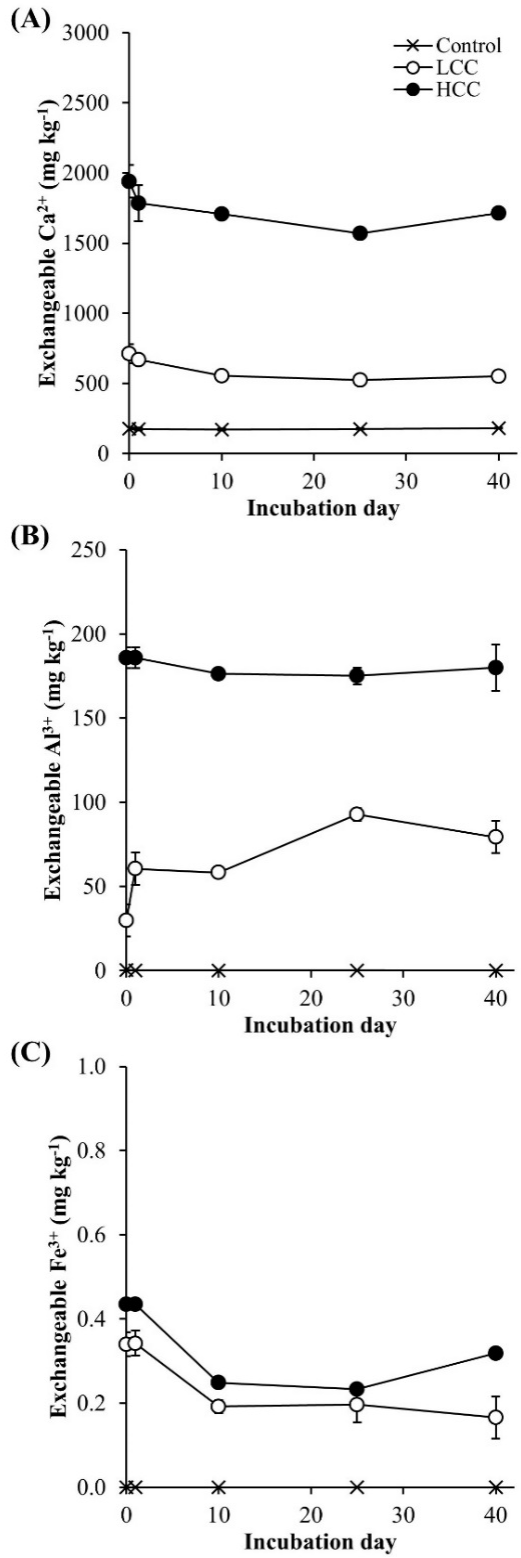

(A)
Control
LCC
HCC
Exchangeable Ca^{2+} (mg kg^{-1})
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
Incubation day
(B)
Exchangeable Al^{3+} (mg kg^{-1})
250
200
150
100
50
0
0
10
20
30
40
Incubation day
(C)
Exchangeable Fe^{3+} (mg kg^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
Incubation day

Figure 4. Temporal variations in the concentrations of inorganic P fractions during batch incubation: (A) Al-P + occluded Fe-P, (B) non-occluded Fe-P + adsorbed-P and (C) Ca-P. The error bars indicate ± standard deviation.

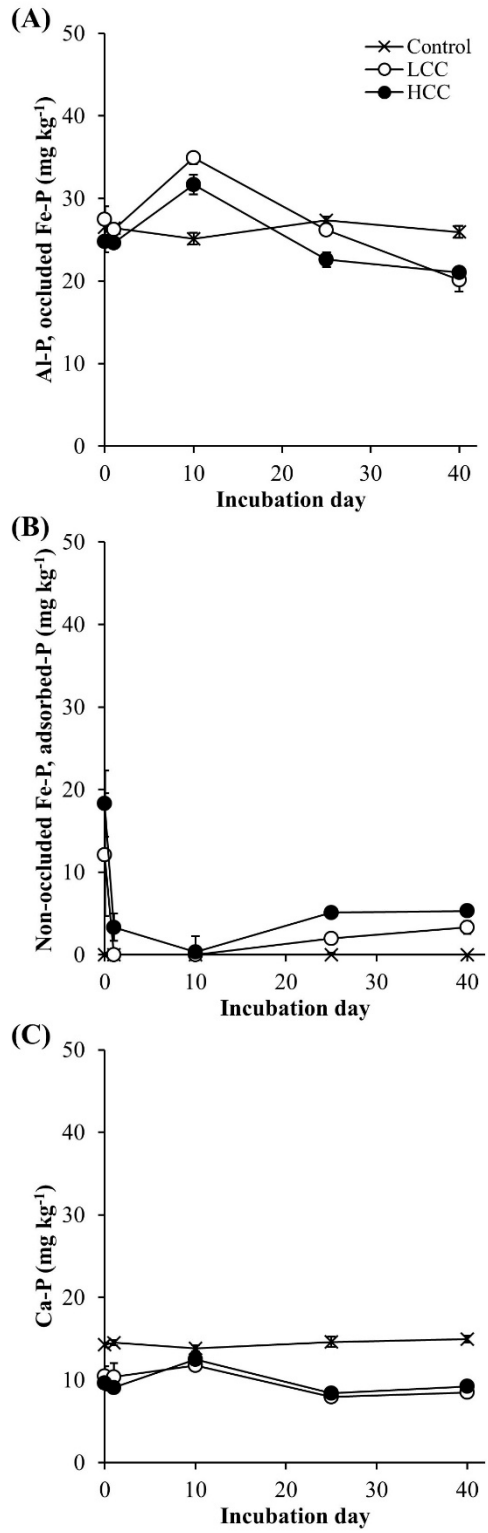

(A)
Control
LCC
HCC
Al-P, occluded Fe-P (mg kg-1)
Incubation day
(B)
Non-occluded Fe-P, adsorbed-P (mg kg-1)
Incubation day
(C)
Ca-P (mg kg-1)
Incubation day

Table 2. Results of two-way analysis of variance (ANOVA) showing the p values for pH, Total-P, Organic-P, Available-P, Inorganic-P and exchangeable cations as affected by $CaCO_3$ treatment, incubation time and their interaction.

Factor	pH	Total -P	Organic -P	Available -P	Inorganic-P			Exchangeable cations		
					Fraction-A[a]	Fraction-B[b]	Fraction-C[c]	Ca^{2+}	Al^{3+}	Fe^{3+}
$CaCO_3$	***	n.s.	n.s.	***	**	***	***	***	***	***
Time	***	**	***	***	***	***	**	**	*	*
$CaCO_3$ × Time	n.s.[d]	n.s.	n.s.	n.s.	***	*	*	n.s.	***	n.s.

[a]Al-P + occluded Fe-P

[b]Non-occluded Fe-P + adsorbed-P

[c]Ca-P of Inorganic P fractions

***: significant at the $p < 0.001$ level; **: significant at the $p < 0.01$ level; *: significant at the $p < 0.05$ level; n.s.: not significant.

4. Discussion

4.1. Effect of liming on P measures and exchangeable cations

Compared with control soils, the pH initially increased in $CaCO_3$-treated soils, and then quickly decreased within 1 day of incubation (Figure 1). In general, the extent of the increase in soil pH due to liming varies with soil buffering capacity, exchangeable ions such as Al^{3+} and Fe^{3+}, and the equilibrium reactions among the inorganic P fractions (Hsu, 1964). Chaplain et al. (2011) ascribed the subsequent decrease in soil pH after liming to the protonation of variable charges and retention of positively charged hydroxyl-Al polymers. However, Ro and Cho (2000) observed that the formation of solid phosphate precipitates resulted in a lowering of soil pH due to H^+ production in both acidic soil systems.

During incubation, the levels of organic-P did not change in control and both $CaCO_3$-treated soils. Several factors such as microbial activity, temperature, humidity, aeration, soil pH, and plant species are known to affect mineralization of organic-P and immobilization of inorganic-P. Mckenzie et al. (1992) found that an increase in microbial activity increased mineralization of organic-P. However, the relative contribution of these factors to the balance between inorganic- and organic-P forms remains unclear, since the mechanisms associated with dynamics of organic-P are difficult to determine due to the limitations in the available analytical procedures and the complexity of mineralization-immobilization turnover (Curtin and Syers, 2001).

In this study, available-P was inversely related to liming (Figure 2C), and this inverse relationship was not consistent with the results of most previous studies. In general, available-P increased due to the application of lime (Fageria, 1989). However, Pailles and Moody (1995) demonstrated a strong negative effect of $CaCO_3$

Figure 5. Time-course mass-balance distribution patterns of organic P, available P, Al-bound P + occluded Fe-bound P, non-occluded Fe-bound P + adsorbed-P, Ca-bound P and unaccounted-for P fractions in soils as affected by the addition of $CaCO_3$.

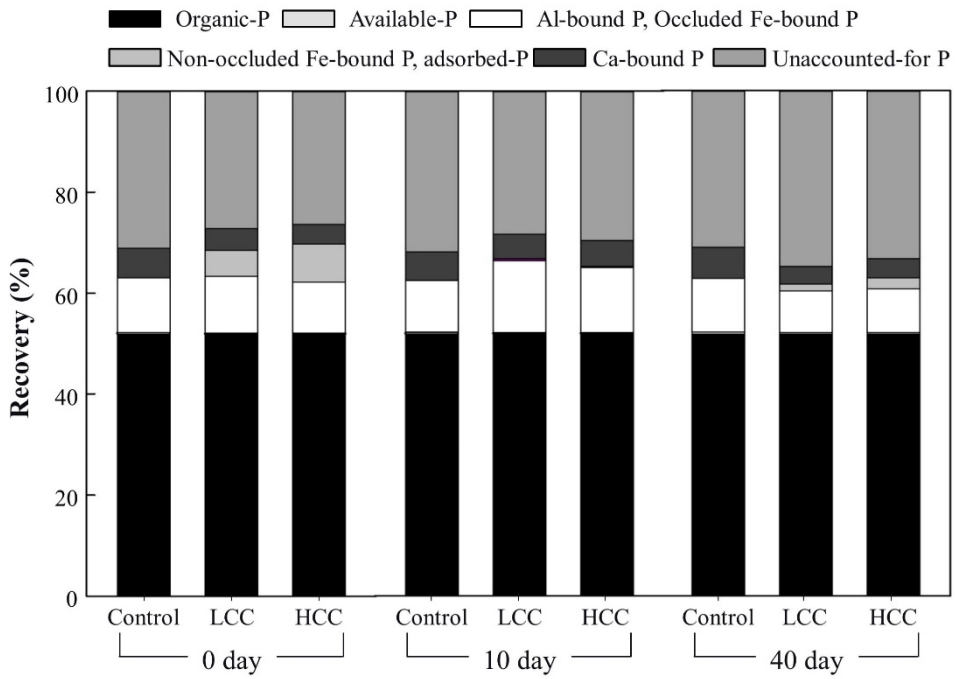
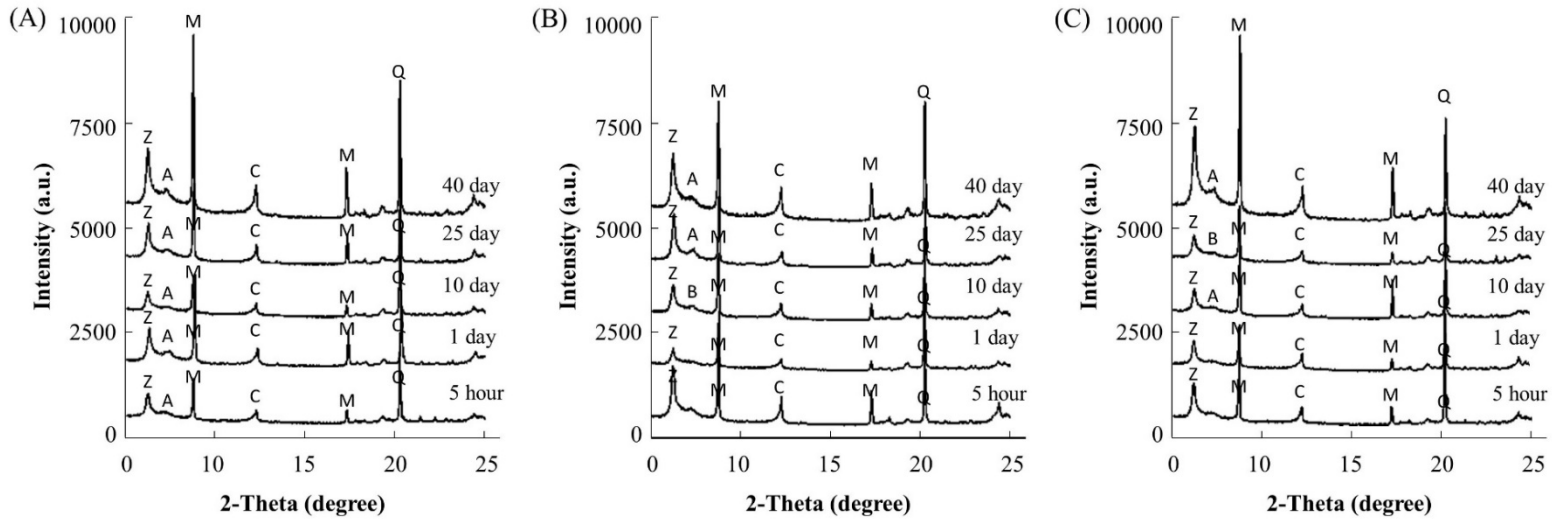

Organic-P
Available-P
Al-bound P, Occluded Fe-bound P
Non-occluded Fe-bound P, adsorbed-P
Ca-bound P
Unaccounted-for P
Recovery (%)
100
80
60
40
20
0
Control
LCC
HCC
Control
LCC
HCC
Control
LCC
HCC
0 day
10 day
40 day

Figure 6. Temporal variations in X-ray diffraction patterns of (A) Control, (B) LCC and (C) HCC soils during batch incubation with spectral reference of the minerals identified. Different codes indicate the minerals from the JCPDS PDF database: (Z) Zeolite X PDF 01-089-8231, (A) AlMePO-α PDF 00-052-1383, (B) Berlinite PDF 00-044-0044, (M) Muscovite PDF 00-007-0042, (C) Clinochlore PDF 00-060-0323 and (Q) Quartz PDF 00-046-1045.


(A)
(B)
(C)
Intensity (a.u.)
2-Theta (degree)
40 day
25 day
10 day
1 day
5 hour
Z
A
B
C
M
Q

content on available-P in estuarine and marine sediments. In contrast, Chang and Jackson (1958) observed that the application of lime increased the level of available-P in high phosphate soils but not in low phosphate soils. Hence, the presence of a causal relationship between Ca^{2+} content and available-P in the soil remains unclear. Several investigations reported that the minimum solubility of P was attained in the pH range between 5.5 and 6.5 due to the interaction with exchangeable cations (Haynes, 1982).

During incubation, the addition of $CaCO_3$ caused an early increase in the levels of exchangeable Ca^{2+}, Al^{3+} and Fe^{3+} ion species (Figure 3) due to dissolution of $CaCO_3$ and the subsequent displacement of Al^{3+} and Fe^{3+} by exchangeable Ca^{2+} (Higgins et al., 2012), thus leading to a significant decrease in the levels of available-P (Figure 2C), due presumably to increased phosphate precipitation and/or adsorption reactions (Brady and Weil, 2010). Unlike the temporal variation patterns of Al^{3+} ions (Figure 3B), the initial increase in the concentrations of exchangeable Fe^{3+} ions decreased within 10 days of incubation and thereafter remained virtually unchanged (Figure 3C), indicating a probable formation of Fe-containing compounds.

4.2. Effect of liming on inorganic P speciation and mass-balance

The levels of Fraction-A (Al- and occluded Fe-P) in both $CaCO_3$-treated soils peaked at 10 days of incubation, and then decreased finally below the control levels (Figure 4A). The initial increase in the levels of Fraction-A during the first 10 days of incubation could be explained in two ways. One is the direct formation of the Al-P compounds as a result of a marked increase in exchangeable Al^{3+} due to displacement and the conversion of hardly extractable Al-P compounds, such as berlinites and variscites, into more extractable P forms due to an increase in the solubility with

increasing soil pH (Lindsay, 1979). The other explanation is the direct formation of the occluded Fe-P compounds, which was evidenced by the concurrent decrease in the concentration of exchangeable Fe^{3+} (Figure 3C). For the decrease in the levels of Fraction-A in the latter part of incubation, however, I could not provide direct evidence and logical explanation. A possible explanation might be the conversion into hardly soluble Al-P compounds as indicated by the equilibrium constants under neutral or alkaline conditions (Lindsay, 1979) or into the non-occluded Fe-P compounds as shown in Figure 4B.

The initial increase in the concentrations of Fraction-B (non-occluded Fe- P and adsorbed-P) for $CaCO_3$-treated soils within a day of incubation (Figure 4B) indicated an early formation of the non-occluded Fe-P compounds by precipitation with displaced Fe^{3+} ions (Figure 3C). However, I could not eliminate the possibility of the conversion of the occluded Fe-P forms into more soluble forms due to an increase in soil pH (Lindsay, 1979), since the levels of Fraction A (Figure 4A) slightly decreased during the same period. In the latter stage of incubation, the levels of Fraction-B slightly increased (Figure 4B) while those of exchangeable Fe^{3+} remained virtually unchanged (Figure 3C), and this phenomenon indicated the possibility of the conversion of the occluded Fe-P forms into the non-occluded forms, as corroborated by the concurrent decrease in the levels of Fraction-A (Figure 4A).

My results obtained for Fraction-C (Ca-P) did not follow the widely-held belief that the level of Ca-P increases with liming. During incubation, the levels of Fraction-C were lower in $CaCO_3$-treated soils than in control soils (Figure 4C), and this could be ascribed presumably to the conversion into more stable Ca-P compounds (not extractable with the extraction scheme herein) as a result of extensive interactions of phosphates with more exchangeable Ca^{2+} ions. The initial decrease in the levels of Ca-P compounds with the concurrent decrease in those of exchangeable Ca^{2+} (Figure 3A) provided experimental support for the conversion

into more stable and less soluble Ca-P compounds. In general, the solubility of Ca-P compounds in the soil decreases as the pH increases (Lindsay, 1979). It is well-known that Ca-P compounds, such as mono-calcium phosphates and di-calcium phosphates, are easily converted into less soluble P compounds in soils at neutral or alkaline pH or Ca^{2+}-rich conditions (Tan, 2000). However, Pan and Darvell (2009) suggested that complete reappraisal of the Ca-P system should be needed to solve the confusion arising from my reliance on the reported solubility because detailed and accurate knowledge of the chemical equilibria between the solid phase and its solubility is lacking. Ro and Cho (2000) observed the initial formation of a very unstable amorphous Al-P [$Al(OH)HPO_4$] and its quick conversion to another amorphous or crystalline precipitate as a result of a series of acid-base titrations. Tunesi et al. (1999) demonstrated that P added to calcareous soils was considerably insolubilized within 5 weeks due to the formation of insoluble Ca-P precipitates, suggesting that P availability is reduced for soils having a high reservoir of exchangeable cations by forming insoluble P compounds. However, I cannot proceed further because relevant information on the solid phosphates and their thermodynamic data that are essential to postulating the course of chemical interconversion processes are mostly unavailable in the literature. In addition, the temporal variations in the mass-balance of each cation species (Al^{3+}, Ca^{2+} and Fe^{3+}) were not directly measured, I cannot fully explain the relative contribution of each cation species to the mass-balance distribution of each P species.

5. Conclusions

My approach challenged the widely-held notion that the portion of Ca-P (Fraction-C) and the availability of P would increase in the presence of increased exchangeable Ca^{2+} ions after liming. My results consistently revealed that a disturbance in chemical equilibria right after liming resulted in an unexpected decrease in Ca-P and P availability and a concomitant increase in exchangeable Al^{3+} and Fe^{3+} cation species, which contradicts current hypotheses on liming and P availability. Furthermore, within a day of incubation, a decrease in Ca-P was observed with a concomitant decrease in soil pH (an indicator of the formation of precipitates in the system) and exchangeable Ca^{2+} ions, indicating a probable formation of thermodynamically more stable solid phosphate compounds (i.e., $AlPO_4$) and their interconversions, as identified in the X-ray diffractograms. However, investigations about the quantification of the solubility isotherms of various solid phosphate compounds using acid-base titrations and the effect of soil types and soil fertility with differing organic matter content on the mass-balance distribution of chemical P species need further study with the aim of confirming my results.

CONCLUSIONS

The purpose of this study is to interpret the forest soil C dynamics and P speciation effect of nitrogen (N) deposition and deforestation. To do so, I hypothesized four research: 1) N deposition to the surface of the forest floor would stimulate the decomposition of litter and SOC pools and affect the mixing of new C substrates released from decomposition into preexisting old SOC pools down the profile, thus causing a difference in the response to N deposition between two oak and pine forest soils, 2) N addition on the soil of two different forest floors (deciduous trees; Qa and coniferous trees; Pk) would result in a different size of soil aggregates through different SOC decomposition patterns, 3) N addition on the soil of two different forest floors (deciduous trees; Qa and coniferous trees; Pk) would result in a different soil aggregate-size through different humic substances and soil organic functional group patterns, 4) the portion of Ca-P (Fraction-C) and the availability of P would increase in the presence of increased exchangeable Ca^{2+} ions after liming.

The conclusions from each study are as follows.

First, I revealed that an increase in total-C and N contents due to N deposition was greater under coniferous forest stands than under deciduous forest stands as a result of greater mixing of new C substrates into the soil profile in this temperate forest. Compared with their respective control soils, the lowering of $\delta^{13}C$ with increasing total-C contents in the surface soil layers (0–20 cm) for both forest soils after N application well reflected the relative contribution of the production of fresh C substrates from litter decomposition and the subsequent physical mixing into old SOC pools down the soil profile. In addition, I found that the incorporation of new C substrates into old SOC pools was greater under coniferous pine tree stands than under deciduous oak tree stands at least in this temperate region. Particularly, an increasing pattern of $\delta^{13}C$ of the soil to a depth of 20 cm indicated deeper penetration

of new C substrates into the soil profile as a result of ^{13}C isotope mixing with old C pools, and this phenomenon was well evidenced by an increase in ^{15}N recovery in this upper region with time.

Second, I revealed that the N treatment affects the decomposition of organic matter and soil aggregate distribution, and that these differences depend on the tree species. Interestingly, different patterns of total-C content between the Qa and Pk soils were observed as a result of the differences in the decomposition characteristics associated with their chemical compositions. Nitrogen treatment decreased the miner N content and DOC content in the soil regardless of tree species. In particular, unlike my hypothesis that the changes in total-C content for the 2000–1000 μm or 1000–250 μm aggregates would be larger, the total-C content mostly changed in aggregates for 250–53 μm and <53 μm. At the end of incubation, N treatment in both of tree species soil decreased aggregate for 2000–1000 μm and the others size of aggregates for 1000–250 μm, 250–53 μm, and <53 μm were increased.

Third, I revealed that the N treatment affects the humic substances and soil organic functional groups and that these differences depend on the tree species. The different patterns of soil organic functional group between the Qa and Pk soils were observed as a result of the differences in the decomposition characteristics associated with their chemical compositions. In particular, unlike my hypothesis that the changes in humic substances for the 2000–1000 μm or 1000–250 μm aggregate-sizes would be larger, the humic substances mostly changed in aggregate-sizes for 250–53 μm and <53 μm, regardless of tree species. However, the soil organic functional groups were changed more significant at 2000–1000, 250–53 and <53 μm aggregate-sizes, and showed different trends for humin and humic acid fractions.

Fourth, I revealed that liming application affects the unexpected opposite data for Al-, Fe- and Ca-P compounds at least in the early stage of equilibrium disturbances. The disturbance in chemical equilibria right after liming resulted in an

unexpected decrease in Ca-P and P availability and a concomitant increase in exchangeable Al^{3+} and Fe^{3+} cation species, which contradicts current hypotheses on liming and P availability. Furthermore, within a day of incubation, a decrease in Ca-P was observed with a concomitant decrease in soil pH (an indicator of the formation of precipitates in the system) and exchangeable Ca^{2+} ions, indicating a probable formation of thermodynamically more stable solid phosphate compounds (i.e., $AlPO_4$) and their interconversions, as identified in the X-ray diffractograms.

However, I could not fully explain that 1,2,3) how differences in nutrient composition between two contrasting tree species affect litter decomposition and the formation of SOC pools, since no direct measurements were made on the kinetics of litter decomposition and microbial activity in both forest floor soils, 4) temporal variations in the mass-balance of each cation species were not directly measured, I cannot fully explain the relative contribution of each cation species to the mass-balance distribution of each P species. Therefore, the kinetics of litter decomposition of leaf-litter types, soil microbial activity, separation of the mixing of new substrates from the decomposition of old C substrates, and the formation and stabilization of SOC pools; and the quantification of the solubility isotherms of various solid phosphate compounds using acid-base titrations and the effect of soil types and soil fertility with differing organic matter content on the mass-balance distribution of chemical P species need further study with the aim of confirming my results.

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ABSTRACT IN KOREAN

산림 생태계는 식물종, 토양 양분 그리고 미생물 활성과 같은 다양한 유기물들의 순환에 영향을 받는다. 따라서 산림 생산성을 높이기 위해 적절한 영양 순환 시스템을 유지하는 것이 필수적이다. 최근 산림 환경은 산업화, 산림업 등 인간 활동의 영향으로 끊임없이 변화하고 있다. 현재 우리가 직면하고 있는 주요 산림 토양 환경의 문제는 대기중 질소 강하물, 과량 시비, 벌채, 개간 등으로 이와 같은 환경파괴 요인을 줄이기 위해 전 세계적으로 연구가 진행되고 있다. 따라서 본 연구의 목적은 질소의 강하 그리고 벌채같이 산림 토양 환경에 영향을 주는 인자들을 이용하여, 토양 내의 탄소와 질소 그리고 인에 대한 연구를 안정성 동위원소비의 변화, 화학종 분획, 토양 입단, 부식 물질 그리고 유기물 작용기를 이용하여 연구하고자 한다. 이러한 연구를 위해 총 4개의 가설을 구상하였으며, 이를 실험실 내 실험과 현장 실험으로 나누어 진행하였다.

먼저, 수종이 다른 두 산림 지역에서, 질소 강하에 의한 토양 탄소와 질소 동태를 연구하기 위해 ^{15}N으로 표지 된 요소를 사용한 실험을 3년 동안 수행하였다. 이 연구를 통해서 질소 처리가 산림 토양의 유기물 분해를 촉진시켜, 토양 내 유기물 함량을 증가시키는 것을 알 수 있었다. 또한 흥미롭게도, 침엽수보다 활엽수의 유기물의 분해에 더욱 영향을 준다는 일반적인 통념과 달리, 본 연구에서는 침엽수림에서 더 많은 토양 유기 탄소의 증가를 확인할 수 있었다. 또한 안정성 동위원소비를 이용하여, 새롭게 유입된 탄소와 이들의 동태를 시간과 토양 깊이 따라 구명할 수 있었다. 앞선 질소의 처리가 유기물의 분해를 촉진시켜 토양 내 탄소를 증가시키며, 이는 수종 별로 차이가 있다는 연구 결과를 기반으로 두 번째 연구를 진행하였다. 질소의 처리가 토양 입단의 분포 그리고 입단 별 서로 다른 유기탄소 분해 패턴을 가질 것이라는 가설을 기반으로 항온 배양 실험을 1년 동안 진행하였다. 이

연구를 통해 선행 연구와 마찬가지로 수종에 따른 토양 입단 별 토양 유기 탄소 분해 차이를 확인할 수 있었으며, 이러한 결과로 인하여 토양 입단의 분포가 변한 것을 확인할 수 있었다. 세 번째 연구는 두 번째 실험의 토양으로 진행하였으며, 유기 탄소의 분해가 수종과 입단 별로 차이가 있다면, 항온 배양 후 그들의 유기물의 부식을 확인할 수 있는 부식 물질과 토양 유기물 작용기에도 차이가 있을 것이라는 가설로 실험을 진행하였다. 이 연구를 통해 질소의 처리로 인하여 시간에 따른 부식 물질의 분포 변화를 확인할 수 있었다. 또한, 입단 별 토양 그리고 부식 물질의 유기 작용기의 변화를 확인 할 수 있었다. 마지막 연구는 산림 환경 파괴 중 벌채에 따른 토양 산도에 대한 연구를 수행하였다. 인은 토양 산도에 영향을 받아 다양한 인산염을 생성하는데, 본 연구는, 산림벌채로 인해 산도가 증가한 토양에 석회를 시용하여 토양 pH를 교정하였을 때, 석회와 토양 내 인의 상호작용과 인의 화학종 형태에 미치는 영향을 구명하였다. 흥미롭게도, 결과는 일반적인 통념과 다르게 초기 산도 증가 시 칼슘 인의 증가를 확인할 수 있었다. 이 연구 결과를 통해 석회 시용 초기 단계에서 토양 내 평형 교란으로 인한 인의 화학종의 변화를 확인할 수 있었다.이러한 4가지의 연구를 통해 질소의 투입, 산도 조절과 같은 외적 인자들로 인한 토양 내의 탄소와 질소 그리고 인의 영향을 제시하였다. 또한, 수종, 토양 입단, 부식 물질, 토양 유기 작용기 그리고 인의 화학종과 같은 토양 물리 화학적 특성을 조사하였으며, 이들의 기작을 해석할 수 있었다. 본 연구를 통해 산림 토양에서 질소 처리에 따른 탄소와 질소 그리고 산도 조절에 따른 인의 화학종 변화와 같은 토양 산림 토양 내 생태계의 반응 및 발생하는 현상들에 대한 정보를 제공할 수 있다고 판단된다.

주요어: 산림토양, 수종, 탄소, 질소, 입단, 유기작용기, 인의 화학종

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먼저 석사·박사 짧지 않은 시간 동안 연구 및 논문 작성에 많은 격려와 지도를 해주신 노희명 교수님께 깊이 감사드립니다. 부족한 저를 인내하시며 가르쳐주시고, 연구와 관련하여 좋은 기회들을 열어주셨습니다. 단순히 지식뿐 아니라 삶을 대하는 자세 그리고 앞으로 살아가는데 있어서 필요한 덕목들을 교수님을 통해 배우고 느낄 수 있었습니다. 또한 석사 박사 학위 기간 동안 많은 가르침을 주신 응용생명화학 전공 모든 교수님께 깊은 감사드립니다. 그리고 박사학위 논문심사를 맡아주신 김정한 교수님, 신찬석 교수님, 임종환 박사님, 윤석인 교수님께 감사드립니다.

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