Journal of
Integrated Field Science

Vol. 7

March. 2010

Field Science Center
Graduate School of Agricultural Science
Tohoku University

Preface to Symposium Papers on "Biological Interactions in Arable Land-Grassland-Forest Continuums and their Impact on the Ecosystem Functions", 7th International Symposium on Integrated Field Science

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Field Science Center of Tohoku University aims at developing sustainable bio-production system in integrated ecosystem from forest, grasslands, arable lands to coastal sea. Field Science Center is comprised by 5 research groups in collaboration with staffs belonging to Graduate School of Agricultural Sciences, Tohoku University. The research groups are referred as Research Cores, which are the Forest-Andisol Core, the Ruminant Production Core, the Rice Production Core, the Marine Bio-production Core and the Integrated Field Control Core. Each core has in turn organized the international symposium annually. In 2009, the Forest-Andisol Core in collaboration with Ecosystem Adaptability Global COE, Tohoku University, organized the international symposium on "Biological Interactions in an Arable land-Grassland-Forest Continuum and their impact on the Ecosystem Functions" as 7th International Workshop on Integrated Field Science.

We now face global and local environmental change due to human activities. It is urgently needed for us to understand how the environmental changes have been caused, how such changes affect our living, how we mitigate such changes and how we adapt ourselves to the changes. Most phenomena in global and local environmental changes are macroscopic. However, these macroscopic phenomena are often caused by microscopic organisms and by their interactions. It is now well recognized that interactions among organisms not only govern dynamics of populations and communities but also affect various phenomena in ecosystems.

From the above viewpoint, we organized the symposium to discuss how biological interactions affect ecosystem functions with emphasis on a continuum of different land-use from forest to arable- and grassland and arable lands. The symposium was held on Oct 10-12, 2009, at Multimedia Education and Research Complex, Kawauchi Campus of Tohoku University. Fifteen scientists, including from Canada, USA, New Zealand and Indonesia, were invited to oral session. Sixteen posters were also presented. The topics included plant pathogens, endophytic and mycorrhizal fungi, earthworm, tubifex and wood mouse. Also terrestrial carbon accumulation in terms of global carbon sequestration was discussed. Field excursion to Terrestrial Field Science Center in Naruko-Onsen, Osaki city, was conducted to look at soil profiles and some field experimental sites. It was an excellent opportunity to discuss the most progressive research topic among the participants. I believe the symposium has contributed to our understanding of significance of biological interactions in integrated field science.

Most of the invited speakers contribute their papers to this issue. On behalf of the organizing committe, I would like to express my sincere thanks to all contributors. My gratitude is extended to Prof. Li, who was invited to the symposium but could not come due to sudden sickness, kindly contributes his paper. All abstracts including poster presentation are also included in the last part of this issue.

Is the Janzen–Connell Hypothesis Valid in Temperate Forests?

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Received 5 November; accepted 1 February 2010

Abstract

Although degradation of biodiversity is a worldwide concern, relatively little evidence is available on the mechanisms of biodiversity. If sustainability of forest ecosystem is closely related to species diversity, recovery of species diversity is important for forest managers. Thus, it is important to understand the mechanisms of biodiversity in forest ecosystems. The Janzen–Connell hypothesis is one of the most important hypotheses explaining species diversity in tropical forests. To examine whether this hypothesis is also valid in temperate forests, we investigated the density, growth, mortality, and agents of mortality of seedlings, and the density, size, and age of saplings of *Prunus grayana* at three distances (0–3, 6–10, and 16–26 m) from conspecific adults in a temperate forest in Japan. The probability of mortality was highest at 0–3 m during the first 2 years of growth. Mortality mainly resulted from distance-dependent attack by two types of pathogen that caused damping-off epidemics and spot symptoms on leaves. Vertical and diameter growth was lowest at 0–3 m and highest at 16–26 m in both seedlings and saplings. The results suggest that seed dispersal enhances the probability of survival by distancing offspring from adults because natural enemies reduce recruitment near conspecific adults in a distance-dependent manner, freeing space for other plant species. Such phenomena affect species diversity within plant communities. The traits demonstrate that the Janzen–Connell mechanism also operates in a temperate forest in Japan. Knowledge of the mechanisms of species diversity and of ecosystem functioning will encourage forest mangers to create mixed hardwood–coniferous forests rather than monocultures.

Loss of species diversity in forest ecosystems

Humans are altering the composition of biological communities through a variety of activities that increase rates of species invasion and species extinctions from local to global scales. These changes in components of Earth's biodiversity lead to ethical and aesthetic concerns, but also have a strong potential to alter ecosystem properties and the goods and services that biodiversity provides to humanity (Hopper et al. 2005).

Increasing evidence is available on the effects of biodiversity on ecosystem properties such as productivity, carbon storage, hydrology, and nutrient cycling (e.g., Tilman et al. 1996; Hopper et al. 2005). Although degradation of biodiversity is a worldwide concern at both global and local scales, relatively little evidence is available on the mechanisms involved. If biodiversity is a consequence of biotic interactions, linkage between biodiversity and ecosystem functioning (services for humans) is inevitable.

In Japan, a large area of the natural virgin forest has been clear-cut and converted to monoculture, such as in Japanese cedar plantations established after World War II. Now, conifer plantations occupy one-quarter of the total area of Japan (10 million ha; Forestry Ag., 2006). Even-aged conifer forests generally have low value for wildlife, are susceptible to pests and strong winds, and may create a more acid soil. If sustainability of forest ecosystems is closely related to species diversity, recovery of species diversity is an important management goal for forest managers, and it is essential that they understand the mechanisms of forest ecosystems.

Hypotheses explaining species diversity

A substantial number of hypotheses have been presented to explain species diversity in forest communities (Fig. 1). In temperate forests, it was thought that species diversity is mainly created by abiotic factors such as heterogeneity of light, water, and nutrients, which are closely related to topography and gap creation. In tropical forests, increasing evidence has demonstrated the effects of biotic agents such as pathogens and herbivores, each of which has an important role in maintaining species diversity. One of the most important hypothesis regarding tropical forests is the Janzen–Connell hypothesis.

Janzen (1970) and Connell (1971) proposed that spatial patterns of juvenile mortality caused by natural enemies (i.e., pathogens and herbivores) could be a key factor in maintaining high tree diversity. They predicted that seed dispersal enhances the probability of survival by distancing offspring from adults because host-specific natural enemies reduce recruitment near conspecific adults in a density-dependent and/or distance-dependent manner, freeing space for other plant species (Fig. 2). Such phenomena affect species diversity within plant communities. In a large number of tree populations, density- and distance-dependent analyses of microbial pathogens and invertebrate or vertebrate herbivores support the Janzen–Connell hypothesis for tropical forests (e.g., Augspurger 1983; Gilbert 2005; Bell et al. 2006). Recent community-level studies have also shown that the density- or distance-dependent mortality of progeny plays an important role in the maintenance of plant

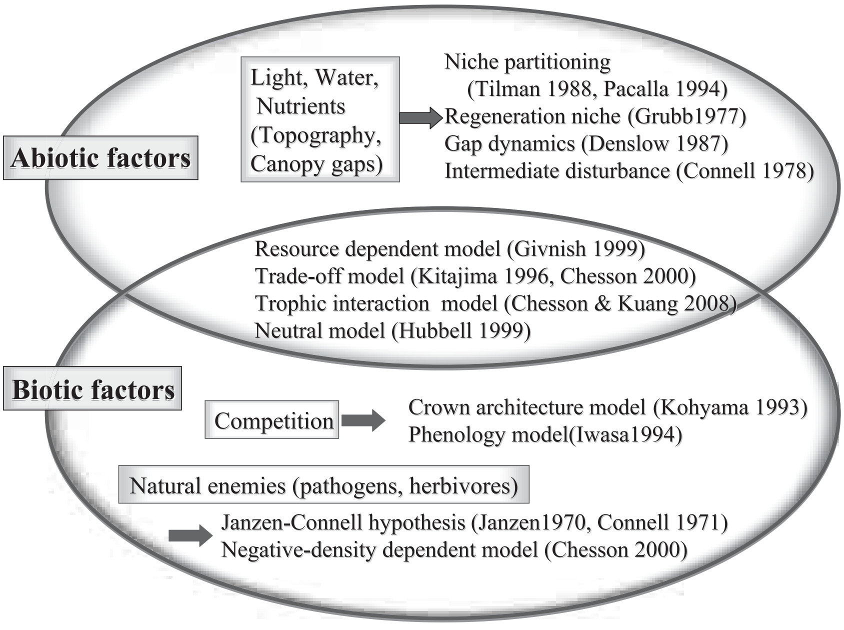

Fig. 1. Hypotheses explaining species diversity in forest communities.

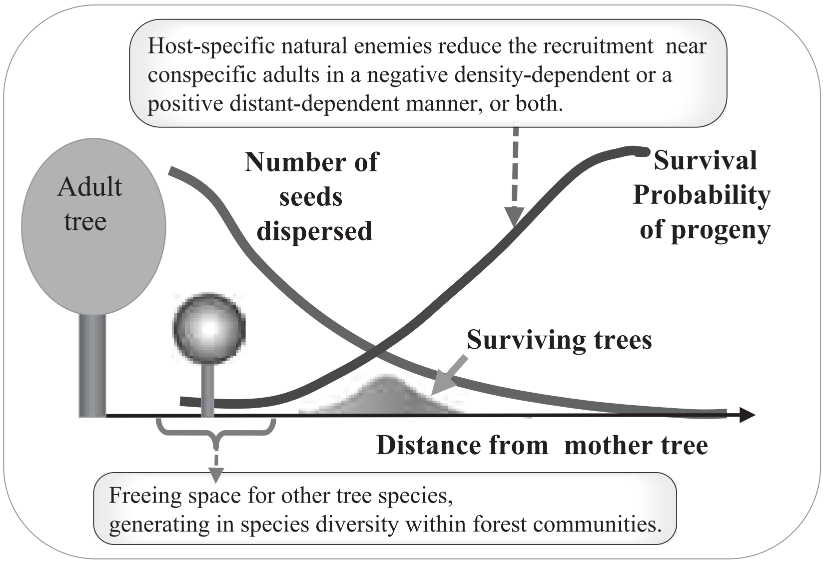

Fig. 2. Janzen–Connell hypothesis

alpha diversity in tropical moist forests (Wills et al. 1997; Harms et al. 2000; reviewed by Wright 2002), but several previous studies found density- or distance-dependent trends in half or fewer of the species examined (Connell et al. 1984; Condit et al. 1992).

Fewer studies have tested the Janzen–Connell hypothesis in temperate forests than in tropical forests. Although several studies in temperate forests have reported the density and distance dependence of tree seed and seedling mortality (e.g., Lambers and Clark 2003), little evidence is available regarding the causes of mortality and the consequent spatial patterns of progeny (but see Packer and Clay 2000; Masaki and Nakashizuka 2002; Tomita et al. 2002; Kotanen 2007). Recently, Seiwa et al. (2008) clearly demonstrated that the Janzen–Connell hypothesis is valid for a deciduous broadleaf tree, *Prunus grayana*, in a temperate forest of northern Japan. In this review, I explain how the cause of seedling mortality changes in type and intensity with distance from the parent and how the relationship between age and juvenile size changes with distance from the parent in a temperate hardwood forest.

Distant- and density-dependent mortality

Although seedling density was highest beneath (0 –3 m) and lowest far (16–26 m) from an adult, the mortality of *Prunus grayana* seedlings decreased with increasing distance from conspecific adults. However, the percentage mortality was higher at 0 –3 m than at 6–10 m or 16–26 m at the end of both growing seasons examined. These results indicate the distance-dependent mortality of seedlings of *Prunus grayana* in a temperate forest.

Causes of mortality

Distance-dependent mortality was caused mainly by pathogen activity on seedlings beneath adult conspecifics (Fig. 3). The percentage mortality from the pathogens that cause damping-off and from *Phaeoisariopsis pruni-grayanae* was greater at 0–3 m than at 6–10 m and 16–26 m, whereas few effects were observed for vertebrate herbivores, invertebrate herbivores, and physical damage (Fig. 3, 4). In *P. grayana*, damping-off accounted for a much greater proportion of seedling mortality than did other factors and was higher close to parent trees. Bell et al. (2006) found that seedling death of a tropical tree was not directly affected by the density itself, but by the density-mediated infection of damping-off pathogens. They conducted a manipulation experiment in which the effect of density was clearly separated

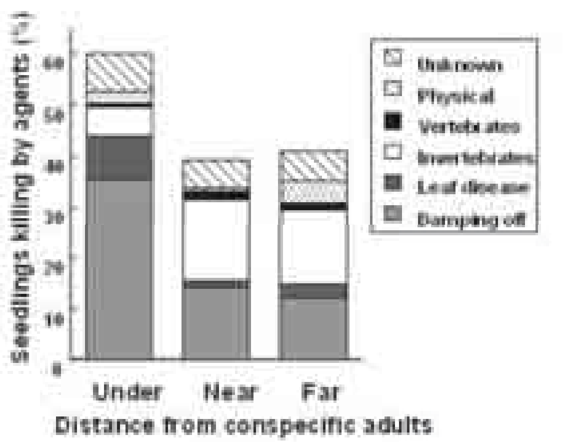

Fig. 3. Seedlings killed by individual agents of mortality during the first growing season: differences among distances from conspecific adults (under, 0–3 m; near, 6–10 m; far, 16–26 m). (Seiwa et al. 2008)

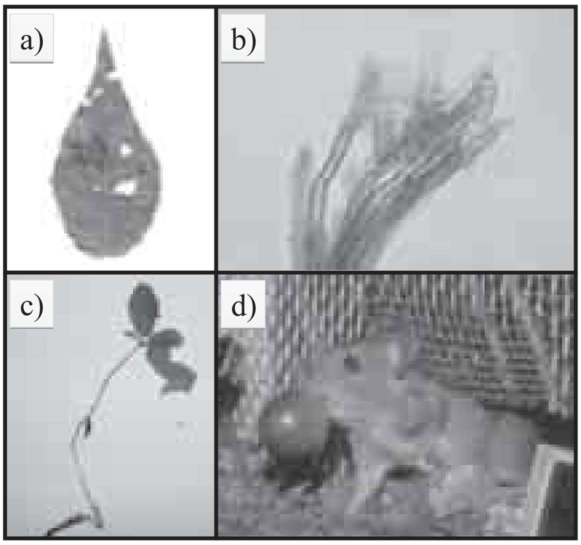

Fig. 4. Kiling agents for the current-year seedlings of *Prunus grayana*. Spot symptoms on the leaves caused by the leaf pathogen, *Phaeoisariopsis pruni-grayanae* Sawada, which infected many more seedlings of *P. grayana* than seedlings of the two other tree species tested in inoculation experiments (a; Seiwa et al. 2008). Conidia and conidiophores characteristic of *Phaeoisariopsis* (b). Damping off epidemics caused by soilborne pathogens such as *Colletotrichum*, *Phoma*, *Cladosporium*, and *Fusarium* (c). Vertebrate herbivore, *Apodemus speciosus*.

from that of pathogens (Bell et al. 2006). Therefore, heavy seed rain may not overwhelm the effects of pathogens, but rather reinforce them. We also found that the leaf pathogen *P. pruni-grayanae* attacked *P. grayana* seedlings in a distance-dependent manner, although the effects were much lower than were those of damping-off pathogens.

In temperate forests, similar traits of distance-dependent morality by pathogens were observed for *Prunus serotina* in North America (Packer and Clay 2000) and *P. grayana* in Japan. Furthermore, in a seed-sowing experiment of eight species co-occurring within a forest community, we found that six species (i.e., *Castanea crenata*, *Cornus controversa*, *Fraxinus lanuginosa*, *Magnolia obovata*, *P. grayana*, *Fagus crenata*) showed distance- and/or density-dependent mortality caused by pathogenic fungi (Yamazaki et al. 2009). These traits, together with evidence of distance-dependent mortality by pathogens for *C. controversa* (Masaki and Nakashizuka 2002) and *F. crenata* (Tomita et al. 2002), suggest that the effects are not specific to the genus *Prunus*, but are more general for tree species in temperate as well as tropical forests.

Reduction of vertical growth beneath the adults

The height of saplings increased with age, and this increase was most at 16–26 m from the adult and slowest at 0–3 m (Fig. 5). What is the most important cause of the reduction of vertical growth beneath conspecific adults? Little difference among the three distances was observed for either relative PPFD or soil water potential, suggesting only slight influence of abiotic factors on sapling growth. Although pathogens that cause damping-off epidemics can affect plants of any age, damage caused by their infection is usually highest during the early stages of seedling growth and decreases with increasing maturity (Sahashi et al. 1995; Martin and Loper 1999). Furthermore, infection of large and old plants is often limited to the fine roots, suggesting that damping-off pathogens become less important growth-inhibiting agents as plants increase in size. In contrast, the pathogen *P. pruni-grayanae* strongly reduces plant performance (growth and survival), irrespective of plant size, because it usually causes early shedding of leaves with necrotic spots. We observed this pathogen over a large area of individual leaves for seedlings, saplings, and adults (Miwa and Seiwa personal observation; see also Kobayashi et al. 1992). Furthermore, a higher number of diseased leaves fall onto conspecific juveniles, particularly in the vicinity of adults. This suggests that continued distance-dependent reduced sapling performance (mortality and growth) could result from pathogen activity, especially that of this leaf pathogen.

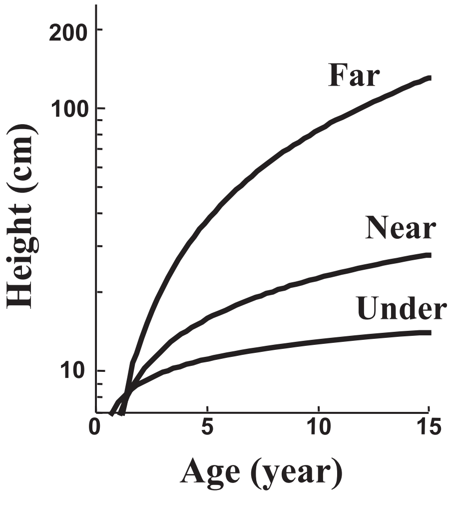

Fig. 5. Relationships between age and height of saplings at three distances from conspecific adults.

Consequences of distance- or density-dependent seedling mortality

An exploration of the consequences of distance- or density-dependent seedling mortality is also needed. Even though density-dependent seedling mortality is observed, the highest density of survivors occurs in the vicinity of adults when most seeds land close to these adults (Nathan and Casagrandi 2004). The full extent of recruitment reduction near fruiting conspecifics has been systematically underestimated in short-term studies focused on the very early stages of regeneration because distance- and density-dependent reductions in performance accumulate as juveniles grow (Wright 2002). Therefore, the spatial distribution of saplings is more important than the spatial distribution of seedlings for determining species diversity within a community.

In *P. grayana*, the smallest and youngest saplings were distributed at 0–3 m, whereas a higher number of larger and older saplings were observed at 16–26 m. *P. grayana* saplings can survive gap-forming events, grow more rapidly in gaps than below the canopy, and reach the canopy in a single gap cycle

(Hara et al. 1991). These characteristics suggest that the larger saplings at 16–26 m have a higher probability of recruitment to the canopy than do smaller saplings at 0–3 m or 6–10 m because larger plants usually out-compete smaller neighbors for resources (i.e., light) under crowded conditions after canopy gap formation. In contrast, competitor-free space near adults of *P. grayana* should promote recruitment of other tree species. As a result, the adults are distributed randomly in the studied forest (Fig. 6).

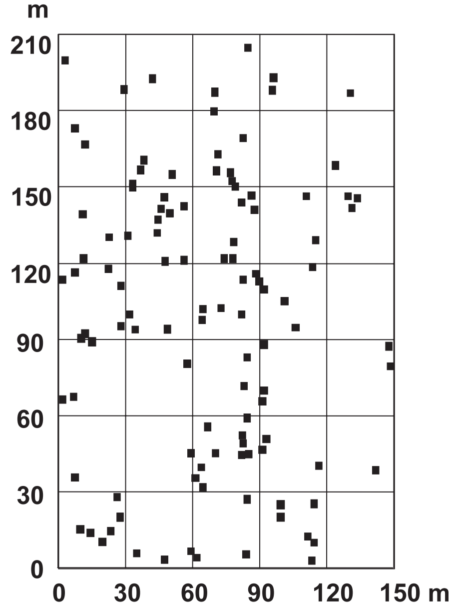

Fig. 6. Spatial distribution pattern of the adults of *Prunus grayana*.

Implications for forest management

If the Janzen–Connell hypothesis is valid in temperate forests, replacement of individual tree species would occur at a fine scale, generating species diversity in the forest communities even in temperate forests. Knowledge of the mechanisms of species diversity and of ecosystem functioning would encourage forest mangers to create mixed hardwood–coniferous forests instead of monocultures because diversity becomes increasingly important as a management goal, from both economic and ecological perspectives.

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JIFS, 7 : 9 − 13 (2010) Symposium Paper

Carbon Allocation and Growth–Survival Trade-Offs in Temperate Tree Species

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Keywords: competition, defense, shade tolerance, species diversity, storage

Received 12 January 2010; accepted 1 February 2010

Abstract

The growth–survival trade-off is an important mechanism in maintaining species diversity in forest communities. The model hypothesizes that a negative correlation between growth rates in gaps and survival rates in the forest understory across tree species allows their coexistence in spatially heterogeneous light environments. Seedling survival in the shaded understory depends on the ability to defend against herbivores and pathogens, and/or ability to recover from biotic damage. However, relatively few studies have demonstrated carbon investment in defense and storage as a mechanism of the trade-off, and no studies have simultaneously examined carbon allocation to both defense and storage. We examined carbon allocation patterns to defense, storage, and growth in seedlings of two temperate species of differing shade tolerance. Carbon allocation to growth was higher for the shade-intolerant species compared to the shade-tolerant species, whereas carbon allocation to defense was greater for the shade-tolerant than shade-intolerant species. This contrasting carbon allocation pattern would result in a growth–survival trade-off. In addition, we found that the shade-tolerant species preferentially invested more carbon in defense than in storage, suggesting that optimal carbon allocation is inherent to individual tree species.

Trade-off model

Trade-off between growth and survival based on tree species specialization along resource gradients can contribute to species diversity in forest communities. This model proposes a trade-off between low-light survival and high-light rapid growth among co-occurring tree species in a forest (Fig.1). Rapid growth in gaps and low survival rates in the forest understory characterize light-demanding species, whereas high survival in the forest understory and slow growth rates in gaps characterize shade-tolerant species. In a heterogeneous environment, the trade-off has a role in equalizing overall recruitment success among tree species, thus generating species coexistence. In both tropical and temperate forests, evidence is increasing for the growth–survival trade-off (Kitajima 1994; Baraloto et al. 2005; Gilbert et al. 2006; Seiwa 2007).

Ability to persist in a shaded forest understory

The term "shade tolerance" is usually used to refer to the ability of plants to survive in low-light environments. It is thought that shade tolerance largely depends on physiological, morphological, and phenological mechanisms for light capture and utilization at low light intensity to maximize present and future

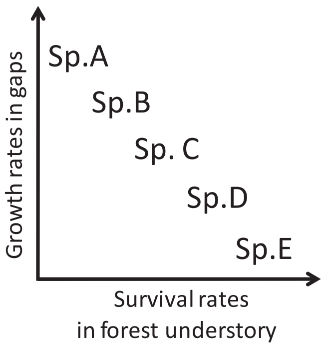

Fig. 1. Trade-off between growth rates in gaps and survival rates in the forest understory among tree species.

growth in the forest understory. These mechanisms include the capacity to maintain a positive carbon balance under low light flux, efficient use of sunflecks, optimal architecture favoring light interception, and phenological avoidance of shade stress (see references in Seiwa 1998). However, recent demographic studies clearly revealed that the most important cause of seedling mortality in the forest understory is attack by herbivores and pathogens (Seiwa 1998; Nakashizuka 2001), whereas seedling death directly caused by a negative carbon balance under low-light conditions is rare. It is likely that a lower ability to capture light under shaded conditions could lead to reduced investment of resources for defense against herbivores and pathogens, resulting in more severe attacks by natural enemies. Thus, increased ability to defend against natural enemies or ability to recover from biotic damage would enhance seedling survival in the forest understory. Previous studies found that shade-tolerant species usually invest more carbon in defense to protect against biomass loss from herbivores and pathogens (Coley 1987; Alverz-Clare and Kitajima 2007) and that shade-tolerant species invest more carbon in storage for recovery following tissue loss (Kobe et al. 1997; Myers and Kitajima 2007; Poorter and Kitajima 2007). Therefore, it seems that shade tolerance (i.e., ability to persist in the shaded understory) is largely determined by the ability to allocate as much carbon as possible to defense and storage in order to resist biotic stresses.

Ability to survive in gaps

In gaps, severe competition for light with neighboring plants usually occurs. Thus, to establish in gaps, carbon allocation to vertical growth is critical, particularly for light-demanding pioneer species (Myster 1993). Because of the limit to total carbon fixation, this can be achieved by preferentially investing carbon into new tissue at the expense of allocation to survival-enhancing traits such as defense and storage. These conflicting selective pressures predict that shade-tolerant species will lack competitive ability under high-light conditions, whereas light-demanding species are unable to persist for a long time in shaded conditions. Therefore, the trade-off between growth and survival is most likely caused by a trade-off between carbon allocation to growth and allocation to defense and storage (e.g., Kitajima 1994; Seiwa 2007; Kitajima and Poorter 2008; Fig.2).

Defense vs. Storage

A growing body of evidence supports the trade-off model in both tropical and temperate forests. However, most studies have focused on a single relationship, either between defense and growth (Coley 1987; Kurokawa et al. 2004) or between storage and growth (Myers and Kitajima 2007; Poorter and Kitajima 2007). Few studies have simultaneously examined relationships among defense, storage, and growth. Carbon allocation to defense and storage is important for the survival of seedlings, and both defense and storage incur carbon costs. Thus, the potential carbon investment to survival-enhancing traits remains to be determined. If preferential carbon investment is made in either defense or storage in plant species, these species may have different survival strategies, i.e., investment in deterrence or investment in damage-recovery mechanisms.

Carbon allocation to growth, defense and storage

Imaji and Seiwa (2010) examined the pattern of carbon allocation to defense, storage, and growth in seedlings of two temperate broadleaf tree species with contrasting shade tolerance (shade-intolerant species: *Castanea crenata*; shade-tolerant species: *Quercus mongolica* var. *grosseserrata*). RGR (relative growth rate) in gaps was higher in the light-demanding species, *C. crenata*, compared to the shade-tolerant species, *Q. mongolica* var. *grosseserrata* (Fig.3). In contrast, concentrations of defense compounds (condensed tannins and total phenolics) were higher in *Quercus* than *Castanea* at both sites (Fig. 4a,b). TNC (total non-structural carbohydrate) pool sizes did not differ between the two species (Fig.4c). Thus, interspecific trade-offs between growth and de-

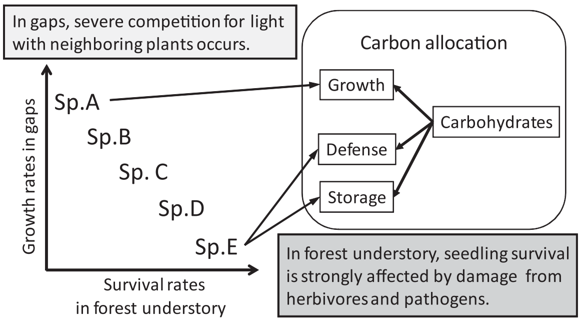

Fig. 2. Relationship between growth–survival trade-off and carbon allocation.

fense were observed in species differing in shade tolerance. These results suggest that *Castanea* allocates more carbon to shoot elongation at the expense of defense, which enables seedlings to regenerate under high-light conditions where rapid vertical growth is required to compete with neighboring plants for light. In contrast, *Quercus* puts more carbon into defense, rather than growth and storage, which enables seedlings to persist in the shaded understory where activity of natural enemies (herbivores and pathogens) is high. From the results of relationships in carbon allocation to defense and storage (Fig. 5a,b), relative allocation to defense against storage was usually higher in *Quercus* compared to *Castanea*. This suggests that *Quercus* preferentially allocates carbon to defense rather than to storage. Therefore, the optimal

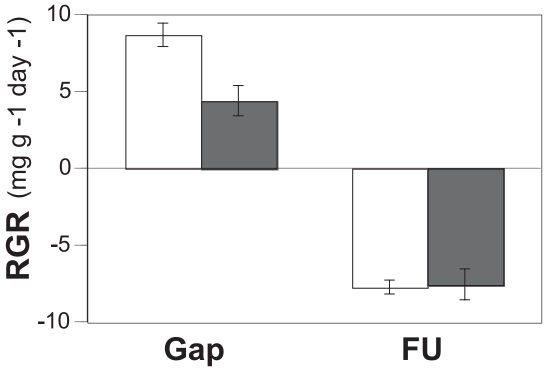

Fig. 3. Relative growth rate (RGR) of the light-demanding species *Castanea crenata* (white bars) and shade-tolerant species *Quercus mongolica* var. *grosseserrata* (shaded bars) in gaps and the forest understory (FU) (Imaji and Seiwa 2010).

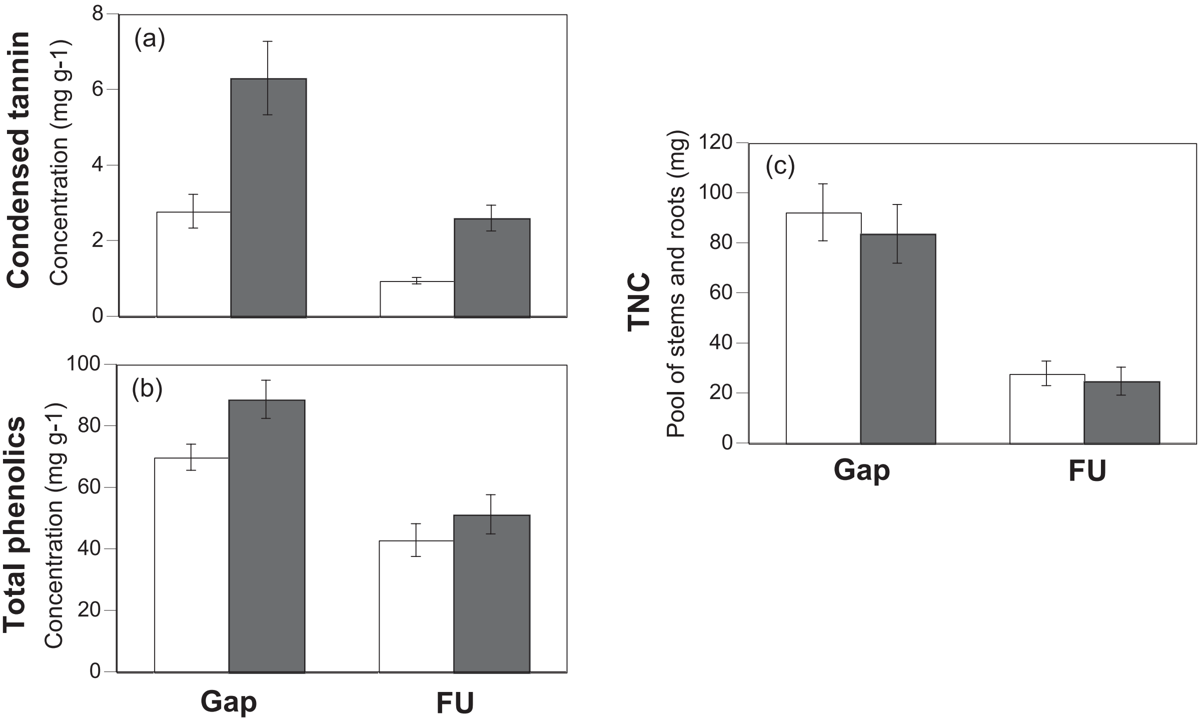

Fig. 4. Concentration of condensed tannins (a), total phenolics (b), and pool of TNC in stems and roots (c) of the light-demanding species *Castanea crenata* (white bars) and the shade-tolerant species *Quercus mongolica* var. *grosseserrata* (shaded bars) in gaps and the forest understory (FU) (Imaji and Seiwa 2010).

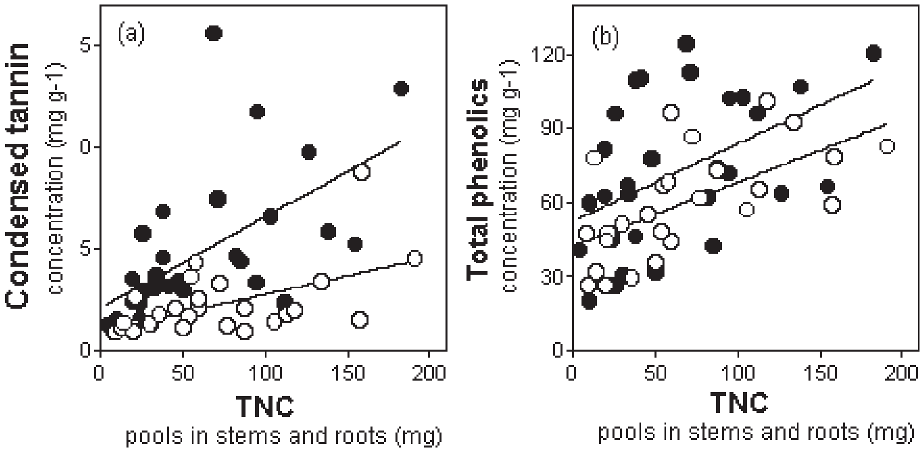

Fig. 5. Relationships between pool of TNC and concentration of condensed tannins (a), and pool of TNC and concentration of total phenolics (b) for the light-demanding species *Castanea crenata* (open circles) and the shade-tolerant species *Quercus mongolica* var. *grosseserrata* (solid circles) (Imaji and Seiwa 2010).

carbon allocation pattern is inherent to individual tree species, and may contribute to persistence in their respective habitats. To understand the carbon allocation strategy more generally in an ecological context, further comparative studies of a large number of species are needed.

Conclusion

Recent studies, especially in tropical forests, have provided evidence for the prediction that differences in carbon allocation underlie species differences in growth and the survival of seedlings, resulting in a trade-off between growth and survival in spatially heterogeneous environments. Our study (Imaji and Seiwa 2010) also supported the contribution of carbon allocation to the growth–survival trade-off in temperate forests. These studies suggest that growth–survival trade-offs, one mechanism of species diversity, are strongly associated with contrasting carbon allocation patterns. However, few studies have addressed carbon allocation patterns in relation to susceptibility to multiple seedling mortality agents. In particular, the relative importance of carbon allocation to defense and storage for survival remains poorly understood. Furthermore, empirical studies examining relationships between carbon allocation to growth, defense, and storage, together with causes of mortality across a large number of species, should lead to better understanding of mechanisms underlying growth–survival trade-offs. Because the trade-off model is based not only on abiotic conditions (i.e., spatially heterogeneous environments) but also on biotic conditions (i.e., interaction between plants and natural enemies), the integrated model should well explain the mechanisms shaping species diversity in forest communities.

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JIFS, 7 : 15 − 18 (2010)

Effects of Seed Size and Chemical Variation on Seed Fates in a Deciduous Oak Species *Quercus serrata*

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Keywords: counter-directional effect, intraspecific variation, seed characteristics, seed predation, tannin

Received 3 December 2009; accepted 1 February 2010

Abstract

Relationships between seed traits (size and tannin content) of individual seeds and their seed fates (dispersal and survival) were examined by the combination of a large-scaled field experiment and a nondestructive tannin estimation method using seeds of a deciduous oak *Quercus serrata*. The field experiment revealed that large and low-tannin seeds tended to be dispersed well and, contrary to this, small and high-tannin ones tended to survive well. These findings demonstrated the presence of two counter-directional effects of seed size and tannin content on seed fates.

Introduction

Plants in stages from seed dispersal to seedling establishment are subject to be damaged by a lot of factors: seed consumers, decomposers, and unfavorable situations, such as shortage of light and soil drought (Moles and Westoby, 2004). These factors may affect seed fates and, subsequently, the recruitment of plant populations. Various seed traits, such as seed size and chemical compositions, are supposed to have considerable impacts on seed fates via interrelations with the biotic and abiotic environmental factors.

Seed traits are not uniform even within species. Indeed large intraspecific variations have been reported in some seed traits. A number of previous studies documented those in seed size and examined effects of seed size on seed fates based on intraspecific variation (e.g. Gómez, 2004). Intraspecific variations in the content of seed chemical constituents have also been reported (e.g. Sork et al., 1983). However, effects of seed chemical content on seed fates have not been examined yet in individual seed scale, though chemical contents may affect their seed fates like seed size.

Tannins are one of the most popular plant secondary metabolites, which act as a defense chemical against herbivory and fungal attack. Some of oak species include high level of tannins in their seeds (Shimada and Saitoh, 2006). Recently we found a large intraspecific variation in acorn tannin content in a deciduous oak species *Quercus serrata* within a local population and, further, within individual mother trees (mean ± SD, 6.6% ± 3.5; range, 0.7-27.1%; Takahashi & Shimada, unpublished data). Such large differences in tannin content may likely have substantial influences on seed fates even in the individual seed scale.

The objective of the present study is to examine the effects of individual seed characteristics, such as size and tannin content, on seed fates using seeds of *Quercus serrata* by the combination of a large-scaled field experiment and a nondestructive tannin estimation method, which we have recently developed.

Materials and methods

This study was conducted in the Takizawa Research Forest of Iwate University (Iwate pref., 39° 46’ 31” N, 141° 9’ 27”E, c.a. 200m a.s.l.). We selected 34 trees of *Q. serrata* in this site, whose crowns did not overlap each other. Preliminary survey revealed that two species of wood mice, *Apodemus speciosus* and *A. argenteus*, inhabited in this site. Both species are major predators and dispersers of acorns (Kikuzawa, 1988).

Seed traps were set up under each sample tree to collect acorns. They were in a rectangle form and covered the crown projection area of each tree as large as not intermixing acorns with ones from neighbor *Q. serrata* trees. Acorns were collected every three or four days from September to November in 2007 and sorted into five categories as sound, damaged by insects, immature, maldeveloped, and infected by fungi. Only sound acorns which were not damaged by consumers and free from deterioration were stored at 5 ºC until the following treatment: measuring seed traits and attaching tapes to trace.

Tannin content of individual acorns were estimated with the near infrared spectroscopy calibration model, which we have developed (Takahashi et al., unpublished data). Each acorn was placed in a water bath maintained at 25 ºC for at least 20 min to bring the sample temperature to 25 °C. After the temperature adjustment, near infrared spectrum of each acorn was obtained with a modified FQA-NIRGUN instrument (FANTEC, Kosei, Japan). This portable near infrared spectrophotometer operates in transmittance mode, in which absorbance of light penetrating through a sample is measured. A standard reference was measured prior to every sample measurement. Transmittance readings were converted to absorbance values. After the pretreatment for the absorbance spectra, tannin content in individual acorns was estimated with the calibration model. All the samples were weighed individually after spectra acquisition.

After measuring seed characteristics these acorns were marked with identifiable number and tape. Then, marked acorns were returned under the seed traps where each acorn was collected. The study site was intensively searched for the marked acorns from May to June in 2008. Status of the seed discovered was recorded. We defined an acorn whose hypocotyl emerged as "survived", that is, success in early establishment. In addition, the places where the marked acorns were found were located for all the marked acorns regardless of their conditions of survival. We defined the acorns found outside the seed traps of their maternal trees as dispersed.

We analyzed relationships between seed characteristics and individual seed fates (dispersal or survival). Relationship between seed traits and dispersal was analyzed using the dataset of all samples. Missing acorns were categorized as dispersed, because they could not be detected under the seed traps in spite of intensive search. Relationship between seed traits and survival (success in early establishment) was analyzed using the dataset of all sample acorns excluding missing ones. To examine the effects of seed characteristics on seed fates in the individual seed scale, we analyzed the relationships between seed fate and seed characteristics (acorn size and tannin content) by multiple logistic regression analysis using maternal trees as a random effect. The null samples whose tannin content failed to be estimated by near infrared spectroscopy were excluded from the data beforehand.

Results

Total number of collected *Q. serrata* acorns was 45,335. Number of sound acorns, infested by insects, immature, maldeveloped, and infested by fungi were 19,606, 19,456, 6,027, 223, and 23, respectively. A half of total sound acorns were used as samples in the seed fate analyses (9,803). After removal of the null samples, 8,988 of the marked acorns were supplied for further analyses. Acorn weight varied from 0.10-4.49 g (2.11 g ± 0.55 SD). Tannin content varied from 0.001 % to 31.48 % (5.19% ± 2.42 SD). The relationship between tannin content and weight of acorns was not significant ($r = -0.0056$, $P = 0.5934$).

We could find 4,280 acorns (47.6 % of all the acorns) among 8,988 of the marked ones. Among them 176 acorns (2.0 %) were proved to be dispersed, and the rest (4,104 acorns, 45.7%) were found at the site where the marked acorns had been returned. A total of 766 acorns (8.5 %) successfully germinated, which consisted of eight acorns (0.1 %) that were dispersed and 758 ones (8.4 %) that were not dispersed. 4,708 acorns (52.4 %) were missing, and they were regarded as being dispersed.

Both of acorn weight and tannin content in individual acorns had significant relationships to whether an acorn was dispersed or not ($n = 8988$; whole model: $\chi^2 = 1623.0$, $P < 0.0001$; tannin content: β coefficient $= 0.1024$, $P < 0.0001$; seed weight: β coefficient $= -0.4288$, $P < 0.0001$). Seed dispersers tended to disperse larger-sized and lower tannin acorns. Similarly, acorn weight and tannin content showed negative and positive correlations with survival of acorns, respectively ($n = 4280$; whole model: $\chi^2 = 651.2$, $P < 0.0001$; tannin content: β coefficient $= -0.0805$, $P < 0.0001$; seed weight: β coefficient $= 0.7843$, $P < 0.0001$). Namely, small-sized and higher tannin acorns tended to survive better.

Discussion

In this study the relationship between seed traits and seed fate was examined in the scale of individual seeds. We discovered that tannin content and seed size of individual acorns have significant impacts on seed fate. This is the first study to demonstrate the effects of seed chemical traits on seed fate in the individual seed scale.

Detection ratio of marked acorns in this study (47.6 %) was similar to those of previous studies (53.9 – 71.5 %, Sork, 1984; 54.4 – 95.0 %, Hoshizaki and Hulme, 2002). Dispersal ratio (54.3 %) was thought to be ordinary comparing with those of previous studies (7.0 – 93.1 %, Sork, 1984; 30.1 – 77.7 %, Hoshizaki and Hulme, 2002), although the ratios largely differed among maternal trees or years. Survival ratio (8.5 %) was higher than that in Hoshizaki and Hulme (2002) (0.08 – 2.9 %). It might result from predator saturation caused by mast seeding in this year.

The field experiment revealed that acorns with lower tannin content and larger size were more likely to be dispersed. This result seems to be reasonable for seed dispersers, because they tend to prefer less tannin and large acorns, which are better in food quality and feeding efficiency. A question occurs from this result: can wood mice detect tannin content without removing pericarps and seed coats? Grey squirrels can distinguish between acorns of red and white oak, which differ widely from each other in tannin content. Grey squirrels may be able to distinguish acorn groups with odor of tannins and /or tannin-related materials (Steele, 2001). The relationship between chemical content and odor of acorns in our study system needs to be examined. The relationship between seed dispersal and seed size has been reported in a number of previous studies. Large sized foods tend to be removed better and hoarded rather than consumed instantly. The result of the present study showed the same tendency.

For seed survival, acorns with smaller size and higher tannin content tended to survive better. This result was consistent with selective consumption by the Japanese wood mouse according to tannin content (Takahashi and Shimada 2008). Tanniferous acorns may have higher probability to survive through selection by consumers, since consumers generally avoid negative effects of tannins.

Large seed size generally brings some benefits for survival from unfavorable conditions, such as competition with existent vegetation (Jakobsson and Eriksson, 2000), drought (Leishman and Westoby, 1994). In these studies, however, changes in predation risk according to seed size were not considered. Actually, predation risk often increases with seed size. In the case of cache dispersal seeds by rodents (*Q. ilex*), large size invites higher predation risk than small size, whereas it increases germination rate and seedling survival in acorns (Gómez, 2004). He suggested that seed size effects work on fitness in two different ways: large seeds suffer high selective pressure of seed consumers before germination; and, in contrast, they have advantage in seedling establishment (after germination). Indeed, in this study we found that larger seeds seemed to suffer higher predation risk than smaller ones.

In this study, seed characteristics that were advantageous for dispersal (large size and low tannin) were opposite to those for survival (small size and high tannin). This finding demonstrated the presence of two counter-directional effects of seed size on dispersal and survival. Similarly, two counter-directional effects of tannin content for dispersal and survival might exist. Namely, acorns with low tannin tended to be dispersed, but to be predated. Advantageous seed traits might be determined through the balance of these two counter-directional effects. In the case of high predation pressure by seed consumers like our study, acorns with higher tannin content and in smaller size would be favorable in the early seedling establishment stage. Contrary to this, in the case of low predation pressure, acorns in larger size would be favorable, and ones with lower tannin content might have some benefits, if tradeoff in resource use exists between tannin and any other traits related with seed survival. We suppose that these counter-directional effects of seed traits on seed fates may generate and maintain large intraspecific variation of seed traits in *Q. serrata*.

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Ecological and Evolutionary Interaction Network Exploration: Addressing the Complexity of Biological Interactions in Natural Systems with Community Genetics and Statistics

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Keywords: community ecology, species covariances, co-occurrence analysis, facilitation, foundation species, *Populus fremontii*, lichens

Received 18 December 2009; accepted 1 February 2010

Abstract

Ecological communities play an integral role in determining ecosystem functions. However, community-level patterns and processes are complex because they are typically comprised of many interacting components. Therefore, pair-wise reductionist investigations of interactions among species are unlikely to reveal the dynamics of the whole community. Here, we present results from a study of the interactions among members of a lichen community associated with different genotypes of a foundation tree species, *Populus angustifolia*. Three key findings emerge. First, null-model based analysis of species co-occurrence patterns suggest that interactions are likely contributing to lichen community structure. Second, the pattern of co-occurrences and pair-wise correlations of lichen species suggest that interactions among lichens are primarily facilitative. Third, the significance and magnitude of co-occurrence patterns vary among genotypes of *P. angustifolia* suggesting that the strength of facilitative interactions among lichens is tree genotype dependent. In combination, direct and indirect plant genetic effects on the interactions of lichens appear to play an important role in defining the lichen community. We believe that a community genetics approach focused on foundation species will allow researchers to better understand the selection pressures that shape communities and that many unexpected outcomes will emerge. From this perspective we discuss future research directions that employ greater analytical power to further quantify the complex network of species interactions within communities.

INTRODUCTION

Ecosystems are changing rapidly in response to anthropogenic pressures, such as climate change (Davis and Shaw 2001) and non-native species invasions (Vitousek et al. 1996). It is imperative to the future well-being of society that ecosystem functions are maintained in the face of mounting ecosystem state-changing forces. Thus, ecologists are charged with the task of understanding the factors that control the stability and resilience of ecosystem functions. In this regard, it is important that we understand interactions among organisms in communities because they contribute both directly and indirectly to ecosystem services (Naeem et al. 1994).

Mechanistic studies of the linkage between community structure and ecosystem function are difficult because of the great complexity of interactions among a myriad of factors (Schmitz and Booth, 1997). One major advance in studying the dynamics of ecosystems has been the direction of studies toward a focus on foundation species (i.e., species that have large ecosystem-wide effects). The foundation species concept introduced by Dayton (1972), encapsulates the concepts of dominant species, keystone species and ecosystem engineers. As such, these species have been shown to direct the dynamics of ecosystems (Ellison et al. 2005a). For example, the loss of foundation species, Eastern Hemolock (*Tsuga canadensis*), from an invasion by an exotic aphid-like pest (*Adelges tsugae*) in Eastern North America has caused rapid shifts in plant and ant community composition (Ellison et al. 2005b). This suggests that understanding how a foundation species is likely to respond to environmental perturbations, such as exotic species invasions or climate change, will provide important insights about the dynamics of the ecosystem as a whole.

The foundation species perspective has enabled the growth of the field of community genetics, which is defined as the study of the genetic interactions that occur between species and their abiotic environment in complex communities (Whitham et al. 2006). From an evolutionary perspective, community genetics can be viewed as the study and quantification of the interspecific sources of natural selection. As one species changes evolutionarily other species that interact with it will likely change as well. This has been documented in pair-wise and multi-species studies (see Ehrlich and Raven 1964, Whitham et al. 2003, Johnson and Agrawal 2005). Studying the community genetics of foundation species makes it possible to begin to quantify the evolutionary forces in communities, including complex interactions among species (see Whitham et al. 2006).

Our view beyond the effects of a foundation species is still limited if we do not begin to incorporate greater details about the effects of the self-organizing process of interactions among the associated community members and their subsequent feedbacks. Evidence suggests that interactions among community members are likely to play an important role in determining community structure. The structure of relationships among species has been shown to be an important property of communities (Dunne et al. 2002, Bascopmte et al. 2006). In addition the presence of feedbacks (Bever 2002) and indirect effects (Ohgushi et al. 2007) have also been shown to play an important role. For example, Johnson et al. (2010) found that the source of arbuscular mycorrhizal communities had an effect on the reproductive output of different ecotypes of a foundation grass species. If interactions among community members associated with a foundation species are weak, then solely focusing on the dynamics of the foundation species can provide robust predictions of ecosystem dynamics. However, if these community interactions are strong or non-linear (e.g., thresholds), then inferences that do not include them will be unlikely to predict ecosystem dynamics. To illustrate this point, consider two hypothetical interaction network structures (Fig. 1): one in which the community is linked together only through the foundation species (A) and another where the foundation species is still central to the community but the associated species interact with each other to form a complicated web (B). In community A, a change in the foundation species can potentially affect all species in the community, but the effect will be direct as long as feedback effects are

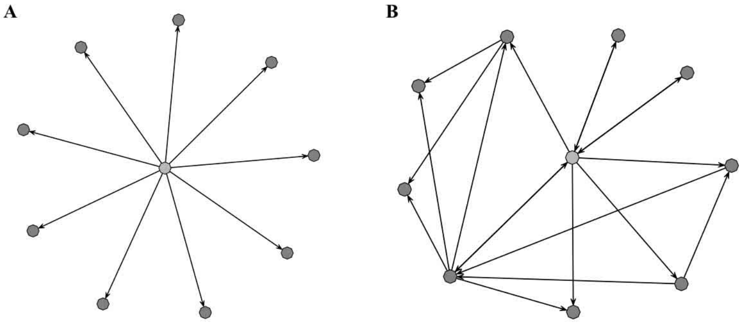

Fig. 1. Two network graphs of how foundation species influence associated organisms and how associated organisms can influence each other to influence community structure. Vertices (dots) and edges (arrows) represent species and interactions, respectively. The direction of the arrowhead indicates the direction of the interaction. In each case the foundation species is the central vertex with associated species radiating around it. Graph (A) shows a situation where there is a unilateral effect of the foundation species on the associated community (i.e., all interactions are direct via the foundation species). (B) shows a more complicated interaction network with the same richness in which some species do not interact directly with the foundation species and some species affect the foundation species to influence other species (e.g., interspecific indirect genetic effects).

small. However, in community B, the change in the foundation species will ripple though the community and could be amplified by interactions among other species creating a community-wide effect that would be much harder to predict. Unfortunately, experimental manipulation of communities to tease apart multi-species interactions are typically intractable. Field-based observational data, however, is often easier to obtain and may provide more reliable information about species relationships, which we utilize here.

We present the results of analyses of communities of epiphytic lichens associated with a foundation tree species, *Populus angustifolia*. This cottonwood species is a dominate tree of riparian habitat found throughout the interior mountains of western North America, including parts of the U. S., southern Canada and northern Mexico (Eckenwalder 1984). Previous studies have demonstrated that tree genotype plays a major role in defining the canopy arthropod community, soil microbial community, trophic interactions and even nutrient cycling (Shuster et al. 2006, Bailey et al. 2006, Schweitzer et al. 2008, Whitham et al. 2006). Although these communities are either known or suspected to strongly interact with the tree, other community members such as epiphytic lichens were not thought to be sensitive to sub-specific variation in tree traits and, thus, not be influenced by the effects of tree genotype. However, recent observations suggest that lichen communities do differ in composition among *P. angustifolia* genotypes (L. J. Lamit et al. unpublished). We use this lichen community dataset to explore how a community genetics approach combined with co-occurrence analyses can reveal novel interactions and unexpected community structure. Two major questions were addressed. First, is there evidence that interactions among species associated with a foundation tree species contribute to community structure? Second, do these interaction effects vary with foundation tree species genetics? In light of the results of these analyses we discuss potential research and modeling methods that could help elucidate community-level patterns of species interactions and their implications for community genetics.

MATERIALS AND METHODS

We quantified the lichen community on the lower trunks of individual *Populus angustifolia* trees of known genotype planted in a common garden in Ogden, Utah, USA. The common garden was initiated in 1991 using cuttings taken from trees growing along the nearby Weber River. Tree genotypes were planted in a fully randomized design to minimize environmental influences. We sampled 16 genotypes with 3 to 8 replicates each for a total of 70 trees. In July 2008, we obtained the percent cover of each lichen species in 10 cm^2 quadrats centered at 15 cm, 50 cm and 85 cm from the ground on both the north and south side of the main trunk of each tree (total sampling area = 60 cm^2 per tree). A total of 5 lichen species were observed: *Xanthomendoza galericulata*, *Physciella melanchra*, *Candelariella deflexa*, *Caloplaca holocarpa* and *Rinodina turfacea*.

Grouping lichen community observations on each tree by genotype, we estimated the net effects of interactions among lichen species on each genotype using null model based co-occurrence analyses conducted in EcoSim (Gotelli and Entsminger 2005). Originally developed as a means to test hypotheses of assembly rules, co-occurrence analysis has developed into a statistical means to estimate the potential effects of interactions among species (Gotelli and Graves 1996). In essence, a co-occurrence statistic from the observed data is compared to the distribution of the same statistic calculated for a large set of communities that are not structured by interactions but simulated by permutation of the original data. By observing the co-occurrences in the common garden on randomly distributed replicates of each tree genotype, we can assume that any factors influencing the co-occurrence patterns other than interactions among species will introduce only random variation.

Although we explored several metrics and permutation algorithms here we present the results using the C-Score, which measures the average co-occurrence among all species pairs, developed by Stone and Roberts (1990) as our metric and a fixed-equiprobable permutation algorithm in which the species totals remain constant (fixed) for each simulation but the total number of occurrences in an observation can vary (equiprobable). This combination has performed well with respect to Type I and Type II errors in simulation tests (Gotelli 2000). We follow that standard method of using a Standardized Effect Size (SES) to compare our observed to simulated (i.e., random) communities. The SES is calculated as the observed C-Score minus the mean of the simulated C-Scores divided by the variance of the simulated data.

To explore the relationship among pairs of lichen species we examined the pair-wise correlations. As with the co-occurrence analyses above, observations were grouped by the genotype of *P. angustifolia*. Using Pearson's correlation coefficient (r) we calculated and then averaged all unique pair-wise correlations within each genotype. The regression lines used in the correlation plot are the product of a linear regression model for each species pair that was observed for *P. angustifolia* genotype RL6. The bivariate plots for the pair-wise correlations and network diagrams were generated using the statistical programming language R (R Development Core Team 2009, Butts 2009).

RESULTS

The lichen communities we examined showed evidence of variation in the degree of species interactions among tree genotypes. In the co-occurrence analysis the SES values for several of the *P. angustifolia* genotypes were significantly different from zero with values less than -2 (Fig. 2). In other words, the average co-occurrence patterns for these lichen communities were at least 2 standard deviations less than the simulated mean from the null model randomizations. Note that SES values less than zero are indicative of species co-occurring together more often than would be expected under the null-model (i.e., random species associations). In addition, the SES magnitude and statistical significance varied among genotypes with the largest being over 3X the smallest SES value.

Patterns of pair-wise correlations among lichen species were primarily positive. The mean correlations for the genotypes that had significant co-occurrence results were all greater than zero (subscripts indicate genotype): $r_{1008} = 0.23$, $r_{10} = 0.47$, $r_{WC5} = 0.94$ and $r_{RL6} = 0.81$. A bivariate plot of the lichen species on tree genotype RL6 shows that all species pairs exhibited positive relationships (Fig. 3).

DISCUSSION

Genetic-based interactions affect community structure - Addressing our two main questions, there is evidence that interactions among lichen species contribute to community structure and that this effect varies among genotypes of *P. angustifolia*. Our evidence is based on the application of null-model based co-occurrence analyses that examine how the genetics of a foundation tree species influences the net effects of interactions among associated species. The co-occurrence analysis showed not only significant co-occurrence patterns, but also variation in the co-occurrence patterns among genotypes. Although the co-occurrence analysis method has been used previously to study the variation in co-occurrence patterns of insect guilds associated with the foundation tree species, *Tsuga canadensis*, (Dilling et al. 2007), it has not been used previously to examine the genetic effects of a foundation species on interactions.

Over the spatial scale of this study, non-random co-occurrence patterns can arise from the common influence of an environmental gradient or interactions among species. However, because the communities were observed in a common garden, environmental effects other than the influence of the variation in tree genetics primarily introduce random variation in species co-occurrence patterns. It is possible that other organisms, cryptic lichen species or non-lichen species (e.g., fungus mites) that were not included in these analysis, may influence the lichen community, and further observation of a broader portion of the community and natural history studies will provide more detailed information on the ultimate cause of these patterns.

A genetic component to facilitative interactions - In addition, the SES values from the co-occurrence analysis and correlations among species suggest that the interactions were primarily facilitative. All of the SES values were less than or equal to zero. Negative SES values arise when species tend to co-occur more often than is predicted by the null-model. Therefore, the negative SES values indicate that species tended to cluster together on average. This pattern is corroborated by the positive average correlation values and the pair-wise correlations within the *P. angustifolia* genotype RL6.

Thus, these results suggest that interactions within the community of lichens contribute significantly to community structure, primarily through facilitative relationships, but this depends on the genetic effects of the tree on which they were growing. Our results are in line with the findings of previous studies of the lichen community in this system which demonstrated that *X. galericulata* and the community as a whole exhibits heritable variation among *P. angustifolia* genotypes (L. J. Lamit et al. in review and unpublished).

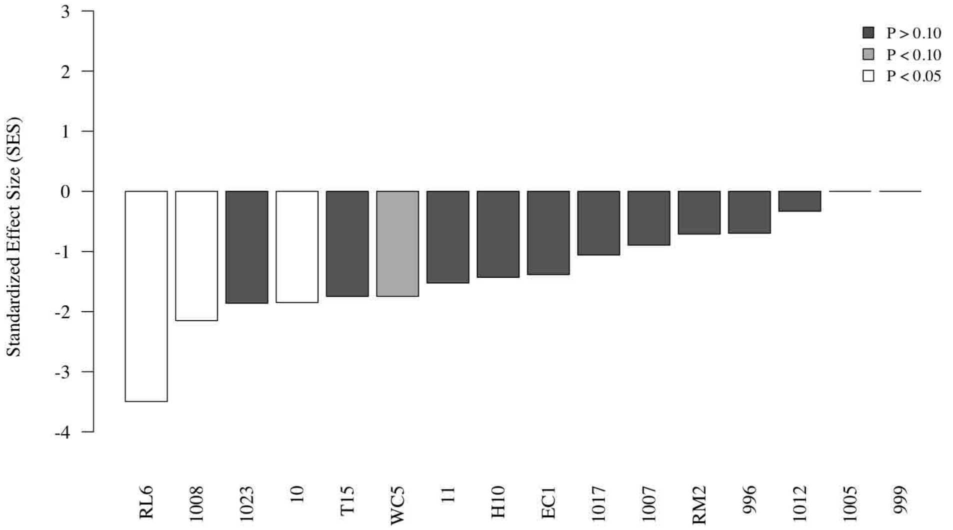

Fig. 2. Plot showing the variation of the Standardized Effect Sizes (SES) for the epiphytic lichen communities on replicated genotypes of *Populus angustifolia* growing in a common garden. Negative SES values are indicative of species being positively associated (i.e., aggregating) beyond what would be expected by chance alone. Bar colors indicate significance levels for the SES of each genotype.

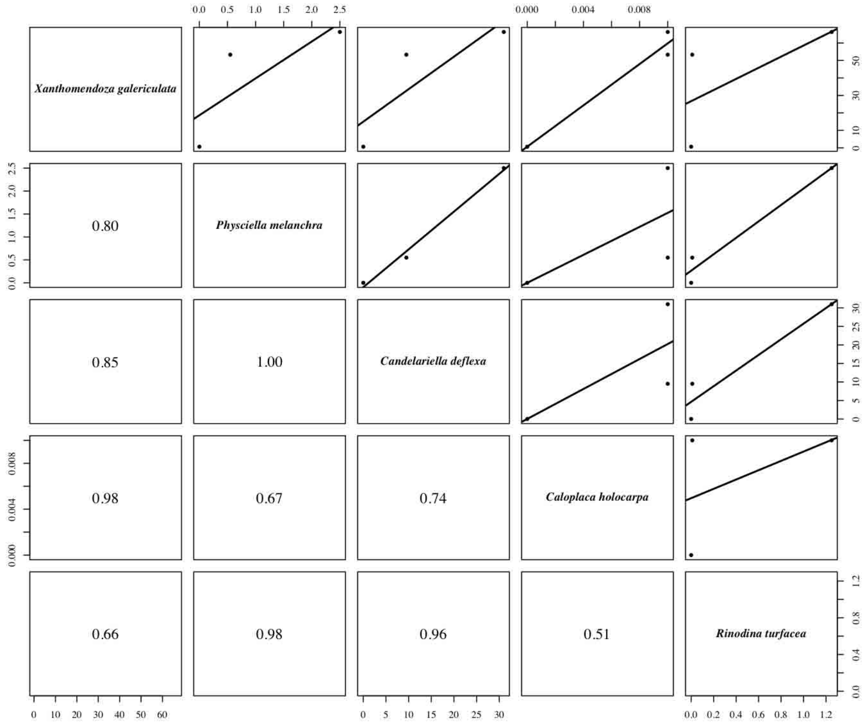

Fig. 3. Bi-variate plots for pair-wise species combinations of epiphytic lichen species present on *Populus angustifolia* genotype RL6 in the common garden. Each species is listed on the diagonal. The upper panels show the bi-variate plots with least squares regression lines. The lower panels have the Pearson's correlation value for each correlation.

Understanding ecological systems in the face of the complexity of numerous interacting species is a long standing problem in ecology (Darwin 1859). Traditionally, studies of biodiversity have primarily focused on the number and proportion of species in ecosystems (Bascompte, 2009); however understanding the web of interactions among species is important for predicting community dynamics. For example, in a study of the interaction between the tree, *Juniperus monosperma*, and the mistletoe, *Phoradendron juniperinum*, the introduction of a third species, a seed dispersing bird, *Myadestes townsendi*, altered the net effect of the interaction between the juniper and the mistletoe from parasitism to mutualism (van Ommeren and Whitham 2002). Although empirical studies have demonstrated that genetic variation within foundation species have strong direct effects on community structure (e.g., insects, fungi and birds - Dickson and Whitham 1996, mycorrhizal fungi - Sthultz et al. 2009), our findings indicate that the genetics of foundation species can have indirect effects on the structure of associated communities by influencing the interactions among species, which as the above example demonstrates, may have unexpected outcomes. Because the interactions among associated species are in part determined by the genetics of the foundations species, understanding these genetic effects on interactions will be important in scaling from local (i.e., genetics of individuals) to community and ecosystem-level patterns, which is important for understanding ecological systems in general (Levin 1992, Brooker et al. 2009).

Focusing on foundation species presents a starting place for studying ecological and evolutionary interaction networks. We can apply the results of the present study to make more refined predictions about the dynamics of the bark lichen communities associated with cottonwoods. For example, evidence from empirical and theoretical studies suggest that facilitation is important to community dynamics, diversity and evolution (Rudgers and Maron 2003, Velland 2008, Bronstein 2009). Specifically, mathematical modeling indicates that facilitation can lead to greater community stability depending on local levels of environmental severity (Butterfield 2009). Thus, those genotypes that promote facilitation among associated lichen species will tend to have more stable communities of lichen and lichen associated species, such as mites (Acarina), springtails (Collembola), slugs and snails (Gastropoda) and endolichenic fungi (see Brodo, Sharnoff and Sharnoff 2001, Arnold et al. 2009).

Statistical exploration of interaction networks - As shown in our lichen community study, statistical analyses of community abundance datasets can compliment experimental approaches for exploring interactions among species and directing further observations and experiments to develop and test theory. Null-model based co-occurrence analysis is not only a useful method to measure and test for the net effects of species interactions but also has a large literature base with a long history (Weiher and Keddy 1999). However, one limitation of the application of co-occurrence analysis to investigate interaction networks is that its focus is on the net effects of species interactions. Thus, other methods are needed to elucidate the structure of the interaction network.

Analysis of correlations among species is a simple means to probe datasets for species interaction information, especially when we already have hypotheses about the interactions (e.g., trophic relationships). Although correlation analysis is limited by the causal interpretability of pair-wise correlations, especially when species relationships are likely to be non-linear, its results still provide useful information in the face of difficulties in experimental manipulation of the many numerous pairs of species not to mention their higher dimensional interactions (Shipley 2000). A promising analysis using temporal, rather than spatial (as is the case with our lichen data), species covariances has recently been developed using reverse engineering mathematics originally developed for detecting the structure of gene interaction networks (Jarrah et al. 2007). This method has been used in other fields, such as gene expression networks, but has only recently been applied to ecological interaction networks (Vera-Licona and Laubenbacher 2008).

In an uncertain future of human induced environmental shifts (Breshears et al. 2005) it is important that environmental scientists have a firm understanding of the structure and dynamics of ecological networks (see Cohen 1978, Pascual and Dunne 2006), especially because network theory is broadly applicable across many fields (Barabási 2009). As ecosystems change, changes in the interactions among species will affect the stability and functioning of ecosystems. By resolving the structure of interspecific interaction networks, we can make more accurate predictions of ecosystem dynamics, such as predicting species extinction risks (Allesina and Pascual 2009) and assessing the viability of whole communities (Ebenman and Jonsson 2005). This will require the greater use of analytical methods, especially when experimental research is limited.

ACKNOWLEDGMENTS

We acknowledge the Cottonwood Ecology Group and Soil Ecology Lab at Northern Arizona University and the participants of the 7th International Symposium on Integrated Field Science for their intellectual input and support. We also thank L.L. St. Clair and R. Rees Naesborg for assistance with lichen identification and the Ogden Nature Center (Ogden, UT, USA) for common garden space and field housing facilities. Our research has been supported by NSF grant DEB-0425908 and NSF Integrative Graduate Education and Research Traineeships to M. K. Lau and L. J. Lamit.

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Seasonal Variation of Arbuscular Mycorrhizal Fungal Colonization for Coexisting Plant Species in *Miscanthus*-Type Semi-Natural Grassland

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Keywords: Arbuscular mycorrhiza, coexisting, Miscanthus-type grassland, phosphorus, seasonality

Received 1 December 2009; accepted 1 February 2010

Abstract

Many *Miscanthus*-type semi-natural grasslands in Japan are located on volcanic ash soil, which is short of available phosphate. Arbuscular mycorrhiza (AM) is a symbiotic association between AM fungi and plant roots, in which fungi are known to play an important role in phosphate uptake. For this study, we investigated seasonal variation in AM colonization of coexisting plant species in a *Miscanthus*-type grassland for two years. We selected three major plant species represented in vertical strata of *Miscanthus*-type grasslands: *Miscanthus sinensis*, a tall grass that was the dominant species in the stand; *Lysimachia clethroides*, a forb found in the middlelayer of the stand; *Potentilla freyniana*, a short forb occupying the lower layer of the stand. Mycorrhizal colonization of *M. sinensis* and *L. clethroides* was low in the early growing season. It subsequently increased concomitantly with increased plant dry weight. The mycorrhizal colonization reached a maximum level when the host plants reached a plateau of growth and entered a reproductive growth phase. During the late growing season, the colonization level decreased. Actually, *P. freyniana* showed a high level of mycorrhizal colonization over its growing season and different patterns of colonization between two growing seasons. These results demonstrate that seasonal variations of mycorrhizal colonization of *M. sinensis* and *L. clethroides* were related to their growth patterns and that *P. freyniana* might be susceptible to environmental fluctuations.

Introduction

Arbuscular mycorrhiza (AM) is a symbiotic association between AM fungi and plant roots. The AM fungi are associated with the vast majority of vascular plants (Maeda, 1954; Harley and Harley, 1987) and promote nutrient uptake of the host plant, especially phosphate acquisition from the soil (Smith and Read, 1997). In return, AM fungi gain photosynthate from the host plant. The AM fungi are an important microbe for nutrient cycling in terrestrial ecosystems (Allen, 1991).

Many native grasslands in Japan are located on volcanic ash soil, which is short of available phosphate. Under such conditions, AM symbiosis is expected to play an important role in phosphate uptake of plants. *Miscanthus*-type grasslands are representative of mesic tallgrass-type semi-natural grasslands in Japan (Numata, 1969). Some grasslands have been used for mowing or grazing. Their productivity and community structure are well characterized. *Miscanthus*-type grasslands form three vertical strata in a community: the upper, middle, and lower layers (Koike, 1969). Plants of the upper layer have a higher elongation rate and relative growth rate (RGR) in the early growth stage and a longer elongation term. The middle-layer plants show a higher elongation rate in the middle growth stage than in the early growth stage. The lower layer plants have a low elongation rate and RGR throughout the growing period and cease elongation in the early growing stage. Plant phenology also differs among layers: the lower the layer species of the community, the earlier the flowering time.

The AM colonizing pattern is closely related to plant growth and phenology (Gay et al., 1982; Brundrett and Kendrick, 1988; DeMars and Boerner, 1995; Johnson-Green et al., 1995; Merryweather and

Fitter, 1995; Allen et al., 1998; Ruotsalainen et al., 2002) as well as environmental factors such as temperature, precipitation, soil moisture and phosphorus content in soil (Rabatin, 1979; DeMars and Boerner, 1995; Johnson-Green et al., 1995; Merryweather and Fitter, 1998; Hartnett and Wilson, 1999). However, these effects on AM colonization are not always consistent, probably because of the complicated interaction among factors (DeMars and Boerner, 1995). In *Miscanthus*-type grasslands, plant species for which characteristics of growth and phenology differ coexist in one place. These plant species might exhibit differential AM colonizing patterns under the same environmental conditions. Knowledge of AM colonizing patterns in response to plant characteristics can contribute to an explanation of the dynamics of AM fungi in plant communities.

This study investigated seasonal variations in AM colonization of several coexisting plant species in a *Miscanthus*-type grassland during two years. We selected three major plant species representing a range of structural characteristics: (i) *Miscanthus sinensis* Anderss. (Gramineae), a tall grass that was the dominant species in the stand; (ii) *Lysimachia clethroides* Duby (Primulaceae), a forb found in the middle layer of the stand; (iii) *Potentilla freyniana* Bornm. (Rosaceae), a short forb occupying the lower layer of the stand.

Materials and Methods

Study area

The experiment was conducted in a *Miscanthus*-type grazing land at an experimental Farm of Tohoku University, Miyagi, Japan, in 1999 and 2000. Soil was nonallophanic andisol (pH 4.7) with available phosphorus concentrations of 33.6 $\mu g\ g^{-1}$ (Bray II method). The 1-ha *Miscanthus*-type grassland was grazed by 10 head of cattle on 16–23 July and 30 September – 4 October in 1999, and 4–11 July and 22 –25 September in 2000.

Sampling procedure

2 m × 2 m plots were situated randomly on the *Miscanthus*-type grassland on every sampling date. Numbers of plots were, respectively, eight and six in 1999 and 2000. One plant was harvested from each plot for *M. sinensis*, *L. clethroides*, and *P. freyniana*. Sampling dates were on 23 May, 23 June, 5 August, 1 September, 6 October, and 2 November in 1999, and 16 May, 16 June, 17 July, 18 August, 15 September, and 16 October in 2000. Plants were removed with a 10 cm × 10 cm × 10 cm soil core. Plant parts were separated from soil and subdivided into roots, rhizomes, stems, leaves, and heads. Roots were used for measurement of the mycorrhizal colonization. Plant parts, except for roots, were dried at 70°C for 48 hr and then weighed. Total phosphorus contents in aboveground parts were determined using the ascorbic acid – molybdate blue method (Watanabe and Olsen, 1965) after digestion with concentrated sulfuric acid and hydrogen peroxide at 200°C for 120 min.

Arbuscular mycorrhizal colonization

Fresh lateral roots were cleared with 10% KOH and stained with trypan blue [a phenol-free modification of the method of Phillips and Hayman (Phillips and Hayman, 1970)]. The percentage of root length colonized (%RLC) by all AM fungal materials was determined using a magnified intersection method at 150 × (McGonigle et al., 1990).

Results

Meteorology

Mean temperature and precipitation during the growing season in 1999 was higher than that of the prior ten years (+1.0°C and +180 mm; Figs. 1A and 1B). In the early growing season of 2000, the mean temperature was lower than that of the prior ten years (-1.0°C; Fig. 1A). It was hotter and drier in the summer of 2000 than in the prior ten years (+1.4°C and -216 mm, June–August; Figs. 1A and 1B).

Dry matter production

Dry weight of *M. sinensis* increased toward August and reached a stationary growth stage (Fig. 2A). Their panicles began to emerge from late July. Subsequently, *M. sinensis* occupied the upper layer of the stand. Most dry matter was allocated to its stem to reach top of the stand (data not shown). Patterns of dry matter production were similar between 1999 and 2000. In 2000, the total dry weight decreased during August–September, probably because of the hot and dry summer. *L. clethroides* rapidly grew toward July –August and blossomed in July (Fig. 2B). Although the growth pattern of *L. clethroides* was similar between the two growing seasons, the total dry weight decreased during August–September in 2000, probably because of the hot and dry summer, as shown for

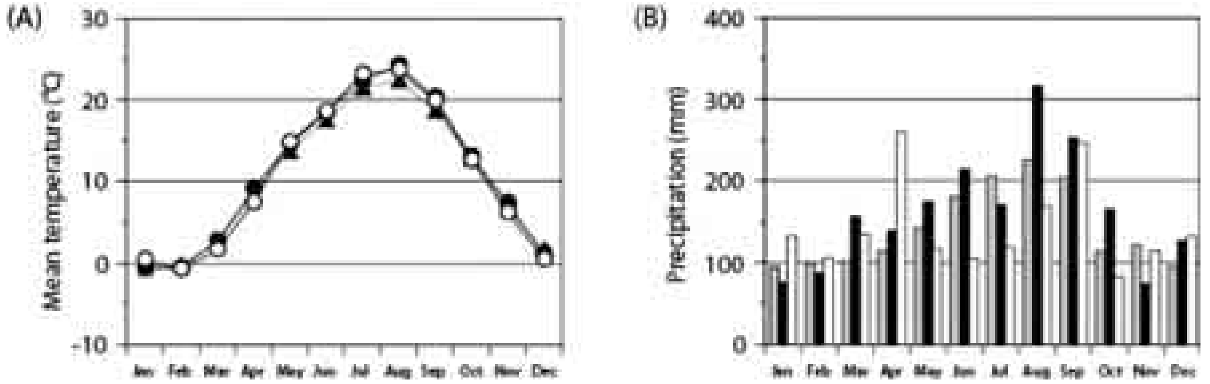

Fig.1. (A) Monthly mean temperatures at the Kawatabi Meteorological Station near the study site in 1999 (black circle), 2000 (white circle), and 1991–2000 (black triangle) and (B) monthly precipitation in 1999 (black column), 2000 (white column), and 1991–2000 (gray column).

M. sinensis. The growth patterns of *P. freyniana* differed between the two growing seasons (Fig. 2C). In 1999, the dry weight of *P. freyniana* increased rapidly during May–June; then it was maintained as almost constant. In 2000, the dry weight was very low in the early growing season (May–July), possibly because of the low amount of precipitation during the season and low temperature in April. The dry weight increased slightly after August. Flowering of *P. freyniana* was observed in May of each year. Actually, *P. freyniana* allocated a large part of its dry matter to rhizome development (data not shown).

AM colonization

The AM colonization of *M. sinensis* showed a similar pattern between two growing seasons (Fig. 3A). The AM colonization was low in the early growing season, but it rapidly increased concomitantly with increasing plant dry weight. The colonization level reached a maximum of 60–70% in August, coincident with the flowering time of *M. sinensis*. In the late growing season, the colonization level decreased markedly. In *L. clethroides*, the AM colonization increased concomitantly with increasing plant dry weight toAugust (Fig. 3B). The colonization level reached a maximum of around 70% in August. During the late growing season, the colonization level decreased in both years, but the decrease was drastic in 1999 and gradual in 2000. Only in 1999 was the high level of AM colonization observed in November when the aboveground part of *L. clethroides* died. In contrast to *M. sinensis* and *L. clethroides*, the AM colonization of *P. freyniana* was high overall during its growing season (Fig. 3C). The patterns of AM colonization differed between the two growing seasons. The AM colonization level in 1999 was maintained at an almost constant of 60–80%, but the colonization in 2000 initially tended to be lower than that in 1999.

Phosphorus content

Phosphorus concentration of shoots was higher in May than in other months for the three plant species, especially *L. clethroides* (Fig. 4). No differences of seasonal variation were found between the two growing seasons, except in May. The phosphorus concentration of *M. sinensis* was 0.11% in May; it gradually decreased thereafter to 0.03% (Fig. 4A). Both *L. clethroides* and *P. freyniana* showed high levels of phosphorus concentration in May, although the levels differed between years (Figs. 4B and 4C). After June, the concentrations in each plant type remained between 0.04–0.09%. The total phosphorus content aboveground was correlated with plant dry weight (Figs. 4D, 4E and 4F).

Discussion

Results of this study demonstrated that seasonal variations of mycorrhizal colonization of *M. sinensis* and *L. clethroides* are related to their growth patterns. The mycorrhizal colonization was low during the early growing season. It subsequently increased as the plant dry weight increased. The mycorrhizal colonization reached a maximum level when host plants reached a plateau of growth and entered a reproductive growth phase. Phosphorus requirements of host plants might be high at the initial growth stages when the rate of phosphorus content increase was high in aboveground. Increased mycorrhizal colonization might reflect the high phosphorus requirements of plants in the early growing season. In the late growing season, the colonization level decreased, which implies a low dependence of the host plants on mycorrhizas. During its growing season, *P. freyniana* showed a high level of mycorrhizal colonization. However, *P. freyniana* is apparently not dependent on mycorrhizae for its phosphorus nutrition because the increased rate of phosphorus content in *P. freyniana* shoots was slight. In *P. freyniana*, AM fungi might be

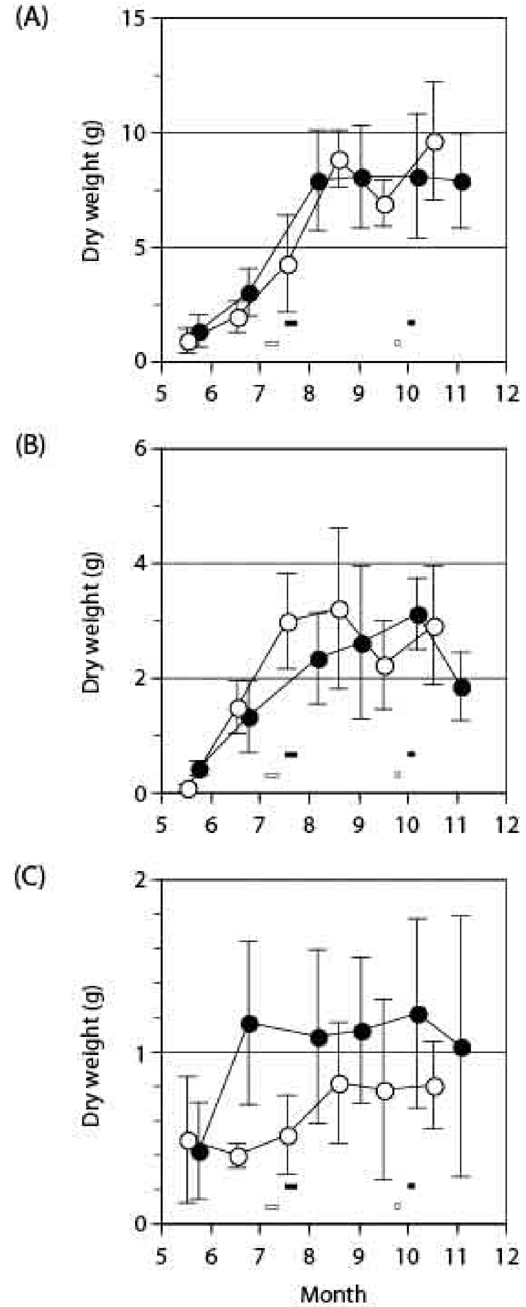

Fig.2. Seasonal variation in total dry matter production (sum of rhizome, stem, leaf, and heads) of (A) *Miscanthus sinensis*, (B) *Lysimachia clethroides* and (C) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle). Values are means ± SD ($n = 8$, in 1999; $n = 6$, in 2000). Horizontal bars represent grazing periods in 1999 (black bar) and 2000 (white bar).

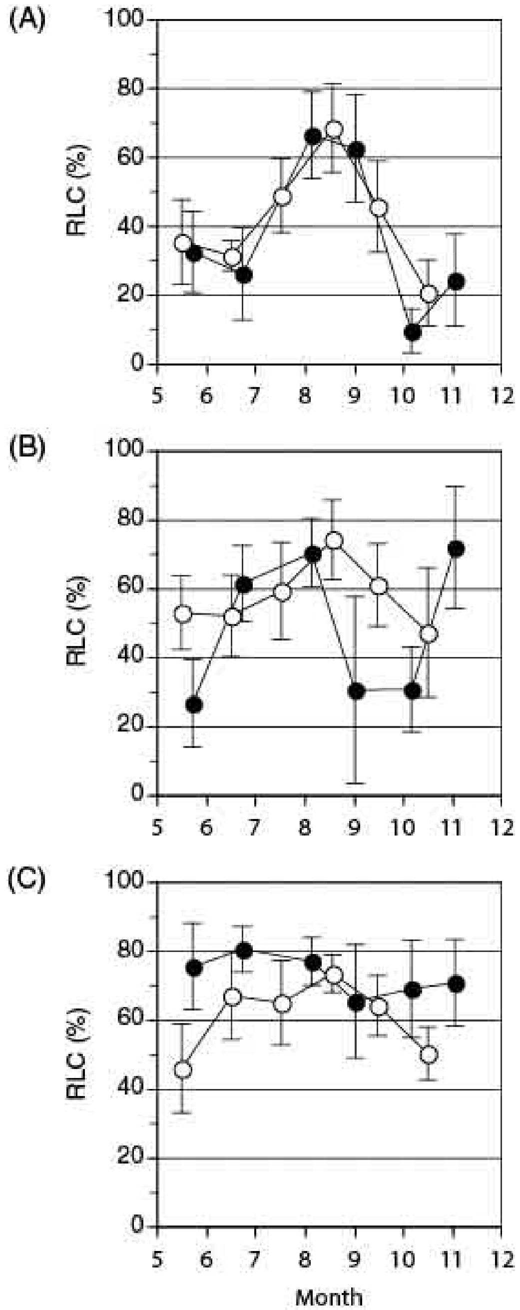

Fig.3. Seasonal variation in mycorrhizal colonization of (A) *Miscanthus sinensis*, (B) *Lysimachia clethroides*, and (C) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle), measured as a percentage root length colonized (%RLC) by all arbuscular mycorrhizal fungal materials. Values are means ± SD ($n = 8$ in 1999; $n = 6$ in 2000).

colonizing parasitically, or have other functions for plants such as micro-nutrient uptake, pathogen tolerance, and drought tolerance.

Mycorrhizal colonization of *M. sinensis* and *L. clethroides* showed little annual fluctuation. Particularly, *M. sinensis* showed almost identical seasonal variation in mycorrhizal colonization in 1999 and 2000, with almost equal dry weight, although the monthly precipitation in 2000 was lower than that of the prior 10 years. In contrast, seasonal variations in mycorrhizal colonization and the dry weight of *P. freyniana* differed between the two growing seasons. Actually, *P. freyniana*, occupying a lower layer of the *Miscanthus*-type grassland, might be susceptible to environmental

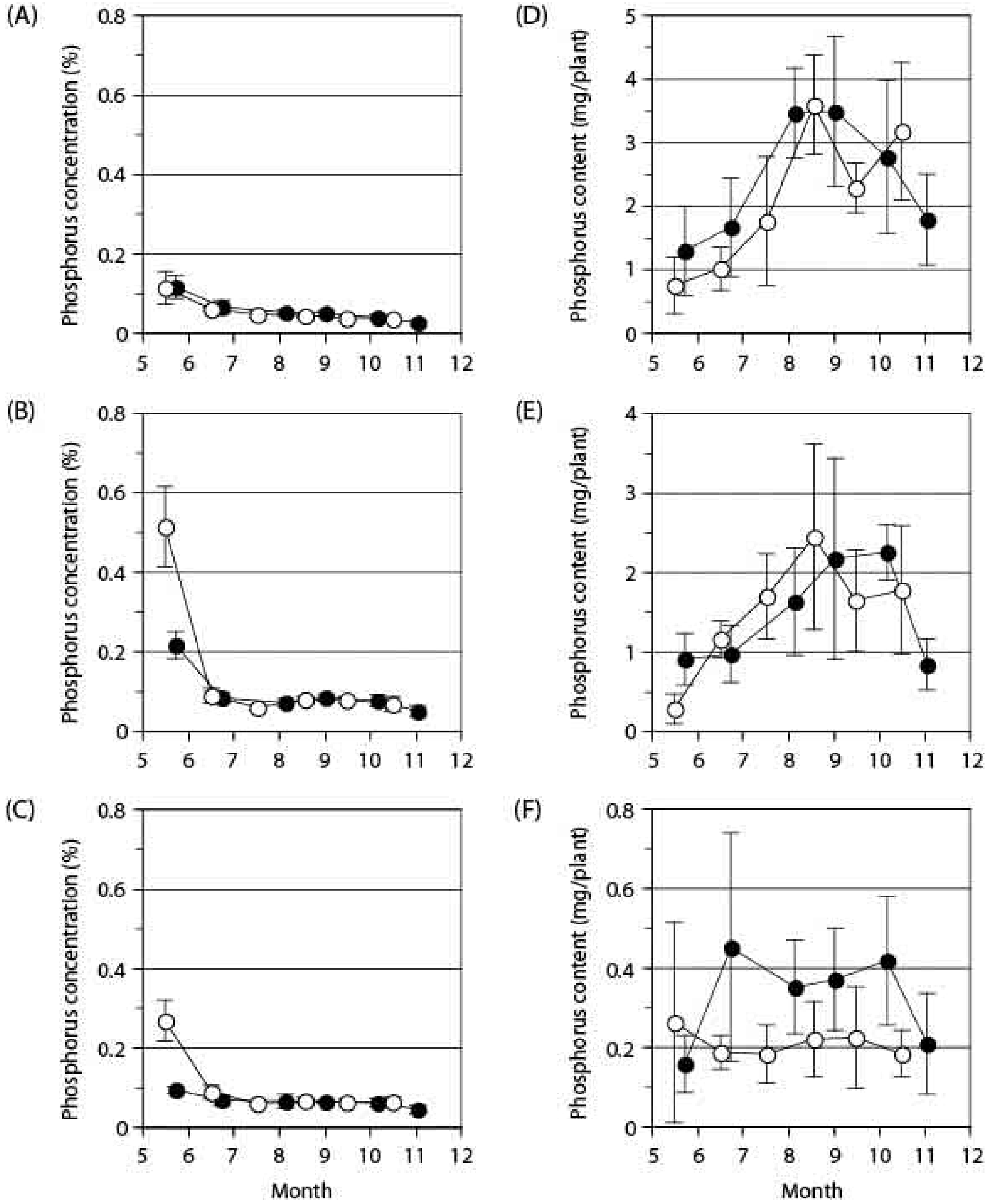

Fig.4. Total phosphorus concentration (A–C) and phosphorus content (D–E) aboveground of (A, D) *Miscanthus sinensis*, (B, E) *Lysimachia clethroides*, and (C, F) *Potentilla freyniana* in 1999 (black circle) and 2000 (white circle). Values are means ± SD ($n = 8$ in 1999; $n = 6$ in 2000).

fluctuations such as temperature and the amount of precipitation. It is necessary to monitor the annual fluctuation of mycorrhizal colonization over the long term.

Mycorrhizal colonization in a semi-natural grassland is higher than that in an artificial grassland where fertilizers have been applied (Wu-en et al., 2002). In this study, three plant species also showed a high degree of mycorrhizal colonization, although there was seasonal and annual variation, implying high dependence of wild plants on mycorrhizal associations to survive in semi-natural grasslands. The three plants co-occur in the *Miscanthus*-type grasslands. The AM fungal community in the grassland soil would be common to the three plant species. However, patterns of AM fungal colonization differ among the plant species. The differences in colonization patterns might originate from difference of AM fungal response to physiological properties of host plants. Mycorrhizal colonization of *M. sinensis* has been shown to decrease by defoliation, indicating that reduction of photosynthate allocation to mycorrhizal roots affects mycorrhizal colonization (Saito et al., 2004). The defoliation of *M. sinensis* has also caused a change in the AM fungal community structure in roots: some AM fungal groups have decreased because of defoliation (Saito et al., 2004). Some studies have revealed ecological specificity through preferential infection of plants by certain AM fungi in natural communities, although it is considered that no specificity exists between plants and AM fungi (Sanders et al., 1999). Different patterns of mycorrhizal colonization of the three plants observed in our study might also be attributable to differences in AM fungal species colonizing in roots. Molecular studies are needed to ascertain whether the host plants are colonized with different AM fungal species.

Acknowledgments

We are grateful to Yasuhiro Yashima for his assistance in grassland management. This research was partially supported by a Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (B) (2), 14360157, 2002.

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JIFS, 7 : 37－40 (2010) Symposium Paper

Significance of Arbuscular Mycorrhizal Fungi in Re-Vegetation Process in Nitrogen-Limited Degraded Ecosystems

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Keywords: degraded soil, hyphae, pioneer plant, succession, ^{15}N

Received 11 January 2010; accepted 1 February 2010

Abstract

It is well documented that arbuscular mycorrhizal (AM) fungi absorb phosphorus (P) from soil and supply P to host plant in exchange for carbon (C) from plants. Currently it is also recognized that AM fungi absorb nitrogen (N) from soil and supply it to plants. We examined possible roles of AM fungi in terms of N acquisition and establishment of plants under N-limited conditions. First, we used a split compartment system composed of root and hyphal compartments. By using the system, we successfully showed that growth of Welsh onion inoculated with AM fungi was increased by supplying N through AM fungi, when movement of soil mineral N to plant roots was limited. Secondly, we investigated how AM fungi were functioning through primary development of vegetation occurring in the lahar (mud flow of volcanic deposit) area of Mt. Pinatubo, Philippines. Pioneer plants in this area were a few gramineous plants, especially *Saccharum spontaneum*. Secondary plant species were wild leguminous plants, such as *Calopogonium muconoides*, which were characterized by co-existence of the gramineous plants. The growth of the gramineous plants was greatly enhanced due to N_2 fixation of leguminous plants. Laboratory inoculation experiments with AM fungi showed that some of the pioneer gramineous species responded to added N only when these were mycorrhizal. These suggest that AM fungi might help their host plants to acquire N efficiently in N-limited soil environments.

Introduction

Arbuscular mycorrhiza (AM) is a sophisticated symbiotic system composed of arbuscular mycorrhizal (AM) fungi and plant roots. It is well documented that AM fungi absorb phosphorus (P) from soil and supply P to host plant in exchange for carbon (C) from plants (Saito 1997). Since 1980's, it has been recognized that AM fungi also absorb nitrogen (N) from soil and supply it to plant (Ames et al. 1983). A main form of mineral nitrogen in soil is nitrate-N under aerobic conditions. In contrast to phosphate, nitrate-N in soil is not adsorbed with soil particles and moves easily along with mass flow of water. Therefore, unlike P, N supply through AM fungi to plant roots may not be critical for plant growth. It is now recognized that AM fungi may not increase plant growth under usual arable conditions (Reynolds et al. 2005). Although metabolic process of N transfer from AM fungi to plant has been clarified (Govindarajulu et al. 2005, Guether et al. 2009), it is still uncertain if N transfer from AM fungi to plant is important under natural conditions.

In this manuscript, first, we examine if AM fungi can enhance plant growth through their N supply to plant using a model system. Secondly, the significance of AM fungi in nitrogen-limited degraded soils is discussed with emphasis on re-vegetation process.

Can AM fungi enhance plant growth by supplying N through their hyphae?

Various compartment systems have been used to study nutrient transfer from AM fungi to plant. In most systems, the roots compartment is separated with fine nylon mesh from extraradical hyphal compartment. In the case of P, such mesh is effective to prevent P transfer between the compartments because of extreme slow mobility of phosphate in soil. Unlike phosphate, mineral N moves easily across the mesh. So it is difficult to quantitatively evaluate the effect of

AM fungi on N transfer. We modified a two-compartment system having roots and hyphal compartments (Tanaka and Yano 2005). In the system, the compartments were separated with fine nylon mesh and air gap so that neither movement of mineral nitrogen nor roots elongation between the compartments occurred. Washed river sand was used as a medium in both plant and hyphal compartments (Fig. 1). A 30 days seedling of Welsh onion, “Naga-Negi” in Japanese, (*Allium fistulos* CV. Motokura) was transplanted and inoculated with *Glomus* sp. R10 (Idemitsu Kosan Co.) in the root compartment (RC). After transplanting, ^{15}N labeled ammonium nitrate was added either RC or hyphal compartment (HC). The treatment was combination of N addition to HC or RC; A) No N, B) N to only HC, C) N to only RC, and D) N to both RC and HC. After 3 weeks, the system was dismantled. Plant growth and fungal growth parameters were measured. Polyphosphate which is a main P storage substance in AM fungi was also determined (Takanishi et al. 2009).

ANOVA analyses showed that “N to RC” treatment increased almost all plant and fungal growth parameters (plant dry weights, N and P concentrations, N uptake, colonization). “N to HC” treatment increased plant dry weight, shoot N and root ^{15}N concentrations, and P uptake. These indicate that AM fungi can enhance plant growth by supplying N under nitrogen-limited conditions (Fig. 2). Interestingly, P uptake and polyphosphate concentration in roots were also significantly increased by “N to HC” treatment. These further suggest that P transfer through AM fungi may be regulated by soil N availability.

Roles of AM fungi in re-vegetation process in nitrogen-deficit volcanic deposit

Secondly, we investigated how AM fungi were functioning through primary development of vegetation occurring in the lahar (mud flow of volcanic deposit) area of Mt. Pinatubo, Philippines (Saito et al. 2002; Oba et al. 2004). Because the lahar is comprised of newly erupted volcanic materials, it contains little organic matter and nutrients available for plants. Most of the area was sparsely vegetated with only a few gramineous plants, especially *Saccharum spontaneum*. However, some densely vegetated areas could be found in patch. These patches were characterized by co-existence of the gramineous plants and leguminous plants such as *Calopogonium muconoides* and *Centrosema pubescens*, which were well nodulated. Growth of the gramineous plants in these patches was greatly favored.

The $\delta^{15}N$ values in wild legumes in densely vegetated (DV) sites were near to atmospheric $\delta^{15}N$ (0 ‰), suggesting that these legumes actively fixed atmospheric nitrogen. *S. spontaneum* in sparsely vegetated (SV) sites showed much lower $\delta^{15}N$ values, probably because it absorbed N from a small amount of N in precipitation and/or in the soil extremely poor in organic N. On the other hand, the same plants in DV sites showed the medium values between the two formers, suggesting that *S. spontaneum* in DV sites used the N fixed by the legumes and its supported its vigorous growth in DV sites.

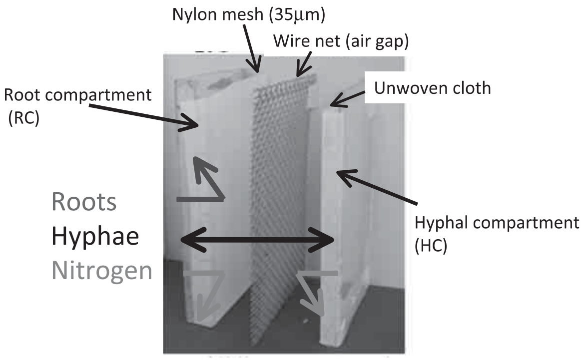

Fig. 1. Split compartment system for nitrogen addition experiment. Nitrogen was added to from the top of root compartment or hyphal compartment. Arrows indicate movement of nitrogen and elongation of roots and hyphae, respectively.

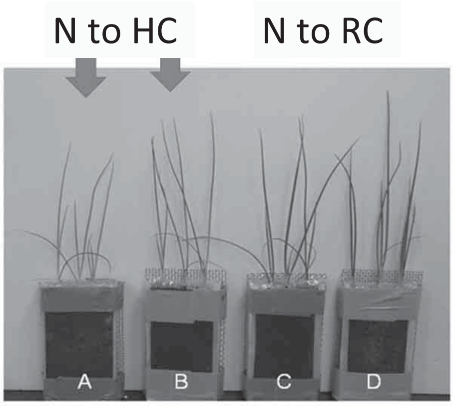

Fig. 2. Effect of nitrogen addition to different compartment on shoot growth of Welsh onion. A) No N, B) N to only HC, C) N to only RC, and D) N to both RC and HC.

The plant roots were colonized with AM fungi. The wild legumes such as *C. muconoides* were well colonized and the mycorrhiza was typical VA-type. *S. spontaneum* was less colonized, and the coiled hyphae were characteristics. In the dry season, the colonization, especially occurrence of arbuscule, was much less in both gramineous and leguminous plants than in the rainy season, suggesting the very weak activities of mycorrhiza in dry season.

The inoculation experiment indicated that *S. spontaneum* was not responsive to inoculation at least under nitrogen deficit conditions such as the lahar soils but increased the spore numbers of AM fungi. Some of other gramineous species responded to added N only when these were mycorrhizal (Fig. 3). This findings suggest that some gramineous plants may contribute to increase the population of AM fungi in soil although the AM fungi are not beneficial to the host plants, and further suggest that AM fungi might help their host plants to acquire N efficiently in N-limited soil environments.

The present study indicated that the re-vegetation process in the lahar area of Mt. Pinatubo involved the complex biological interaction among plants, microorganisms and environment. Based upon the results, we propose a picture of re-vegetation in the lahar soil. First, small seeds of the gramineous plants such as *S. spontaneum* are easily dispersed by wind from the area whose vegetation was not affected by volcanic activity, and they start to grow in the lahar. Secondly, spores of AM fungi are also dispersed by wind or flooding to the lahar area. The fungi colonize the gramineous plants, and host plants increase the fungal density. AM fungi might improve N nutrient of some gramineous species under such nitrogen-limited conditions, although we were not able to show direct evidence. Thirdly, seeds of legumes are dispersed into the lahar. The high population density of AM fungi supports the growth of the legumes, because leguminous plants are known to be highly mycorrhiza dependent (Crush,1974) and require higher P absorption for their nitrogen fixation. Vigorous growth of the legumes promotes the growth of associated gramineous plants through the supply of the fixed nitrogen. Then, once the vegetation is established, the erosion of the soil may be reduced so that organic matter starts to accumulate in the lahar soil, which will support the further growth of other plant species.

In conclusion, AM fungi have a potential to promote plant growth by supplying N to their host. Promoting effect of AM fungi on plants in a soil deficit of N was evident for some plant species when N was added to the soil. In re-vegetaion process in a volcanic deposit, AM fungi may have a role to facilitate vegetational succession.

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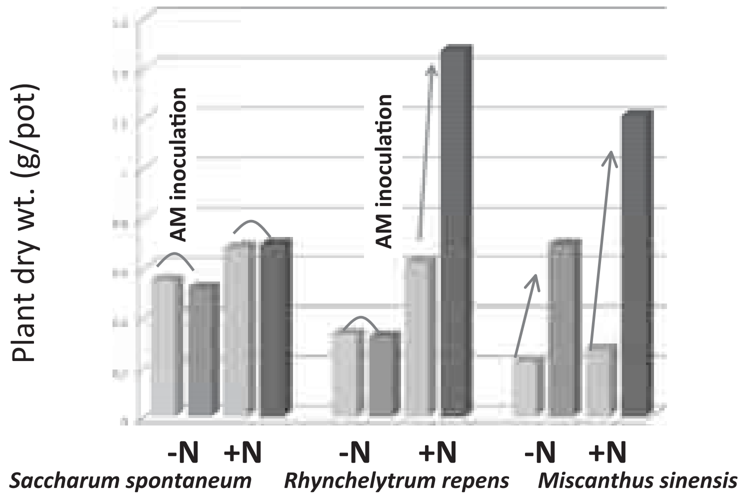

Fig. 3. Effect of AM fungal inoculation on wild gramineous plant species growing in a volcanic deposit deficit of nitrogen. Plants showing no-response with AM fungal inoculation were also colonized.

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Arbuscular Mycorrhizal Fungi Diversity and Contribution to Crop Growth in Agricultural Fields

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Received 17 November 2009; accepted 1 February 2010

Abstract

Arbuscular mycorrhizal (AM) fungi are important soil microorganisms in agroecosystems by enhancing host plant survival and growth. In our survey of AM fungi in the rhizosphere of various crop plants in north and north-west China, thirty three AM fungal species representing seven genera were detected and species richness averaged 2.2 per sample. *Glomus etunicatum*, *G. mosseae* and *G. intraradices* were the dominant species. We also obtained 61 pure isolates, of which 11 pure cultures were registered in the European Bank of the Glomales (BEG). Temporal and spatial dynamics of AMF were studied in the high yield maize system and the result showed that arbuscular colonization, hyphal density, spore density were all reached the maximal at the milk stage of maize (R1 stage). AM fungal community was in a trend of decreasing with increasing soil depth. AMF species richness was decreased significantly only at the depth of 60-90 cm in maize root zone. To estimate the effect of AM fungi on crop growth in agricultural fields, sweet potato, maize, rice, spring onion, taro, strawberry, pimiento, melon, cucumber, tomato and celery were selected as host plants. After pre-inoculation or supply AM inoculum directly in the field, the yield and quality of the crops could be improved. The fertilizer was averaged saving 21%. Moleculer and isotope method were also tentatively used in our present study to monitor of introduced AM fungi, to discriminate the contribution of introduced AM fungi to the crop growth.

1. Introduction

Agriculture is an important economic sector of China. China has to raise agricultural productivity in its limited and shrinking farmland to guarantee food security for its huge and ever-growing population. Sustainable soil nutrient management is of paramount importance.

Arbuscular mycorrhizas are formed by a close association between roots and arbuscular mycorrhizal fungi (AMF), which are ubiquitous in natural and agricultural ecosystems. AMF have long fascinated Chinese agricultural researchers due to their ability to enhance host plant uptake of nutrients, especially phosphorus (P) (Smith and Read 2008). AMF may also be necessary for the long-term sustainability of ecosystems, particularly due to their role in the maintenance of soil structure, and plant community structure and diversity (van der Heijden and Scheublin, 2007). We have done some research on AMF investigation, isolation, efficiency selection and field test. Here, we will focus on the AMF diversity and contribution to crop growth in north China

2. Material and methods

The AMF diversity investigation was conducted in north and north-west China, including Hebei, Beijing, Shandong, Liaoning, Qinghai, Gansu, Xinjiang and Tibet. Soil samples were collected from the root zones of 30 staple crop plants. Isolation and identification was carried out in pot culture and spore morphological method.

The study on temporal and spatial dynamics of AMF was conducted in high yield maize systems of Quzhou, China. Two nutrients input treatments were included, high input (HP) and optimum input (OP). Root and soil samples were collected at the V6, VT, R1, R6 growth stage. Ten soil cores in a Z-line were taken to a depth of 90 cm. The cores were separated into sections corresponding to 0-30, 30-60 and 60-90 cm soil depth. Then AMF colonization, spore density, hyphal density and species richness were determined. The percentage of root length colonized was calculated according to the method of Trouvelot et al. (1986). The colonization data for specific AM structures are expressed as percentage of root length. Spores and sporocarps were extracted from 100 g air dried subsamples of each soil sample in triplicate by wet sieving followed by flotation–centrifugation in 50 % sucrose (Dalpé 1993). The finest sieve used was 38 µm. AM fungal spores were counted on a grid-patterned dish under a binocular stereomicroscope.

The field trial was carried out in Hebei and Beijing. The tested plants included sweet potato, maize, rice, spring onion, taro, strawberry, pimiento, melon, cucumber, tomato and celery et al.

SAS 8.2 software was used to conduct all statistical analyses. To analyze the inoculation effect of AMF on different crops, one way analysis of variance was used and means were compared by least significant difference (LSD) at the 5% level.

3. Results and Discussion

3.1 AMF diversity in agricultural field

In order to study AM fungal diversity, representative soil samples were collected from fields in which various crop plants were grown in north and northwest China. Isolation and purification of the mycorrhizal fungi led to the identification of 33 species in seven genera (Table 1). Sixty-one pure isolates and eighteen pure cultures were obtained, of which 11 pure cultures were registered in the European Bank of the Glomales (BEG). They are *G. mosseae* BEG167, *G. etunicatum* BEG168, *Glomus clarodieum* BEG180, *G. etunicatum* BEG181, *G. constrictum* BEG182, *G. mosseae* BEG189, *G. mosseae* BEG190, *G. mosseae* BEG191, *G. intraradices* BEG192, *G. intraradices* BEG193 and *A. morrowiae* BEG194. A germplasm stock was established for storage of the resources in the target area.

The results from analysis of the ecological diversity in the field soils showed that the frequency of occurrence of the genus *Glomus* was highest and *Acaulospora* was the second most abundant genus. *Glomus etunicatum*, *G. mosseae* and *G. intraradices* were the dominant species and species richness averaged 2.2 per sample. It was consistent with the former survey in arid and semiarid zones of north China (Zhang et al. 1994; Gai et al., 2006).

3.2 Temporal and spatial dynamics of AMF in high yield maize system

There were no significant differences in the temporal dynamics of root AM colonization between high input (HP) and optimum input treatments (Fig. 1). However, there was some difference among the arbuscular, vesicular and total colonization trend. It was obviously that arbuscular colonization was highest at R1, while vesicle formation reached maximum at V6. Spore density was in a similar temporal dynamics to arbuscular colonization, which reached maximum at R1, and then decreased.

Some AMF acquire nutrients near the roots while

Table 1. AM fungus isolated from North China

Genus	Species				
Acaulospora	*A. lacunosa*	*A. laevis*	*A. mellea*	*A. rugosa*	*A. scrobiculata*
	A. spinosa	*A.* sp.			
Archaespora	*Ar. gerdemannii*	*Ar. leptoticha*			
Entrophospora	*En.* sp.				
Gigaspora	*Gi. margarita*				
Glomus	*G. aggregatum*	*G. caledonium*	*G. claroidem*	*G. clarum*	*G. constrictum*
	G. diaphanum	*G. etunicatum*	*G geosporum*	*G. intraradices*	*G. macrocarpum*
	G. mosseae	*G. reticulatum*	*G. rubiforme*	*G. versiforme*	*G. fragarioides*
	G. sprum like	*G. 'cluster* sp'	*G.* sp.		
Paraglomus	*Pa. Occultum*				
Scutellospora	*S. calospora*	*S. gilmorei*	*S. pellucida*		

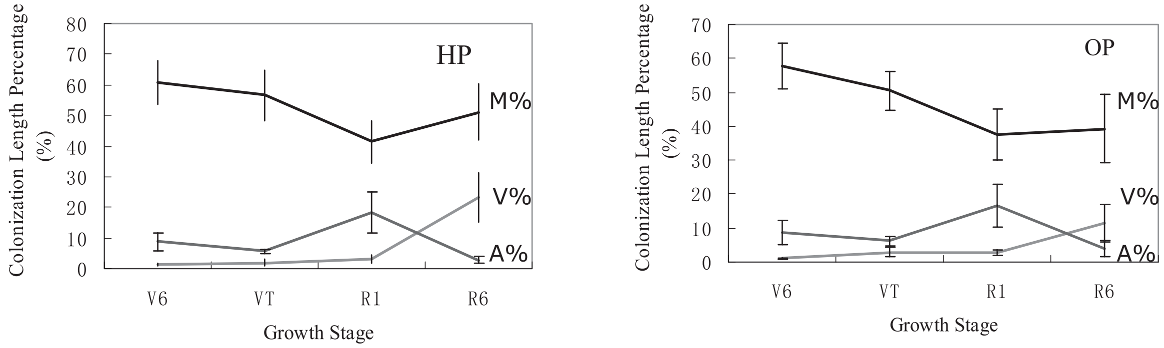

Fig. 1. Root Colonization Rates in HP and OP treatments. The bar shows the difference error, same as below.

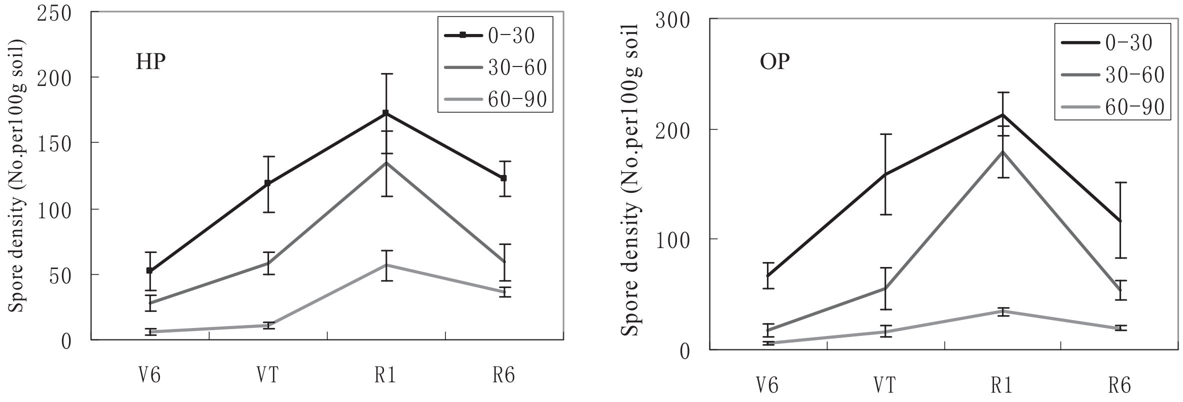

Fig. 2. Spore density of AMF at different soil depth categories in HP and OP treatments

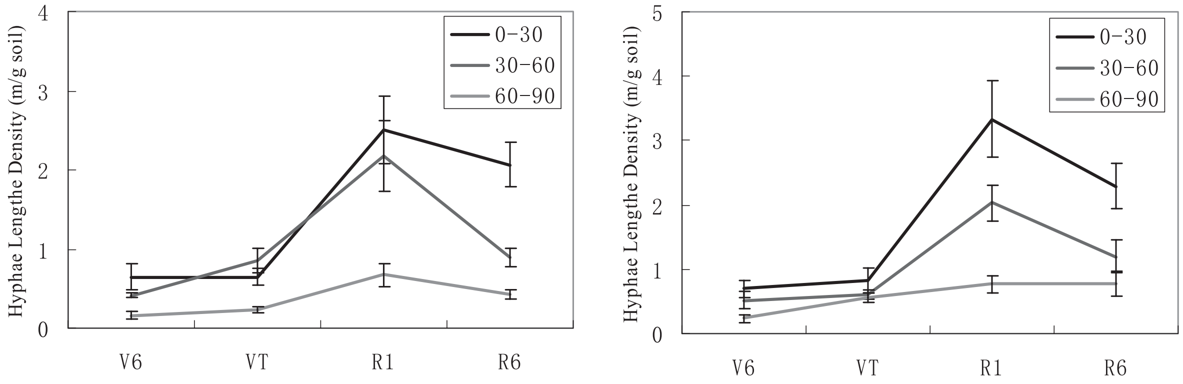

Fig. 3. Hyphal density of AMF at different soil depth categories in HP and OP treatments

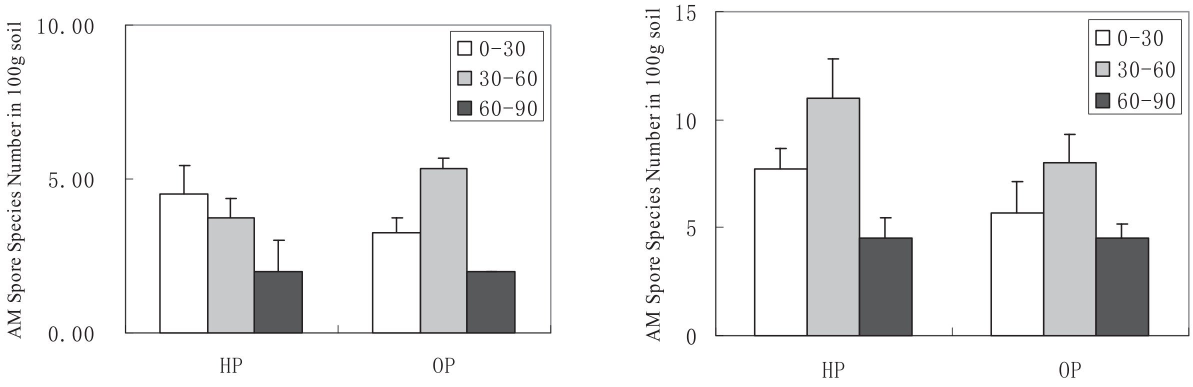

Fig. 4. Species richness of AMF at different soil depth categories in HP and OP treatments

others forage further away and explore different soil volumes (Smith et al., 2000). This can lead to increased productivity through the complementary use of resources. A similar phenomenon has been observed in plants with different rooting depths. Host preference and host range are two functional traits that are potentially very important. The present study showed that spore density were highest in the topsoil (at 0-30 cm) and showed a decreasing trend with increasing soil depth at both nutrient treatments (Fig. 2). Hyphal length and species richness (Fig. 3 and Fig. 4) was in a similar trend. Similar results were obtained in studies by Jakobsen and Nielsen (1983) and Rillig and Field (2003). Physical and chemical properties of soil change with depth and influence the distribution of soil organisms (Abbott and Robson 1991; Entry et al. 2002). Fungi are especially sensitive to low partial pressures of oxygen which prevail at depth (Brady and Weil 1996). AMF are also likely to be scarce where roots are sparse (Anderson et al. 1987).

3.3 Effect of AM fungi on staple crop growth

The inoculaton experimental results showed that the yield and quality of the tubers were improved by inoculation with mycorrhizal fungi. The isolates BEG168, GSP4 and BEG141 increased sweet potato tuber yield by 10% (Table 2). Reducing sugar content

Table 2. Tuber yield and yield index of different inoculation treatments

Treatments	FW (g/tuber)	Tuber no. per plant	Tuber yield (kg/plot)	Tuber yield (kg/hm^2)	Increasing (%)
CK	238.9b	4.5a	33.2b	34912b	0
BEG141	268.8ab	3.9a	36.6a	38488a	10
GSP4	247.8b	4.6a	37.0a	38949a	11
BEG168	304.5a	3.9a	36.8a	38739a	11
M3	280.0ab	3.6a	33.9b	35686b	2
Endo1	267.0ab	3.5a	35.5ab	37370ab	7

Table 3. Tuber quality index of different inoculation treatments

Treatments	Dry percentage (%)	Total starch content (%FW)	Reducing sugar content (%DW)	Total carotenes content (ug/gDW)
CK	22.3a	14.9a	15.78b	22.5b
BEG141	23.5a	14.5a	16.125ab	28.3a
GSP4	24.1a	15.2a	19.93a	22.1b
BEG168	23.4a	16.1a	17.11ab	26.9ab
M3	24.1a	14.8a	16.25ab	22.1b
Endo1	22.9a	14.9a	15.30b	23.8ab

Table 4. Yield of horticultural crops of different inoculation treatments

Treatments	Pimiento (kg/120seedings)	Melon (kg/84seedlings)	Watermelon (kg/40seedlings)	Celery (kg/20seedlings)	Green Chinese Onion (kg/hm^2)
AM	69.0a	31.45a	384.5a	16.2a	10472a
CK	65.5a	28.35b	342.0b	13.7b	12361b
Increasing (%)	5.5	10.9	12.4	18.3	15.3

and total carotenes were increased in isolates GSP4 and BEG141 by mycorrhizal inoculation (Table 3).

Inoculating AMF also increased the yield of some horticulture crops. The increasing rate varied from 5.5% to 18.3% (Table 4).

The experiment results indicate great potential of applying mycorrhizal biotechnology in sustainable agriculture in the future.

New methods such as quantitative molecular techniques were quite important for the investigation on the relative abundance of various AMF types and their impact on ecological processes. Moleculer and isotope method were also tentatively used in our present study to monitor of introduced AM fungi, to discriminate the contribution of introduced AM fungi to the crop growth. It still should be strengthened in the furtue study.

Acknowledgments

We thank the British Council and the UK Department for International Development through their Development Partnerships in Higher Education program (Project DelPHE 1.64) and the Special public sector research (Project 200803034) for generous financial support.

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JIFS, 7 : 47 − 50 (2010) Symposium Paper

Impact of Tubificid Worm on Nutrient Dynamics in Paddy Field

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Keywords: Paddy soil, Overlying water, Nitrogen mineralization, Phosphorus release

Received 1 February 2010; accepted 1 February 2010

Abstract

The soil-water-tubificid microcosm experiments with various densities of tubificid worm (*Branchiura sowerbyi*) were conducted for examining quantitative impact of tubificid on nitrogen and phosphorus dynamics in paddy soil. Tubificid worm increased bioavailable nutrients (nitrogen and phosphorus) in submerged paddy soils and the release rates from soils onto overlying water in proportion to the densities. These effects are presumed to be caused by acceleration of soil organic matter decomposition and enhanced diffusion of the nutrients from soil onto the overlying water. Tubificid worms influenced nutrient dynamics and will impact ecosystem in paddy fields.

Introduction

Tubificid worms (aquatic oligochaete) are known to be one of the dominant zoobenthos in eutrophic lake and influence lake ecosystems through increasing nutrient release to water from the bottom sediments (Fukuhara and Sakamoto, 1987; Fukuhara and Yasuda, 1989; Risnoveanu et al., 2004). Tubificids exist widely in paddy fields as the dominant macroinvertebrate group and showed higher densities in the fields with greater contents of soil organic matter and soil moisture (Simpson et al., 1993). It is also known that tubificids exist at high density in the ricefield with organic farming, and phytoplankton and zooplankton in the overlying water increase in the paddy soil with high density of tubificids more than in that without tubificids (Kikuchi and Kurihara, 1982). The effects on the nutrient dynamics are recognized to be responsible for the bioturbation of tubificids. Tubificid worms are known to be conveyor belt type feeders (Rhoads, 1974). The worms construct burrows and feed on surface sediment or soil and excrete into the surface of the material-water interface. The bioturbation activity of tubificid enhances not only nitrogen and phosphorus dynamics in sediments or soils but also the release into the overlying water and influences the ecosystems of lakes and paddy fields. The effects of tubificid on the formation rates and release rates into surface water of ammonium nitrogen and labile inorganic phosphorus have been measured for lake sediments (Fukuhara and Sakamoto, 1987; Fukuhara and Yasuda, 1989; Risnoveanu et al., 2004). The effects of tubificid worms on formation and release rates of ammonium nitrogen and labile phosphorus in paddy soils have been determined by comparison between the laboratory experiment with and without tubificids (Kikuchi and Kurihara, 1982). However, the quantitative effects of tubificid worm on nutrient dynamics in paddy soils have not been evaluated yet.

In this study, we determined quantitatively the tubificid impact on nitrogen and phosphorus release in paddy soil and onto the overlying water using laboratory microcosm with various densities of tubificid (*Branchiura sowerbyi*).

Materials and methods

We measured the population density of tubificids in the winter-flooded and organically managed ricefield of Oosaki city, Miyagi, Japan.

The soil-water-tubificid microcosm experiments were conducted under continuous dark and dark/light (12/12 hours) conditions at 20 ˚C for 4 weeks using 300 mL glass vials with 7 cm depth of paddy soil and 5 cm depth of overlying water. The luminosity was set to 7,500 lux for the light period. The soil used was alluvial paddy soil, which was collected from plow layer of the winter-flooded and organically managed ricefield of Oosaki city. Air-dried soil samples (<2 mm) and deionized water were used in the experiment. The soil with dry weight of 170 g was packed to each vial. Tubificid worm (*Branchiura sowerbyi*), which was collected from the above-mentioned paddy field, was added to the vials at three levels (0, 110 and 220 mg/vial on the basis of wet weight) with three replications. Because some of tubificid worms died in some treatments with continuous dark condition, we treated all data obtained from nine vials as independent values with no replications.

Concentrations of exchangeable ammonium, available phosphorus and ferrous iron were measured in the submerged soil after 4 weeks. They were extracted with 2M KCl solution, Bray 2 solution (soil to solution ratio of 1:20) and acetate buffer solution (1M, pH2.8), respectively. The values increased from the initial state were calculated. The ammonium-nitrogen and dissolved inorganic phosphorus released onto overlying water from soils were estimated for three weeks. Ninety percent of overlying water was collected weekly and the concentrations of ammonium and dissolved inorganic phosphorus were measured.

Results and discussion

The major species of tubificids were *Limnodrilus socialis* and *Branchiura sowerbyi* in the ricefields surveyed. The population densities of tubificids were higher in the ricefields with organic farming than those of the control ricefields with application of agricultural chemicals (data were not shown).

Amounts of ammonium nitrogen and available phosphorus formed in the soils significantly increased with the increasing of tubificid densities under both of continuous dark and dark/light conditions (Figs. 1 and 2). Tubificid accelerated soil nitrogen mineralization with the rates of 2.3 and 1.4 µg N/mg animal wet weight/kg soil/day in the continuous dark and dark/light conditions, respectively. Also, tubificid increased soil available phosphorus contents with the rates of 11 and 7.2 µg P/mg animal wet weight/kg soil/day in the continuous dark and dark/light conditions, respectively. In comparison with the continuous dark treatment, formation rates of ammonium nitrogen and available phosphorus reduced in the dark/light treatment. Phytoplankton will grow better and release more oxygen in the overlying water for the dark/light treatment than the continuous dark treatment. The surface soil was more oxidized in the dark/light vials than the continuous dark vials. This is suggested by the lower values of ferrous iron contents in the dark/light treatment (Fig. 3). In such environment, ammonium will be easily transformed into nitrate and be lost through denitrification, and released inorganic phosphorus will be rapidly absorbed by ferric iron oxide precipitate produced by oxidation of ferrous iron in the upper layer soils. These processes

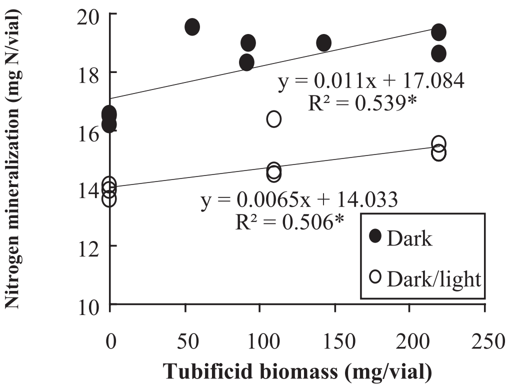

Fig. 1. Relationship between nitrogen mineralization in soil and tubificid biomass

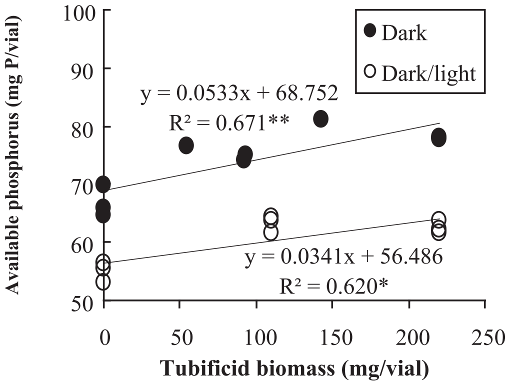

Fig. 2. Relationship between available phosphorus (Bray 2) content in soil and tubificid biomass

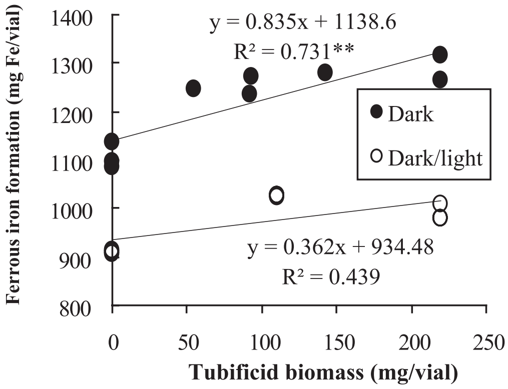

Fig. 3. Relationship between ferrous iron formation in soil and tubificid biomass

will cause the lower values of ammonium nitrogen and available phosphorus for the dark/light treatment.

Figure 3 showed that ferrous iron contents formed in the submerged soils increased with the increment of tubificid biomass. This means that tubificid activity stimulated development of reduced condition in the soil through the acceleration of soil organic matter decomposition. From the results mentioned above, the increment of soil nitrogen mineralization and available phosphorus content is presumed to result from hastening decomposition of soil organic nitrogen and dissolution of phosphorus absorbed by ferric iron oxide with development of reduced condition.

Amounts of ammonium and dissolved inorganic phosphorus released from the soils onto the overlying water were significantly increased with the increasing of tubificid densities under continuous dark condition at the rates of 0.22μg N/mg animal wet weight/day and 0.0095μg P/mg animal wet weight/day, respectively (Figs. 4 and 5). The results of the present study agrees with the study conducted on lake sediments (Fukuhara and Sakamoto, 1987). Tubificid worms construct burrows, and feed on and excrete surface soil. Such a physical disturbance of soil by tubificid worm enhances soil-water interface area and accelerates nutrient diffusion from soil into overlying water. The enhanced release rates of inorganic nitrogen and phosphorus are inferred to lead to a multiplication of phytoplankton and other organisms in the overlying water.

On the other hand, no nitrogen and phosphorus releases were found in the dark/light condition. The reason for absence of nitrogen and phosphorus in the overlying water under the dark/light condition can be explained as follows. In the overlying water of the dark/light vials, phytoplankton uptakes inorganic nitrogen and phosphorus. In addition to phytoplankton uptake, ammonium is transformed into nitrate and is lost with denitrification, and released inorganic phosphorus is rapidly absorbed by ferric iron oxide precipitate produced with oxidation of soluble ferrous iron in the oxygen rich overlying water of the dark/light vials.

From the results of this study, it is concluded that bioavailable nutrients in submerged paddy soils increased in proportion to the densities of tubificid worm due to the acceleration of soil organic matter decomposition by tubificid activity, and tubificid enhanced the nutrients (N and P) release from soils onto overlying water in proportion to the animal densities. Tubificid worms influence nutrient dynamics and will impact ecosystem in paddy fields.

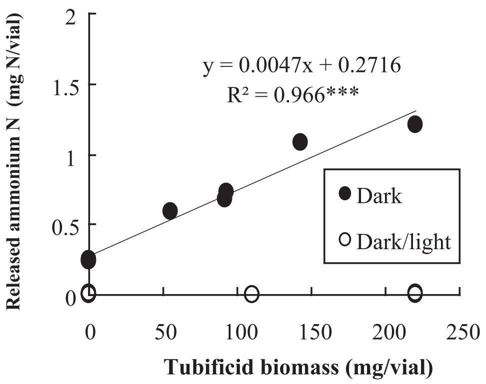

Fig. 4. Relationship between ammonium nitrogen release onto overlying water and tubificid biomass

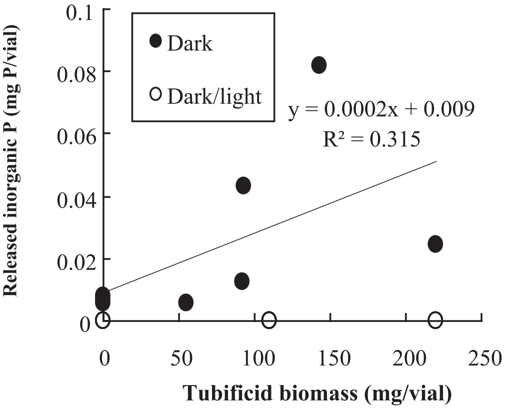

Fig. 5. Relationship between inorganic phosphorus release onto overlying water and tubificid biomass

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JIFS, 7 : 51 − 58 (2010) Symposium Paper

Earthworm and Soil Carbon Sequestration after Twenty One Years of Continuous No-tillage Corn-Legume Rotation in Indonesia

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Keywords: Conservation tillage, microbial biomass and plant residues

Received 6 October 2009; accepted 1 February 2010

Abstract

Soil carbon and earthworm are important components in sustainable tropical agro-ecosystem. Long-term experiment was initially conducted in February 1987, at experiment farm of *Politeknik Negeri* Lampung, Sumatra, Indonesia. The soil is a Typic Fragiudult with slope ranging from 6 to 9%, located at 105˚13'E, 05˚21'S, with elevation 122 m. The experiment was a factorial, randomized complete block design, with 4 replications. Tillage treatments were conservation tillage (no-tillage, NT and minimum tillage, MT), and conventional tillage (CT); while nitrogen fertilization rates were 0, 100 and 200 kg N ha^{-1}. Cropping pattern of the long-term experiment was cereal-legume-fallow rotation. Due to soil compaction in 1997 and 2002, all plots of conservation tillage were plowed. After eleven years of cropping, the soil became acid, therefore; in 2004 all plots were limed with 4 Mg ha^{-1} of $CaCO_3$. To determine the effect of long-term no-tillage and N fertilization on earthworm, the soil samples were collected at depth of 0-10 cm in 2001 and 2008; and for soil microbial biomass C and soil organic C, samples were taken at depth 0-20 cm in 2008 after 21 years of continuous experiment.

The only treatment that affected earthworm population in both 14th and 21st year of cropping was tillage treatment. After 14 years of cropping (2001), NT had earthworms averaged of 101m^{-2}, 46% and 39% ($p<0.05$) higher than CT and MT, respectively. In 21st year of cropping, however, the response showed a little change; NT had earthworm with averaged of 99 m^{-2}, 251% higher ($p<0.05$) than CT, but 40% lower than MT. In rhizosphere samples, MT had microbial biomass 228 mg C-CO_2 $kg^{-1}day^{-1}$, or 45.5% higher ($p<0.05$) than NT and 70.7% higher than CT; while 100 kg N ha^{-1} N treatment had 227 mg C-CO_2 kg^{-1} day^{-1}, 28% and 103% higher ($p<0.05$) than 0 and 200 kg N ha^{-1} treatments, respectively. After 21 years of cropping, soil organic carbon of MT in rhizosphere at 0-20 cm soil depth was 16.5 g kg^{-1}, or 9.3% and 13.0% higher ($p<0.05$) than NT and CT, respectively; but in non-rhizosphere was not significantly ($p<0.05$) affected by any treatment.

Introduction

No-tillage is one of few revolutions in agriculture that has greatly impacted agriculture throughout the world (Triplett and Dick, 2008). In fact, no-tillage is an academically modified ancient land preparation technology namely '*slash and burn*' that successfully has been practiced in modern agriculture (Utomo, 2004). Due to the ability to conserve soil and to increase efficiency, worldwide adoption of no-tillage has been rapidly expanded since about 1990, particularly in the United States, South American countries and Africa. While Asia and Europe have been slower to adopt no-tillage, primarily due to their long history of conventional tillage practices. However, reports from Conference of the International Soil Tillage Research indicate this is changing (Derpsch, 1998; Triplett and Dick, 2008). In Indonesia, no-tillage has been initially promoted by few no-tillage researchers in 1980's, and successfully practiced by farmers just in 1990's, particularly in the region with lack of labors, such as in Sumatra, Borneo and Celebes. Yet in 1998, no-tillage has been explicitly stated in national land preparation policy (Utomo, 2004).

As for conservation tillage, no-tillage requires at least 30% plant residues as mulch covering soil surface (Lal, 1989). Plant residues serve as substrate that is converted to microbial biomass and soil organic matter, and have the potential to enhance carbon sequestration in agricultural soils (Wright and Hons, 2004). The presence of plant residues will create better microclimate that can enhance soil biota activity, especially earthworms (Lavelle, 1984; Brito-Vega, et al., 2009). However, the responses of earthworm population to no-tillage have been variable, and the impact is dependent on soil factors, climatic conditions and the tillage operations. Results of survey in USA showed that eight of the 14 sites had higher earthworm populations in no-tillage than in conventional tillage, with increases ranging from 25% higher to 10 times higher (Kladivko, 1993). The declines in earthworm population often reported in conventionally tilled soils are associated with undesirable changes in soil environmental condition resulting from excessive tillage (Chan, 2001). Research conducted in dry land farming in the Pacific Northwest, USA showed that earthworm populations under no-tillage were more abundance than under conventional tillage (Wuest, 2001). Brito-Vega et al. (2009) reported that in Mexico earthworm populations under conservation tillage at depth of 10-20 cm were 240 individuals m^{-2} in spring and reduced to 60 individuals m^{-2} in winter; while under conventional tillage reduced from 192 in spring to 72 individuals m^{-2} in winter. The survey on nine years of soybean-barley field under different cropping systems in Japan confirmed that, there were no earthworms in the tilled field without mulch, a few in no-tillage without mulch, but there were increasing number of earthworms in no-tillage with mulch and reached the peak in the ninth year of cropping (Nakamura, et al., 2003).

The increasing numbers of earthworm and other soil biota in no-tillage can contribute to carbon sequestration in agro-ecosystem. Earthworm (*Lumbricus rubellus* and *Aporrectodea caliginosa*) for instance, enhanced total C efflux. The interaction between earthworms and soil microbial processes has important implication for soil C turn over and in turn, C sequestration (Zhang and Hendrix, 1995). Overcoming soil organic matter decline through C sequestration therefore, is important way to sustain agro-ecosystem in the tropics. Shift from high to lower soil disturbance such as conventional tillage to no-tillage, often promotes the accumulation of otherwise labile soil organic carbon that is less available to microbial attack, controls carbon decomposition rates and increases total microbial biomass (Paustian et al., 1997; Six et al., 2006). Assessment of the impact of long-term no-tillage on soil carbon sequestration conducted in the eastern United States showed that no-tillage increased soil organic carbon in the upper layer of soil, but it did not store soil organic carbon more than conventional tillage for the whole soil profile (Blanco-Canqui and Lal, 2008). Similar to this study, after 20 years of study in Texas, it was showed that soil organic carbon storage under no-tillage for all cropping sequence at 0-5 cm depth was 64% greater than conventional tillage, but at 5-15 cm, organic carbon storage was only 28% higher (Wright and Hons, 2004).

The beneficial effect of long-term no-tillage over conventional tillage has been published mostly in temperate regions, but very few published in tropical region such as Indonesia. The objective of this experiment was to determine the effect of 14th and 21st year of consecutive no-tillage corn-legume rotation on earthworm population and carbon sequestration in a Typic Fragiudults in Sumatra, Indonesia.

Materials and Method

Site characteristics

A consecutive twenty one years of no-tillage experiment (37 crop seasons) was initiated in February 1987, at the experiment farm of *Politeknik Negeri* Lampung, Sumatra, Indonesia (105˚13'45.5"- 105˚ 13'48.0"E, 05˚21'19.6"- 05˚21'19.7"S; 122 m elevation). The soil is a Typic Fragiudult with slope ranging from 6 to 9%. Soil particle sizes composition in the 0-20 cm layer before this long-term experiment (1987) was 160, 320 and 520 g kg^{-1} of sand, silt and clay, respectively; while bulk density 0.90 Mg m^{-3} and total porosity 65.7%. The other initial soil chemical characteristics are presented on Table 1. The climate of the site is humid tropical with average rainfall of 1800 to 2250 mm yr^{-1} for 20 years. The site of experiment was previously a *ladang* (a local land rotation with period of fallow), which was abandoned for more than four years and covered by *alang-alang* (*Imperata cylindrica*) grass. Before the 21st of this experiment, the plot was left fallow for a year and weeds were still dominated by *alang-alang* (*Imperata cylindrica*) with average dry matter weight 13.3 Mg ha^{-1}.

Method and analysis

The experiment was a factorial, randomized complete block design, with 4 replications. Plot size was four by six meters. Tillage treatments were conservation tillage (no-tillage, NT and minimum tillage, MT), and conventional tillage (CT); while nitrogen fertilization rates were 0, 100 and 200 kg N ha^{-1}. In NT, weed dominated by *Imperata cylindrica* was sprayed with glyphosate of 4.8 L a.i. per hectare, and the deadly weed covered the soil surface as mulch; in MT, it was the same as NT except soil surface which was slightly tilled; while in CT before plowing *Imperata cylindrica* was removed and the soil was plowed at depth 0-20 cm. Cropping pattern of the long-term experiment was cereal (corn or upland rice)-legume (soybean or cowpea)-fallow rotation (Utomo, et al., 1989). For cereal crop, hybrid corn (*Zea mays* L.) was planted each year at spacing of 75 X 25 cm. Nitrogen fertilizer treatment was not applied when legume was planted. Due to the upper layer soil compaction, all plots of conservation tillage were plowed in 1997 and 2002. After 14 years of cropping, the soil became acid (Table 1); therefore, in 2003 all plots were limed with 4 Mg ha^{-1} of $CaCO_3$.

To determine the effect of long-term no-tillage and N fertilization on earthworm, the monolith samples with size of 25 by 25 cm^2 were collected at depth of 0-10 cm in 2001 and 2008 prior to flowering time; and earthworm was counted by hand sorting. Due to technical problem, however, plots treated with N rate of 100 kg N ha^{-1} were not sampled. Soil samples for microbial biomass carbon and soil carbon and other chemical characteristics were taken at depth of 0-20 cm within rhizosphere and outside of rhizosphere at harvest time in May 2008. Microbial C content of the incubated soil was determined using the chloroform fumigation-incubation method. Soil organic C was analyzed with Walkey and Black method, soil organic N with Kjeldahl method, and pH with pH meter.

The analysis of variance and means test (LSD 0.05) were run using the statistics package of SAS (Statistical Analysis System).

Results and Discussion

Earthworm population, biomass and cast weight

Earthworms can have significant impacts on soil properties through their feeding, casting and burrowing activity. Their activities are influenced very much by soil management that affects their habitat. With crop residues as mulch, conservation tillage will create conducive habitat to attract earthworm (Kladivko, 1993; Lal, 1989).

Based on analysis of variance, the only treatment

Table 1. Soil pH, organic C and total N at depth of 0-20 cm prior to experiment after long-term (14 years) continuous tillage systems and N fertilization (2001)

Treatments		pH		Organic C	Organic N
Tillage	N Fertilizer	H_2O	KCl	(g kg^{-1})	(g kg^{-1})
1987 (initial)	-----------	6.8	5.8	16	2.0
2001 (prior to experiment)					
Conventional tillage	0 kg N ha^{-1}	5.0	4.6	15.2	0.9
	100 kg N ha^{-1}	4.8	4.3	15.2	1.1
	200 kg N ha^{-1}	4.7	4.6	16.8	0.8
Minimum tillage	0 kg N ha^{-1}	5.2	4.2	17.8	1.5
	100 kg N ha^{-1}	5.0	4.7	17.6	0.9
	200 kg N ha^{-1}	4.8	4.5	16.8	1.3
No-tillage	0 kg N ha^{-1}	5.1	4.7	15.2	0.9
	100 kg N ha^{-1}	5.0	4.6	16.5	0.9
	200 kg N ha^{-1}	4.7	4.4	16.1	1.0

that affected ($p<0.05$) earthworm population both in 14th and 21st years was tillage treatment. After 14 years of cropping (2001), NT had earthworms averaged of 101 m^{-2}, 46% and 39% higher than CT and MT, respectively. After 21 years of cropping, however, the response showed a little change; NT had earthworm averaged of 99 m^{-2}, 251% higher than CT, but 40% lower than MT. It turned out that in 14th year of cropping, NT had the highest earthworm population among other tillage system, but in 21st year of cropping NT had similar effect with either MT or CT (Table 2). Similar effects were shown in long-term (25 years) dry-land tillage practices on earthworms in southern Alberta, Canada (Claperton et al., 1997), and in 30 years experiment of tillage system in the Pacific Northwest, USA (Wuest, 2001). The lower of earthworm population in CT was associated with undesirable changes in the soil environmental conditions resulting from excessive tillage (Chan, 2001). In this experiment, all plant residues in CT treatment were removed and soil was plowed; while in conservation tillage all plant residues remained on the soil surface as mulch. The present organic plant residues with respect of conservation tillage (NT and MT) had significant effect on the population of earthworm (Kladivko, 1993; Chan, 2001; Brito-Vega, 2009). Tillage system such as conservation tillage which leaves surface residue is one of the important ways to influence the earthworm population. The presence of plant residues will create better microclimate that can enhance earthworm activity (Lavelle, 1984; Brito-Vega, et al., 2009). Conservation tillage has higher earthworm populations than conducting moldboard system, due to increased food supply and mulch protection. With residues on surface, the food supply is available to the earthworms for a longer time than residues removed (Kladivko, 1993). Previous plant residue weight which was used as mulch for conservation tillage (NT and CT) in this experiment averaged of 13.3 Mg ha^{-1}; created better habitat for the earthworm. Nutrient contents of crop residues were N 10.1 g kg^{-1}, P 1.1 g kg^{-1}, K 2.5 g kg^{-1}, Ca 3.8 g kg^{-1}, Mg 1.4 g kg^{-1}, C 322.6 g kg^{-1} and C/N 31.9. Under conservation tillage systems, earthworms can potentially play an important role than under conventional tillage in the functioning of the farming systems because of their abilities to modify soil physical environment and nutrient cycling. The earthworms genus identified in this experiment were *Pheretima* and *Lumbricus*.

Contrary to earthworm population, although earthworm biomass and cast weight under NT were relatively higher than other treatments, it seems that both earthworm biomass and cast weight were not significantly ($p<0.05$) affected by any treatment both in 14th (2001) and in 21st (2008) long-term experiments. The relatively higher soil strength due to long-term conservation tillage at 0-10 cm soil depth tended to decrease the size of earthworms with respect to NT and MT, resulted in the decreased of their biomass. Less responses of N fertilization to earthworm biomass and cast weight were also shown in this experiment

Table 2. Effect of long-term (14 and 21 years) continuous tillage systems and N fertilization on earthworm population, biomass and cast weight at soil depth 0-10 cm

	Population		Biomass		Cast weight	
Treatments	2001*	2008	2001*	2008	2001*	2008
	(indiv.m^{-2})		(g m^{-2})		(g m^{-2})	
Conventional tillage	69.33 b	28.40 b	6.16 a	0.16 a	7.13 a	12.57 a
Minimum tillage	72.89 b	167.15 a	6.11 a	6.69 a	5.34 a	105.36 a
No-tillage	101.33 a	99.56 ab	6.97 a	1.53 a	7.77 a	121.81 a
0 kg N ha^{-1}	78.22 a	119.12 a	6.86 a	2.23 a	5.28 a	40.09 a
100 kg N ha^{-1}	85.33 a	---	6.56 a	---	5.09 a	---
200 kg N ha^{-1}	83.55 a	77.62 a	5.83 a	3.36 a	9.86 a	119.73 a

* In 2001, all plots of conservation tillage were plowed, and N treatments were not applied.
Values within a column followed by the same letter are not significantly different at 0.05 levels

(Table 2), but there were no negative effects of N fertilizer to the earthworm's activities.

Carbon sequestration

As labile component of soil organic C, soil microorganisms can contribute to microbial biomass C (Wang, et al., 2001), and in turn contribute to C sequestration. Carbon sequestration was measured as soil microbial biomass C and soil organic C both in rhizosphere and non-rhizosphere at depth 0-20 cm of the 21st year of cropping (at harvesting time in May 2008).

Based on analysis of variance it was showed that microbial biomass C both in rhizosphere and non-rhizosphere was significantly (p<0.05) affected either by tillage system or by N fertilization, but not affected by their interactions. It turned out that both in rhizosphere and in non-rhizospher, MT had microbial biomass C higher than other tillage system, while NT tended to have soil microbial biomass higher than CT, but in fact its effect is statistically the same as CT.

Generally, conservation tillage with high surface residue results in higher C sequestration than CT. Every season, all previous plant residues in conservation tillage were used as mulch covering the soil surface. Previous plant residue weight which was used as mulch for conservation tillage (NT and MT) in the 21st year experiment averaged of 13.3 Mg ha^{-1}, while in CT there was no surface mulch. Additions of plant residues on the surface and less soil disturbance will increase soil organic C particularly in upper layer of the soil. Conversely, with no mulch on the surface and because of soil disturbance, conventional tillage will decrease soil organic C due to erosion and decomposition. Loss of soil organic C caused by tillage has been estimated to be 16 to 77% (Mann, 1986). Nitrogen fertilization with medium rate (100 kg N ha^{-1}) had the highest (p<0.05) microbial biomass C both in rhizosphere and in non-rhizosphere, while N fertilization with the highest rate (200 kg N ha^{-1}) had the lowest microbial biomass C.

Different from microbial biomass C, the only treatment that affected (p<0.05) soil organic C in rhizospher was tillage system. Minimum tillage had the highest soil organic C, but it was not different from NT (Table 3). It seems that soil microbial biomass C and organic C in rhizosphere are consistently higher than those in non rhizosphere. This is related to the higher root exudates as substrate for microorganism activity resulting in more microbial biomass (Wang, et al., 2001), and higher soil organic matter with respect to rhizosphere. In contrast, soil organic C in non-rhizosphere at depth of 0-20 cm was not significantly (p<0.05) affected by any treatment, even by tillage system. This was attributable to a dilution effect of sampling depth on soil organic carbon. In fact, there is a mark stratification of soil organic matter with soil under long-term NT (Blevins et al., 1984; Unger, 1991). After 20 years of study in Texas it was showed that soil organic carbon storage under no-tillage for all cropping sequence at 0-5 cm depth was 64% greater than conventional tillage, but at 5-15 cm,

Table 3. Effect of long-term (21 years) continuous tillage systems and N fertilization on soil microbial biomass C and soil organic C at soil depth 0-20 cm (2008)

Treatments	Soil Microbial Biomass C: Rhizosphere	Soil Microbial Biomass C: Non-Rhizosphere	Soil Organic C: Rhizosphere	Soil Organic C: Non-Rhizosphere
	(mg C-CO_2 kg^{-1} day^{-1})		(g C kg^{-1})	
Conventional tillage	156.91 b	48.78 b	14.6 b	12.6 a
Minimum tillage	227.64 a	74.80 a	16.5 a	13.8 a
No-tillage	182.11 b	56.10 b	15.1 ab	13.3 a
0 kg N ha^{-1}	177.24 b	62.61 b	15.7 a	13.2 a
100 kg N ha^{-1}	277.23 a	75.61 a	15.9 a	14.2 a
200 kg N ha^{-1}	112.20 c	41.46 c	14.6 a	12.2 a

Values within a column followed by the same letter are not significantly different at 0.05 levels

organic carbon storage was only 28% higher (Wright and Hons, 2004). Clap et al. (2000) also reported that a 14% increase was observed in soil organic in the top 15 cm of NT soil, but it decreased in the 15-30 cm depth.

Soil Organic N and pH

Soil N and pH are among factors that can influence earthworm and soil C dynamic (Kladivko, 1993; Brito-Vega, et al., 2009). Soil organic N and pH were measured at depth 0-20 cm in the 21st years of cropping as those of microbial biomass C were done. Soil organic N and soil pH both in rhizosphere and non-rhizosphere were significantly (p<0.05) affected by tillage system or by N fertilization treatment; except for soil organic N in non-rhizosphere that was only affected by N fertilization (Table 4). It turned out that in rhizosphere MT system had soil organic N higher than CT, but had no difference from NT. Similar responses of soil organic N to tillage system were reported by Zibilske et al. (2002), and Brito-Vega, et al. (2009). Significant effect of the treatment is attributable to mineralization N from plant residues which returned it back each year. In this experiment, decomposition rate of plant residue under conservation tillage is 65% season^{-1} (Utomo, et al., 1989), a potential released of N from plant residues under MT and NT system is about 87 kg N ha^{-1}yr^{-1}.

Nitrogen fertilization with rate 200 kg N ha^{-1} had the highest soil organic N both in rhizosphere and in non-rhizosphere, while no N fertilization had the lowest soil organic N (Table 3). It means, 200 kg N ha^{-1} had residual N effect higher than with no N fertilization. Referring to initial soil organic N content in 1987 (Table 1), both conservation tillage and higher N rate can retain more soil organic N from depletion than other treatments. Long-term loss reductions and greater retention of N fertilizer due to N immobilization in conservation tillage may improve crop N use efficiency by subsequent re-mineralization of the N in better synchrony with crop need (Utomo, et al., 1989; Zibilske et al., 2002).

Different from soil organic N response, CT had the highest (p<0.05) pH both in rhizospher and in non-rhizosphere, while NT had the lowest pH but similar to MT; and 200 kg N ha^{-1} had the lowest pH than other N rate. It indicates that, either conservation tillage or N fertilization has acidifying effect higher than CT or with no N fertilization. In long-term crop production, however, any tillage has similar acidifying effect. The surface acidifying effect of long-term no- tillage, especially when it is combined with high N rates, is consistent with data in temperate region reported by Thomas and Frye (1984). Data on Table 4 show that after 14 years of cropping, pH of soil reduced from 6.2 (initial pH in 1987) to 4.7 (after 14 years of cropping) in any tillage with 200 kg N ha^{-1}, while any combination with 0 kg N ha^{-1} reduced to pH around 5.0. This is the reason why in 2004 all plots were limed with 4 Mg $CaCO_3$ ha^{-1}.

Table 4. Effect of long-term (21 years) continuous tillage systems and N fertilization on soil organic N and soil pH at soil depth 0-20 cm (2008)

Treatments	Soil Organic N (g kg^{-1}) Rhizosphere	Soil Organic N (g kg^{-1}) Non-Rhizosphere	Soil pH Rhizosphere	Soil pH Non-Rhizosphere
Conventional tillage	1.5 b	1.4 a	6.4 a	6.5 a
Minimum tillage	1.8 a	1.6 a	6.2 ab	6.3 b
No-tillage	1.6 ab	1.5 a	6.1 b	6.2 b
0 kg N ha^{-1}	1.4 b	1.3 b	6.4 a	6.5 a
100 kg N ha^{-1}	1.6 ab	1.5 ab	6.2 a	6.4 a
200 kg N ha^{-1}	1.8 a	1.7 a	6.0 b	6.1 b

Values within a column followed by the same letter are not significantly different at 0.05 levels

Conclusion

After 14 years of cropping (2001), NT had earthworms averaged of 101 m^{-2}, 46% and 39% higher ($p<0.05$) than CT and MT, respectively. In 21st year of cropping, however, the response showed a little change; NT had earthworm averaged of 99 m^{-2}, 251% higher than CT, but 40% lower ($p<0.05$) than MT. The earthworm genus identified was *Pheretima* and *Lumbricus*.

In rhizosphere samples, MT had microbial biomass 228 mg $C\text{-}CO_2$ kg^{-1} day^{-1}, or 45.5 % higher ($p<0.05$) than NT and 70.7% higher than CT; while 100 kg N ha^{-1} N treatment had 227 mg $C\text{-}CO_2$ $kg^{-1}day^{-1}$, 28% and 103% higher than 0 and 200 kg N ha^{-1} treatments, respectively.

After 21 years of cropping, soil organic C of MT in rhizosphere at 0-20 cm soil depth was 16.5 g kg^{-1}, or 9.3% and 13.0% ($p<0.05$) higher than NT and CT, respectively. At depth of 0-20 cm in non-rhizosphere, there was less response of soil organic C to NT.

Long-term continuous no-tillage corn-legume rotation in Indonesia has potentially to increase earthworm activity and to sequester C better than conventional tillage.

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Radiocarbon Evidence for Contrasting Soil Carbon Dynamics in a Andisol and Non-Andisol Pasture Soil Comparison

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Keywords: C-14, soil organic matter turnover, carbon models, sequestration, grassland, resampled sites

Received 19 February 2010 ; accepted 26 February 2010

Abstract

In 1959, Athol Rafter began a substantial programme of monitoring the flow of ^{14}C produced by atmospheric thermonuclear tests through New Zealand's atmosphere, biosphere and soil. A database of over ~400 soil radiocarbon measurements spanning 50 years has now been compiled. A key comparison within the dataset is described here, providing quantification of the differences in soil C dynamics between Andisols and non-Andisols. We use ^{14}C to quantify soil carbon turnover parameters in deforested dairy pastures under similar climate in the Tokomaru silt loam (non-Andisol) versus the Egmont black loam (Andisol), originally sampled in 1962, 1965 and 1969. After adding surface soils sampled to a similar depth in 2008, we use a 2-box model to calculate that the residence time of stabilized soil C in the Tokomaru soils is ~9 years compared to ~17 years for the Egmont soils. This difference represents nearly a doubling of soil C residence time, and roughly explains the doubling of the soil C stock. With three measurements in the 1960s, and an assumption of a 1000 year residence time for passive soil C in the surface layer, the data is of sufficient resolution to estimate that passive soil C comprises 15% of the soil C pool in Tokomaru soils versus 27% in Egmont soils. The Tokomaru/Egmont comparison is necessarily illustrative since neither site was replicated extensively, but does provide globally unique data. The comparison supports evidence that C dynamics does differ in Andisols versus non-Andisols, as a result of both the mineral allophane and Al complexation.

Introduction

In 1959, Athol Rafter began a substantial programme of monitoring the flow of ^{14}C produced by atmospheric thermonuclear tests through New Zealand's atmosphere, biosphere and soil. The "bomb-^{14}C" augments the natural cycling of ^{14}C, overlaying annual and decadal resolving power onto the centennial and millennial resolution obtained from radioactive decay (half-life = 5730 years). The programme produced important publications (e.g. O'Brien and Stout, 1978) and leaves a legacy of unpublished data critical for understanding soil C dynamics. A database of over ~400 soil radiocarbon measurements spanning 50 years has now been compiled. This time-series ^{14}C data provides an opportunity to quantify soil C dynamics in the C pools that interact with atmosphere at decadal timescales. These pools have been included in most terrestrial ecosystem biogeochemistry models, yet concerns remain that conceptual pools used in models cannot be isolated and quantified (Baisden and Amundson, 2003; Bruun et al., 2009). Multi-decade ^{14}C datasets and modeling provide a clear and compelling approach that allows the dynamics of conceptual soil C pools to be quantified without the chemical or physical isolation of C fractions specifically representing the conceptual soil pools. The use of time-series ^{14}C datasets therefore creates the opportunity to quantify how models should represent proposed differences between C dynamics in Andisols and non-Andisols (Torn et al., 1997; Parfitt, 2009).

This work builds on research showing that bulk soil and the separation of 2 soil fractions on the basis of density quantifies soil C turnover for the main soil C pool with approximately the same degree of certainty as a more complex separation into 5 density fractions (Baisden et al., 2002a). Model estimates of

C turnover calculated using time-series ^{14}C data as a function of depth under pasture in a New Zealand silt loam soil (Dystrochrept) found that 'active', 'stabilized' (decadal) and 'passive' (inert) pools comprise 10, 75 and 15% of total soil organic C (Baisden and Parfitt, 2007). The nature of radiocarbon as a decadal and millennial tracer, combined with the relatively small size of the active pool and large size of the stabilized pool in New Zealand soils emphasizes the opportunity to undertake modeling that assumes the active pool is negligible, and calculates residence times and pool sizes for the large stabilized and passive C pools.

Key aspects of time-series soil ^{14}C datasets are described here, with an emphasis on quantifying the differences in soil C dynamics between Andisols and non-Andisols (as defined by USDA soil classification). In particular, we focus on comparison of soil carbon dynamics in deforested dairy pastures under similar climate in the Tokomaru silt loam (non-Andisol) versus the Egmont black loam (Andisol), originally sampled in 1962, 1965 and 1969. Here, we add measurements for surface soils sampled to a similar depth in 2008 and calculate residence times for the major pools of C present in surface soil. We examine the calculated residence times in terms of observed flow of carbon through the pastures and the size of soil C stocks.

Methods

In this work, we collate information obtained over nearly 50 years from an "experiment" effectively identified during the 1960s by researchers interested in using ^{14}C to identify differential rates of C cycling in pasture soils with and without substantial presence of volcanic mineralogy delivered by andesitic tephra (Figure 1). Egmont loam soils (Hapludands) were sampled within 50 km of the tephra source, Mt. Taranaki (also known as Mt. Egmont). Tokomaru silt loam soils (Fragiaqualfs) were sampled 150-200 km to the southeast where the soil parent material was dominated by quartzo-feldspathic loess, and the soil contains no allophane. Climate and pasture production are similar in the two locations (Roberts and Thompson, 1984; Radcliffe, 1976). Mean annual soil temperature for both sites is ~12˚C and mean annual rainfall in the range of 1000-1300 mm. During the period 1962-1969, soils were collected using 12 2.6 cm soil cores per site, to a depth of 8 cm, as reported in Jackman (1964) and Schipper and Sparling (2009). Later soils were collected from soil pits either as part of New Zealand Soil Bureau soil survey activities, or using similar methods (Schipper et al., 2007) and are part of ^{14}C studies as a function of soil depth to be reported elsewhere. The 2008 Egmont sample is 0-5 cm.

All Δ^{14}C data is reported as defined in Stuiver and Polach (1977) and is the result of combusting soil samples to CO_2 and subsequent ^{14}C determination at the Rafter Radiocarbon Laboratory and its predecessor. Values reported are for <2 mm soil with live roots removed and no chemical treatment. For samples collected prior to 1980, gas proportional counting was used, while more recent samples were analyzed using accelerator mass spectrometry (EN-Tandem). All Δ^{14}C values obtained from gas proportional counting have been recalculated using original counting statistics. In all cases, analytical error is ≤6‰. Additional samples from the Tokomaru and Egmont pastures established in 1870 and 1900, respectively, were collected and measured in 1971 but have been excluded from

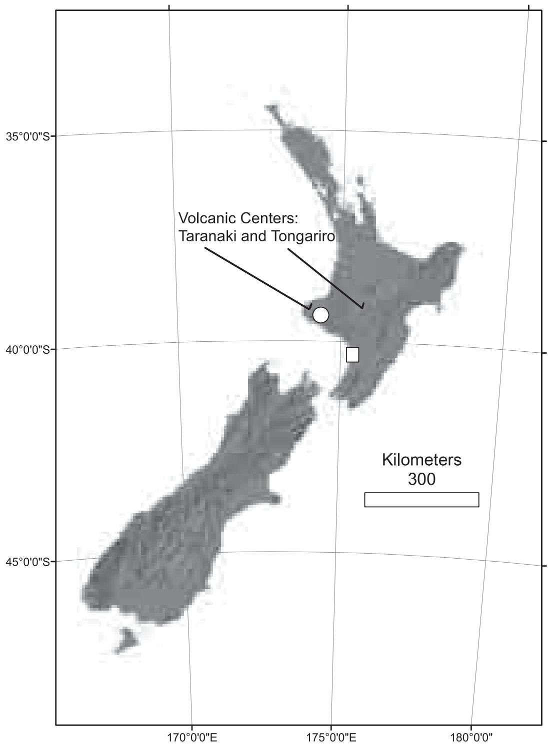

Fig. 1. The location of Egmont (circle; Andisol) and Tokomaru (square; non-Andisol) soils relative to the main volcanic centers generating tephras.

this work because these samples alone were treated with hot 2% phosphoric acid and the Tokomaru data shows a pronounced depression (223‰) while the Egmont data shows a mild depression (124‰) relative to the data shown in Figure 2. The comparison between the Egmont and Tokomaru soils was initially made as a comparison between pastures established in 1870-1900 versus 1945. Where ^{14}C data existed for sites with different ages of pasture establishment, the data were averaged for modeling as shown in Figure 2.

We estimated the turnover rate of SOM based on measured Δ^{14}C values using an approach identical to Baisden et al. (2002a) and Prior et al. (2006). The approach recognizes that two 'pools' of SOM with different residence times can exist within the same soil or soil fraction. We assume one pool (C_{pool}) has annual to decadal residence times while the other pool ($C_{passive}$) is passive (millenial turnover times). The model assumes that both pools have reached steady state, meaning that the inputs, *I*, are equal to the outputs, defined as the residence time, *k*, multiplied by C_{pool}. Starting in 1909, the model numerically incorporates C with a ^{14}C/^{12}C ratio representing the atmospheric CO_2 incorporated in plant biomass, taken from Southern Hemisphere atmospheric data (Currie et al., 2009).

$$\frac{\Delta C_{pool}}{\Delta t} = \left(I - kC_{pool}\right)\Delta t \quad (1a)$$

$$\frac{\Delta\left({}^{14}C_{pool}\right)}{\Delta t} = \left(A_{year-lag} I - (k+\lambda)^{14} C_{pool}\right)\Delta t \quad (1b)$$

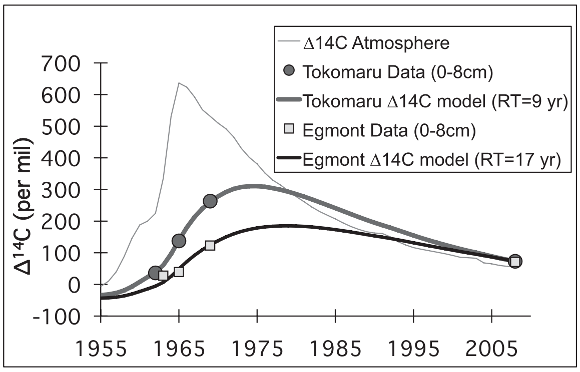

Fig. 2. Variation in Δ^{14}C in the Egmont (Andisol) and Tokomaru (non-Andisol) soils over the last 50 years.

In these equations, C_{pool}, and $^{14}C_{pool}$ represent SOC mass and ^{14}C mass in a portion of the soil fraction, while Δt represents a timestep and is generally one year. Note that Δ in these equations applies the difference operator and not isotope notation. The ^{14}C/^{12}C ratio of the atmosphere, $A_{year\text{-}lag}$, is lagged behind the atmospheric data by 0.25 year to represent the approximate residence time of C in plant biomass. The decay constant for radiocarbon (λ) is set to 1.21×10^{-4} y^{-1}. The Δ^{14}C of the passive pool was calculated based solely on radioactive decay (λ) and assumed residence time, and the Δ^{14}C of the modelled fraction is calculated as the mixture of the passive pool ($C_{passive}$) and C_{pool} in equations 1a and 1b according to the following equation.

$$\Delta^{14}C_{soil} = (1 - P_{passive})\Delta^{14}C_{pool} + P_{passive}\Delta^{14}C_{passive} \quad (2)$$

$P_{passive}$ is the fraction $\left[C_{passive} / \left(C_{passive} + C_{pool}\right)\right]$ of the SOC in the sample which is passive C. The Δ^{14}C value of the passive fraction was poorly constrained by the model, and therefore set for each soil to a residence time of 1000 y (Δ^{14}C = -110‰). The model was implemented in Microsoft Excel with an annual time step. The values of k and $P_{passive}$ were fitted to the data using Microsoft Excel's 'solver' (www.solver.com). The optimized fit minimizes the sum of squared errors between the modelled and measured fraction Δ^{14}C obtained for each year a sample was available. Testing for impact of possible changes in soil C content as reported in Schipper et al. (2007) using the non-steady state model described in Neff et al. (2009) showed that where time-series ^{14}C data are available, small or negligible (<10%) impacts on calculated residence times occur. Varying the residence time chosen for passive C causes the size of the passive fraction to vary proportionally, but has a small effect on the residence time of the stabilized pool for passive residence times of 1000 years or more.

Results

Considerably greater bomb-^{14}C uptake is observed in the 0-8 cm layers of the Tokomaru silt loam than in Egmont silt loam (Figure 2). The simple 2-box model calculates that the residence time of stabilized soil C in the Tokomaru soils is 9 years compared to 17 years for the Egmont soils. This difference represents nearly a doubling of soil C residence time, and roughly explains the doubling of the soil C stock from approximately 45 to 75 Mg C ha^{-1} in the Tokomaru and Egmont 0-8 cm layers, respectively, as sampled by Jackman (1964). With three measurements in the 1960s, and an assumption of a 1000 year residence time for passive soil C in the surface layer, the data is of sufficient resolution to estimate that passive soil C comprises 15% of the soil C pool in Tokomaru soils versus 27% in Egmont soils. The range of values calculated is broadly consistent with soil C models (e.g., Parton et al., 1987), and previous ^{14}C studies (e.g., Baisden et al., 2002a,b). The greater quantity of passive soil C in the Andisol is consistent with previous New Zealand studies, which indicated the differences in the proportion of passive C between an Andisol and non-Andisol were more dramatic when summed over the upper 20 cm of soil (Parfitt et al., 1997; Parfitt et al, 2002). Both enhanced stabilized pool residence times and passive soil C pool sizes are consistent with the view that Andisols or soils with allophanic mineralogy enhance soil C storage (Torn et al., 1997; Parfitt, 2009).

Based on the soil C pool sizes and calculated residence times, the throughput of C through the stabilized pool is relatively more similar across the soils, calculated as 3.4 and 4.4 Mg C ha^{-1} y^{-1} for the Egmont and Tokomaru soils, respectively. This is a substantial fraction of net primary productivity (NPP), based on figures for aboveground NPP obtained by repeated pasture clipping of 5 Mg C ha^{-1} y^{-1} for both sites (Roberts and Thompson, 1984; Radcliffe, 1976).

Discussion

The difference between rates of bomb-^{14}C uptake in the surface layer of the Andisol (Egmont) and non-Andisol (Tokomaru) is clear, and can be inferred to correspond directly to the rate of C turnover through the soil. Using a simple 2-box model, excellent fits are obtained to the data (Figure 2) suggesting a near doubling of residence time in the Andisol relative to the non-Andisol, which corresponds to a near doubling of the C stock. It is notable, however, given suggestions that stabilization of soil C by the mineral allophane may be responsible for enhanced soil C stocks in Andisols (Torn et al., 1997) that the effect on stabilized C residence times is not more dramatic. Indeed, allophane may account for a larger passive pool in the Andisol. Because of the larger C stock in the Andisol and the near doubling of the passive fraction calculated using ^{14}C data, the size of the passive pool in the Andisol appears to be 3-4 times that in the non-Andisol. In contrast, the enhanced residence time of stabilized soil C in the Andisol may correspond to the higher exchangeable Al levels associated with Andisols, which contribute to soil C stabilization through complexation (Parfitt, 2009).

The contrasting C dynamics observed in the Andisol and non-Andisol emphasize the potential of ^{14}C to constrain C dynamics, and elucidate directions for further research. First, compiling and understanding datasets spanning 50 years requires considerable effort to compile and reconcile information. Efforts should be made to better understand the differences between results obtained on carefully resampled sites and those obtained across soil mapping units. At present, both approaches appear valid. Second, efforts to understand sources of variability affecting soil Δ^{14}C would be valuable. At this stage, it appears that the soil C pool may not always be at steady-state (Schipper et al, 2007; Bellamy et al., 2005). While long-term trends affecting soil C stocks have been tested and found to have little impact on residence times calculated with time-series ^{14}C data, year-to-year variation in pasture production may be worthy of exploration as a means to improve model fitting following suggestions that it may cause transient variations in soil C stocks (Schipper et al, 2009), and therefore potentially Δ^{14}C data obtained during years immediately following the bomb-^{14}C spike. Finally, although modeling soil C dynamics as a function of depth involves greater complexity (Baisden and Parfitt, 2007), this surface 0-8 cm dataset represents only a beginning to true understanding of soil C dynamics. Efforts to partition NPP between respiration and stabilization of C in soil, with subsequent transformations and transport of stabilized soil C, remains an important requirement of integrated C-cycle studies.

The Tokomaru/Egmont comparison is necessarily illustrative since neither site was replicated extensively, but provides globally unique data. Moreover, the Tokomaru/Egmont comparison supports evidence that C dynamics does differ in Andisols versus non-Andisols. Additional lines of evidence include emerging theories of soil organic matter stabilisation processes, rates of soil organic matter change following land-use change, and chemistry data. The results presented here suggest C turnover parameters representing soil mapping units can be compiled empirically using ^{14}C, and that time-series samples may present a more useful alternative to physical or chemical fractionation schemes. This finding is particularly useful given that the contrasting soil C dynamics in these different soils appear to have implications for land-use change and management schemes that could be eligible for "C credits".

Acknowlegements

We thank Dawn Chambers for assistance with Rafter Radiocarbon Laboratory databases and archives. Louis Schipper has provided invaluable guidance, energy and discussion in the ongoing recovery of archived data and resampling programme. WTB thanks Rota Wagai and others at Japan's National Institute for Agro-Environmental Sciences and the Tohoku University Conference for discussions that improved this work. We thank R.H. Jackman and Athol Rafter for their pioneering work and the documentation that has allowed us to continue their work. Funding has been provided by NZ FRST.

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JIFS, 7 : 65 – 67 (2010) Symposium Paper

Organic Carbon Accumulation in Andosols: (1) Unaccounted Andosols in Japanese Forest Soil

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Keywords: Japanese forest soil, carbon storage, Brown Kuroboku soils, Fulvic Andosols, Fulvudands

Received 26 December 2009; accepted 1 February 2010

Abstract

Andosols cover 17% of the national land in Japan and largely accumulate organic carbon, and therefore they could play an important role in the domestic carbon storage. Unfortunately, there are some Andosols which remain in an unaccounted state in Japanese forest soil. Brown Kuroboku soils, which would be correlated with unaccounted Andosols, were newly defined in the Unified Soil Classification System of Japan –2nd Approximation (2002). For an accurate estimate of the carbon storage in Japanese soil, a further study is necessary to demonstrate the distributional area of Brown Kuroboku soils in Japan.

Organic carbon accumulation in Andosols

Soils are major absorbers, depositories, and releasers of organic carbon (OC) on the earth's surface. The amount of OC in soils is more than four times that of carbon in terrestrial biota and three times that in the atmosphere. The OC content in soils significantly varies, from less than 1% by mass in some arid-zone soils to 50% or more in waterlogged organic soils (Hillel and Rosenzweig, 2009).

Andosols, which occur in volcanic regions all over the world, show the highest OC density aside from the organic soils according to the result of the OC mass estimation in soils around the world (Eswaran et al., 1993). Rapid weathering of porous volcanic ejecta or glasses results in the accumulation of stable organo-mineral complexes and/or short-range-order minerals, such as allophane, imogolite and ferrihydrite (Shoji et al., 1993). Due to the stability of organo-mineral complexes, Andosols largely accumulate OC. Andosols, whose area is estimated at about 975,000 km^2 or 0.8% of the world soils, contain approximately 1.8% of the global soil carbon (Hillel and Rosenzweig, 2009). Even though the Andosols contribution of the OC stock is not significant on a global level, it is relatively important in Japan. Andosols cover 17% of the national land in Japan (Committee for Soil Classification and Nomenclature, 2001) and therefore they do play an important role in the domestic carbon storage.

Other Andosols in Japanese forest soil

An Andosol with a thick dark-brown horizon called the fulvic horizon is classified as a Fulvic Andosol. Fulvic Andosols have been identified in Chile, Indonesia, Japan and New Zealand (Shoji et al., 1993). In Japan, Fulvic Andosols commonly form under forest ecosystems and often show a biosequential relationship with Melanic Andosols which have a thick black horizon called the melanic horizon. Fulvic horizons are rich in OC but their colors are lighter than the melanic ones, have andic properties, and 30 cm or more cumulative thickness, therefore Fulvic Andosols also significantly store OC like other Andosols.

Unfortunately, Fulvic Andosols in the Japanese forest soil remain in an unaccounted state. The forest soil covering 60% or more of the national land in Japan has been classified into 15 soil units by the Classification of Forest Soil in Japan (1975). In this classification, about 70% of the forest soils are occupied by Brown forest soils containing mostly Cambisols and some Andosols, while Black soils (Andosols) are the second largest group at 13% (Morisada et al., 2004). It is difficult to distinguish the area of Andosols from that of Cambisols within the area of Brown forest soils due to the lack of chemical analysis data for classifying Andosols. Since most Andosols included in Brown forest soils will be potentially classified as Fulvic Andosols, they should be counted as superior OC accumulators.

Brown Kuroboku soils in Brown forest soils

Fig. 1 shows the distributions of Brown forest soils and Kuroboku soils in Japan. The gray-colored areas indicate the Brown forest soils and occupy 53% of the land surface in Japan. The black-colored areas denote the Kuroboku soils and occupy 17%. In this map, the Brown forest soils may include some Fulvic Andosols. Fulvic Andosols are not counted among the Andosols group in this soil map based on the traditional soil classification system.

Brown Kuroboku soils, which were correlated with Fulvic Andosols (WRB) or Fulvudands (Soil Taxonomy), were newly defined in the Unified Soil Classification System of Japan –2nd Approximation (2002)– (The Fourth Committee for Soil Classification and Nomenclature of the Japanese Society of Pedology, 2003). However, the distributed area of the Brown Kuroboku soils is almost still unaccounted for in Japanese forest soil. Since most of the Andosols included in the Brown forest soils will be potentially classified as Brown Kuroboku soils, they should be counted as superior organic C accumulators. For an accurate estimate of the OC stock in Japanese soils, a further study is necessary to distinguish their area from that of the Brown forest soils and assign map units in the soil map of Japan (Kanno et al., 2008).

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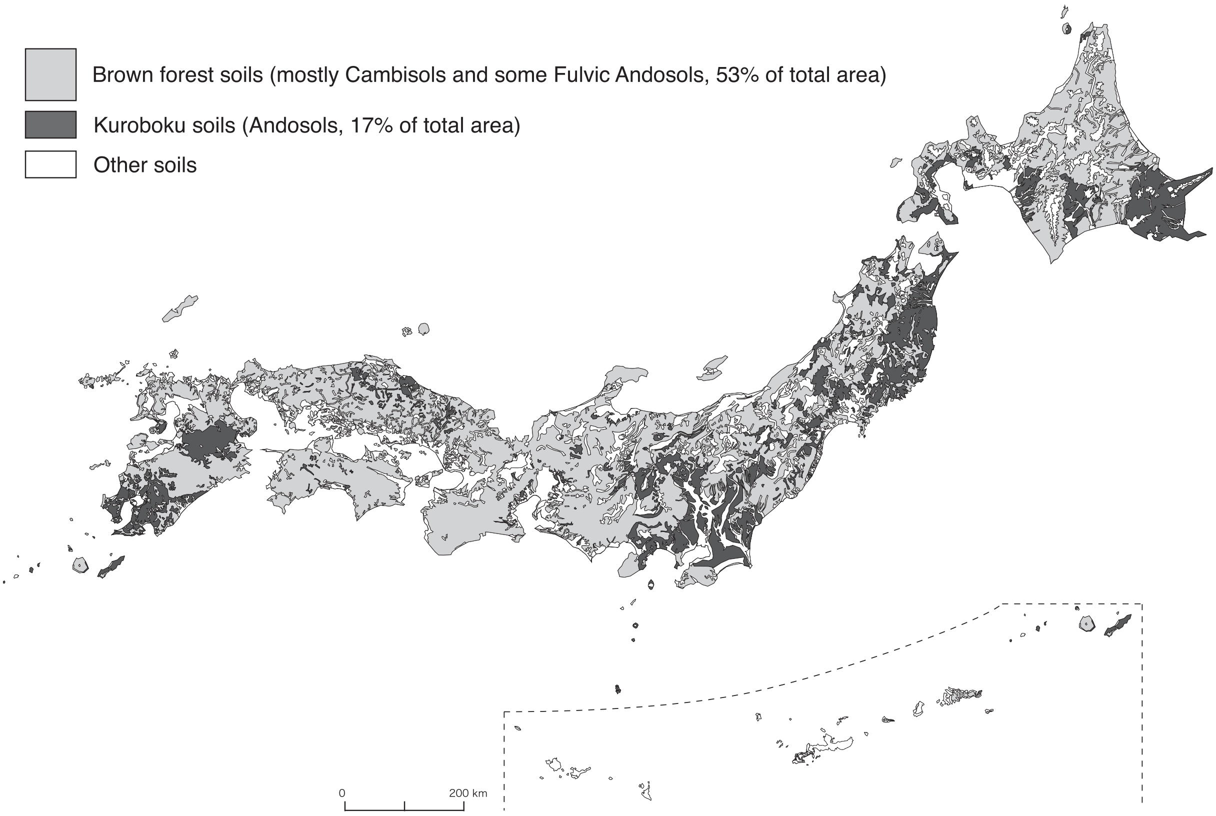

Fig. 1. Distributions of Brown forest soils and Kuroboku soils in Japan based on the soil regions map of Japan (Kanno et al., 2008).

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Organic Carbon Accumulation in Andosols: (2) Contribution of Aluminum-humus Complexes to Carbon Accumulation in Non-allophanic Andosols

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Keywords: activity of microorganisms, allophanic Andosols, aluminum solubility, aluminum toxicity, exchangeable aluminum, organo-metallic complexes

Received 8 December 2009; accepted 1 February 2010

Abstract

Andosols, especially non-allophanic Andosols, accumulate organic carbon (OC) in large amounts mainly due to stabilization of the OC by formation of aluminum-humus (Al-humus) complexes, low soil pH and high Al toxicity. These factors can be easily changed by soil management, and this may lead to a considerable decrease in the OC.

Introduction

Andosols accumulate large amounts of soil organic matter (SOM). Andosols cover only 0.8% of the earth's surface (Kimble et al., 2000), but they contain approximately 1.8% of the global soil carbon (Hillel and Rosenzweig, 2009). This is because not only of vigorous vegetation growth due to the high fertility of the soils, but of the high stability of the SOM against decomposition. It is generally considered that the SOC stabilization is related to 1) formation of the SOM in organo-mineral and/or organo-metallic (Al-humus) complexes (Torn et al., 1997; Inoue and Higashi, 1988; Nanzyo et al., 1993; Percival et al., 2000), 2) low activity of soil microorganisms due to the low soil pH and the high level of toxic Al (Tokashiki and Wada, 1975; Tonneijck, 2009), 3) physical protection of the SOM by the high porosity of the soils (Baldock and Nelson, 2000), and 4) the presence of charcoals (especially in melanic Andosols) (Nishimura et al., 2006). In this paper, we will briefly review the role of the Al-humus complexes, soil pH and toxic Al in the OC accumulation, and introduce our recent studies showing that these factors are rather easily controlled by soil management.

Importance of Al-humus complexes on OC accumulation in Andosols

Among the factors listed above, Al-humus complexes are considered to be the most important regarding the accumulation of OC. Inoue and Higashi (1988) showed that accumulation of OC in the upper 35 cm depth is strongly related to the pyrophosphate-extractable Al ($r = 0.89$, $P < 0.01$) or Al + Fe ($r = 0.88$, $P < 0.01$) using the data of Andosols that form under widely different climatic conditions around the world. Nanzyo et al. (1993) also confirmed the close positive correlation ($r = 0.84$, $P < 0.01$) between the content of pyrophosphate-extractable Al and the OC concentration using the A horizon data of the Andisol Tohoku University Database that consists of soil mostly from the Tohoku and Hokkaido districts in Japan and Alaska and Oregon in the United States. Similarly, in New Zealand soils, the pyrophosphate-extractable Al strongly correlated to the OC, whereas allophanic materials were not related to the OC (Percival et al., 2000). It is considered that the complexation of multivalent cations (e.g., Al^{3+} and Fe^{3+}) by humic substances results in functional groups becoming more condensed and less susceptible to biological attack (Baldock and Nelson, 2000).

With a lower soil pH, organic matter may be protected against decomposition by the Al toxicity to microorganisms (Tokashiki and Wada, 1975). In Andean Andosols, the KCl-extractable Al (toxic Al) concentration and soil pH value were also closely related to the OC amounts (Tonneijck, 2009).

High SOM accumulation in non-allophanic Andosols

Andosols are divided into two major groups on the basis of their colloidal compositions, i.e., allophanic Andosols and non-allophanic Andosols. Non-allophanic Andosols are characterized by the dominance of Al-humus complexes in their active Al. They usually show low soil pH values (pH (H_2O) of about 5 or less) and a high Al toxicity. Therefore, as compared to allophanic Andosols, non-allophanic Andosols tend to accumulate larger amounts of SOM as summarized by Nanzyo et al. (1993). Even in allophanic Andosols, when soils are strongly acidified (such as observed in Japanese tea gardens), the SOM significantly increases in comparison to the inorganic non-crystalline materials (Mitamura, 2005; Takahashi et al. 2008). This is due to the increase in the Al-humus complexes, namely the non-allophanic-andosolization of allophanic soils.

Stability of Al-humus complexes – are all of them highly stable?

As mentioned above, the Al-humus complexes are highly stable and contribute to the OC accumulation. However, it has been revealed that a part of the complexes can be rather unstable as described below.

We determined the Al solubility in A horizon soils of allophanic and non-allophanic Andosols based on an equilibrium study (Fig. 1) (Takahashi et al., 1995). For the allophanic Andosol, the solubility was identical to that of gibbsite, indicating that the solubility is controlled by the soil minerals. On the other hand, the solubility of the non-allophanic Andosols deviated from that of gibbsite (Fig. 1). This indicates that the solubility is mainly controlled by the Al-humus complexes, or the exchange reaction of Al ions and H^+ on the charges of humus. Accordingly, it was shown, using many A horizon samples of non-allophanic soils from Japan, that the amounts of easily exchangeable Al from soils (an indicator of Al toxicity) are closely related to the soil pH and the organically-complexed Al (pyrophosphate-extractable Al) concentration (Fig. 2) (Takahashi et al. 2003).

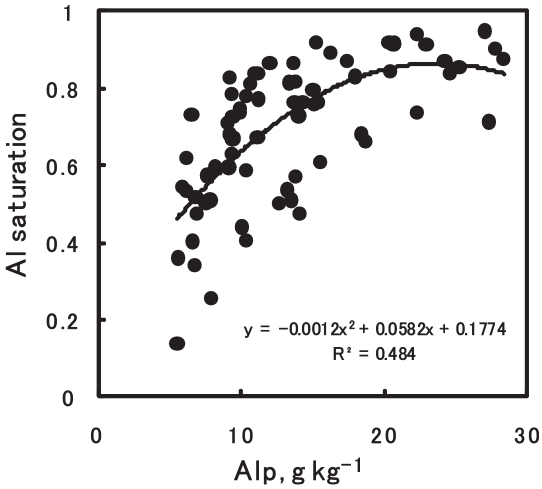

Fig. 2. Relationship between the concentration of pyrophosphate-extractable Al (Al_p) and Al saturation (KCl-extractable Al / effective cation exchange capacity) for A horizon samples of non-allophanic Andosols from northeastern Japan (Takahashi et al., 2003).

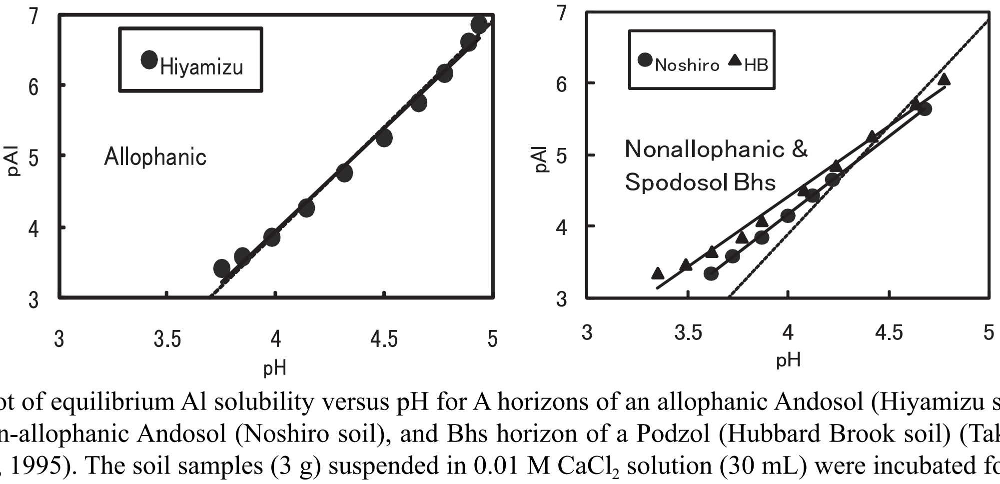

Fig. 1. Plot of equilibrium Al solubility versus pH for A horizons of an allophanic Andosol (Hiyamizu soil) and a non-allophanic Andosol (Noshiro soil), and Bhs horizon of a Podzol (Hubbard Brook soil) (Takahashi et al., 1995). The soil samples (3 g) suspended in 0.01 M $CaCl_2$ solution (30 mL) were incubated for 30 days at 25 ˚C, and the supernatant was obtained after centrifugation for the analyses. The dotted line indicates the solubility of synthetic gibbsite.

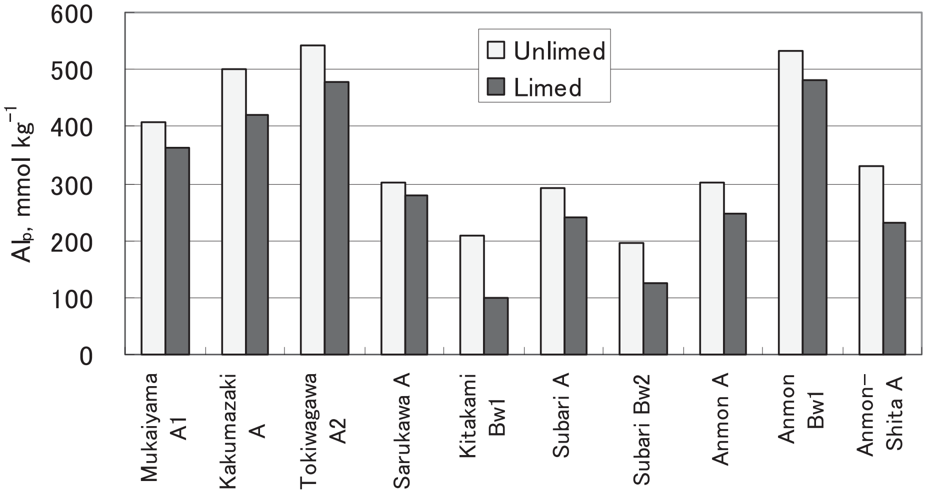

Fig. 3. Amounts of pyrophosphate-extractable Al from unlimed and limed soil samples (Takahashi et al., 2006). The limed soil samples were prepared by the incubation of a mixture of soil samples and calcium carbonate at field water capacity at room temperature (approximately 25 ˚C) for 30 days.

The root growth of Al sensitive plants in synthetic Al-humus complexes showed that the complexes directly cause Al-toxicity to the plant roots (Takahashi et al., 2007). These results indicate that the toxic Al of non-allophanic Andosols, at least, partly originates from the Al-humus complexes.

These facts about the non-allophanic Andosols demonstrate that a part of the Al-humus complexes is rather unstable. This is supported by the fact that even liming significantly decreases the organically complexed Al (pyrophosphate- and $CuCl_2$-extractable Al fractions) (Fig. 3) (Takahashi et al. 2006).

Changes in the land-use of non-allophanic Andosols will affect the extent of carbon accumulation. For example, liming increases the soil pH and decreases the Al toxicity, and thus the activity of microorganisms will increase as shown in the result of the denaturing gradient gel electrophoresis (DGGE) band pattern of eubacterial 16s rRNA (Fig. 4). Liming may accelerate the decomposition of the highly humified SOM due to the liberation of a part of the Al-humus complexes (Fig. 3). Fertilizer application will also activate the activity of microorganisms as observed for the nitrogen and phosphate treatments (Fig. 4). The effects of the soil pH change, the associated change in the Al-humus complexes, and fertilizer application on the OC accumulation potential of soils require further investigation.

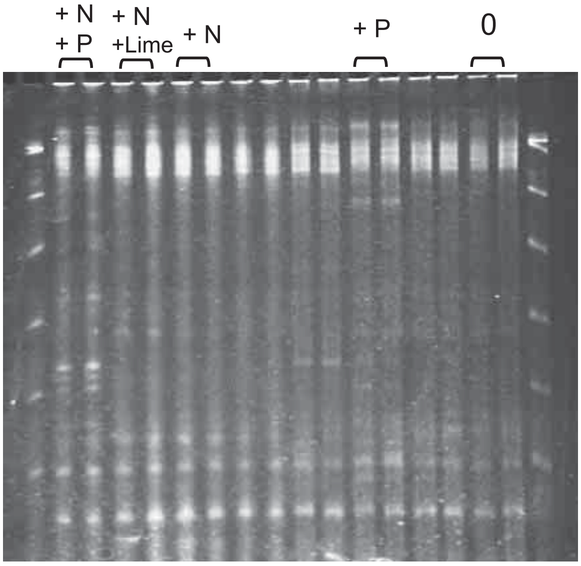

Fig. 4. Denaturing gradient gel electrophoresis (DGGE) band patterns of eubacterial 16s rRNA genes for A horizon samples of Kawatabi soil (non-allophanic Andosol) after several treatments and incubation (30 ˚C) (Yamada et al., unpublished data). +P: 30 days after the treatment of 40 g KH_2PO_4 kg^{-1} soil, +N: 14 days after the treatment of 1.18 g $(NH_4)_2SO_4$ kg^{-1} soil, +N+Lime: 14 days after the treatment of 1.18 g $(NH_4)_2SO_4$ kg^{-1} soil plus liming with $CaCO_3$ (pH 6.5), +N+P: 14 days after the treatment of 1.18 g $(NH_4)_2SO_4$ kg^{-1} soil plus 40 g KH_2PO_4 kg^{-1} soil, 0: 0 day after no treatment.

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Organic Carbon Accumulation in Andosols: (3) Occurrence of Apatite and Biotite in Young and Matured Volcanic Ash Aoils, and Discussions on Nutrient Supply for Ecosystems

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Keywords: Andisol, phosphorus, potassium, stabilization of humus, C sequestration

Received 10 December 2009; accepted 1 February 2010

Abstract

Carbon sequestration in soil is one of the current topics in the soil ecosystem. Andosols show a high C content, especially in the A-horizons, among mineral soils. The major C source of the soils is plant biomass and sufficient nutrient elements are indispensable for rich plant biomass production. Phosphorus and K are the major essential elements for plants that must be supplied from the soils under natural conditions. Thus, the contribution of the P and K content to C sequestration in Andosols was evaluated using soil chemical data. The P content showed a significant positive correlation (r=0.60***, n=53) with the C content of the A-horizons in Andosols, suggesting the contribution of P to C sequestration under natural conditions. Ap horizons were not included in this correlation analysis because abundant P fertilizer is applied to maximize agricultural production. In contrast, the K content of Andosols tended to correlate weakly and negatively with the C content possibly due to leaching loss from the A-horizons with Andosol formation under a humid climate.

Introduction

Volcanic ash soils are located near volcanic areas in the world. The soils are derived from nearly pure ash in the areas with abundant recent ash deposits. In other volcanic or peri-volcanic areas, the volcanic ash soil is more or less a mixture with other soil materials. Although apatite and biotite are minor components in volcanic ash, these minerals contain important nutrients, P and Ca in the former (Nanzyo et al., 1997; Nanzyo and Yamasaki, 1998), and K, Mg, Fe, etc., in the latter (Nanzyo et al., 1999). Weathering of these minerals is relatively rapid under humid and warm climatic conditions. These nutrients are considered to play an important role in the development of natural ecosystems at least at the initial stage of volcanic ash soil formation (Nanzyo et al., 2000; 2003) and also in forming a thick, humus-rich A-horizon of the volcanic ash soils.

Apatite and biotite in tephra and Andosols

Fresh tephra contains 1 – 3 g kg^{-1} or more of P_2O_5 in some cases. These P_2O_5 contents are greater than those for sedimentary rocks. The major P-bearing mineral in the tephra is apatite (Nanzyo et al., 1997). Although the particle size of apatite in volcanic ash is mostly small, the particles are crystalline and much less soluble than those of sedimentary origin.

In spite of being hardly soluble, apatite in the tephra must be an important P source for plants and other living organisms in the tephra-affected areas where P-containing fertilizers are not applied (Nanzyo et al., 2003). Apatite particles can be found in the heavy fine sand fraction of the fresh tephra using an element mapping technique under a scanning electron microscope and energy dispersive X-ray analysis. Fresh apatite particles show a smooth surface (Fig. 1) while those in the volcanic ash soils show a partially dissolved rough surface (Fig. 2).

A small amount of macroscopic biotite is often found in the new tephra as dark-colored platy particles (Fig. 3). Biotite in the new lahar deposit from Mt. Pinatubo, Philippines, was easily converted to vermiculite during lowland rice-cultivation in pots. The K_2O content gradually decreased, and the peak intensity of the X-ray diffraction at 1.4 nm increased during seven cultivations of the paddy rice (Nanzyo et al., 1999). The weathered biotite particle shows a frayed edge (Fig. 4). The vermiculite found in the clay fractions of the soils derived from the old lahar deposits in this area was the tri-octahedral type and it appears to be of biotite origin (Nanzyo, et al., 2001).

Factors affecting the element concentration in Andosols

The process of Andosol formation is primarily characterized by accumulation of active Al and Fe (Shoji et al., 1993). The active Al materials are allophane, imogolite and Al-humus complex. The major active Fe material is ferrihydrite. To evaluate the contribution of nutrient elements to C accumulation in Andosols, changes in element concentrations during Andosol formation were examined focusing on the C content. Soil chemical data were chosen from those of allophanic Andosol areas all over Japan (Saigusa and Matsuyama, 1998) and were cited from Wada (1986).

With an advance in Andosol formation, Al, Fe and other immobile elements are concentrated as exemplified in Fig. 5, while mobile elements such as Si, Ca, Na, etc., are depleted (Nanzyo et al., 2007; 2009). However, the C and N contents in Andosols show no correlation with indices related to Andosol formation (Fig. 6 and 7) because these elements are mostly incorporated in soil by biological activities. In contrast, the N content shows a strong correlation with the C content (Fig. 8) as in other soils, although the C/N ratio is higher than that of other soils. Thus, there are at least three different factors affecting the element concentration in Andosols, weathering, biological ac-

Fig. 1. An apatite particle in a new pyroclastic flow deposit at Mt. Unzen, Japan.

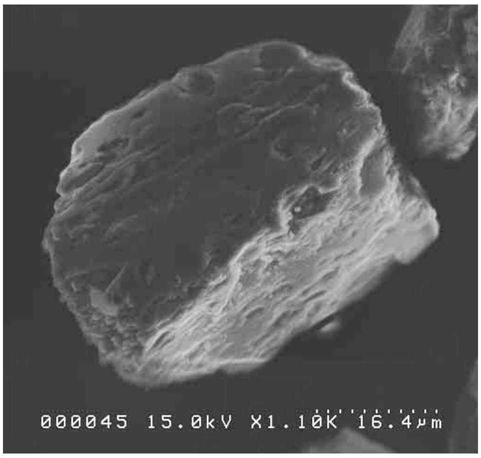

Fig. 2. A partially weathered apatite particle in a soil derived from an old lahar deposit in Central Luzon, Philippines.

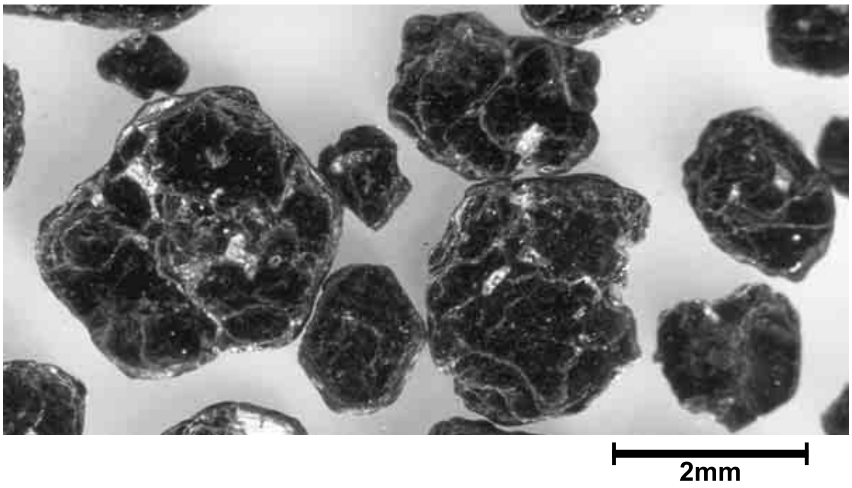

Fig. 3. Biotite particles in a new pyroclastic flow deposit from Mt. Unzen, Japan.

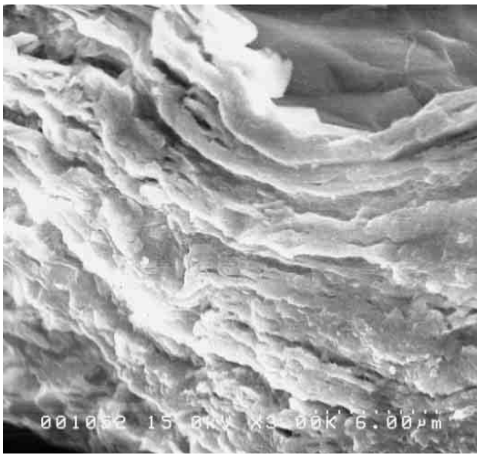

Fig. 4. An edge face of a weathered biotite particle in a soil derived from an old lahar deposit in Central Luzon, Philippines.

tivities, rock type of parent tephra, etc.

Although Al_o and the C content show no significant correlation in Andosols, a pyrophosphate-extractable fraction (Al_p), which is a part of Al_o, significantly correlates with the C content of the A-horizons, except for those existing at the uppermost layers. Because pyrophosphate extracts Al from an Al-humus complex, little from allophane and imogolite, the weathering product of volcanic glass partly contributed to the accumulation of C in the volcanic ash soil under natural conditions. According to the relationship between the contents of Al_p and C, one mole of Al_p is complexed with a unit of humus having 13 moles of C. A rational formula for the Al-humus complex can be estimated as shown in Fig. 9, assuming that every

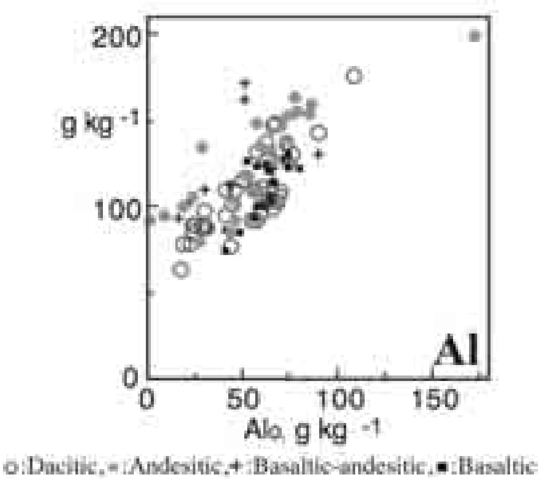

Fig. 5. Relationship between Al_o and the Al content in allophanic Andosols.

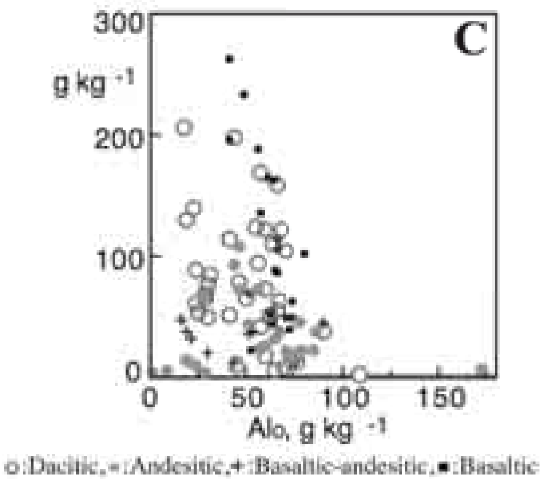

Fig. 6. Relationship between Al_o and the C content in allophanic Andosols.

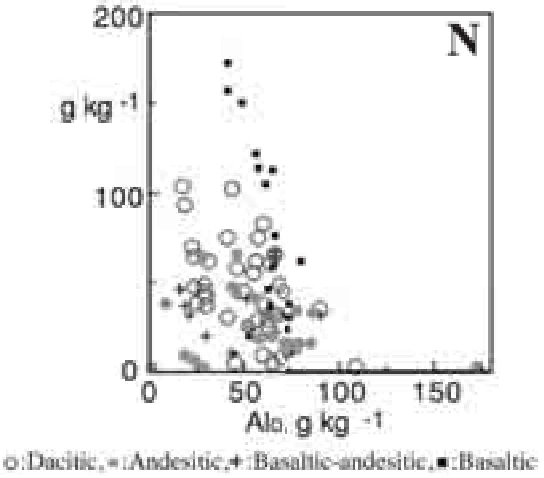

Fig. 7. Relationship between Al_o and the N content in allophanic Andosols.

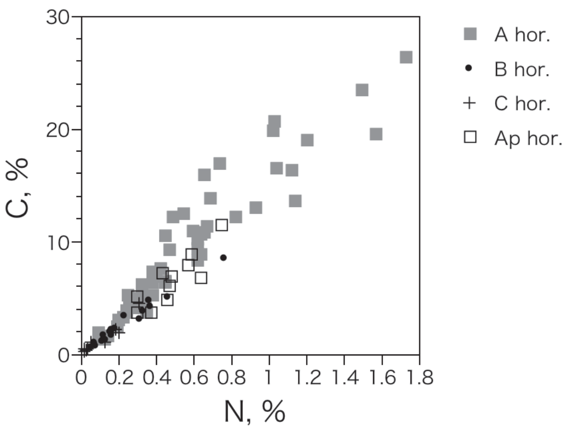

Fig. 8. Relationship between the N and C contents in allophanic Andosols.

40 carbons have 4 carboxyl groups (Yonebayashi and Hattori, 1982), 3 Al_ps and one negative charge at pH 7 (Nanzyo and Shoji, 1993).

Charred materials are also included in the C content of Andosols (Fig. 10). The C from the charred materials was reported to range between 3.8 and 32.7% of the C content in Andosols (Honma et al., 2002; Nishimura et al., 2006). The charred materials can be supplied by burning vegetation through human activities and natural fire during the soil formation process, and also by fire during volcanic eruption. The role of charred materials in the accumulation of highly humified humic acid in Andosols is under discussion. The charred material was not considered in constructing the formula shown in Fig. 9.

Relationship between the P and C contents in Andosols

Although P originates in the parent tephra and is strongly sorbed by active Al and Fe materials, the correlation between the P content and Al_o, even after grouping the volcanic ash soils with rock types of the original tephra, is not significant. However, significant correlation can be found between the P and C contents in the A-horizon soils (Fig. 11) excluding the Ap horizons that have P from fertilizers. Thus, it is considered that P weakly contributes to C accumulation in the A-horizon soils of natural Andosols probably through P supply from volcanic ash for vegetation. Thus, the biological activities may be affected by the content of P in Andosols.

Relationship between the K and C contents in Andosols

Biotite is easily weatherable, it releases K, and K

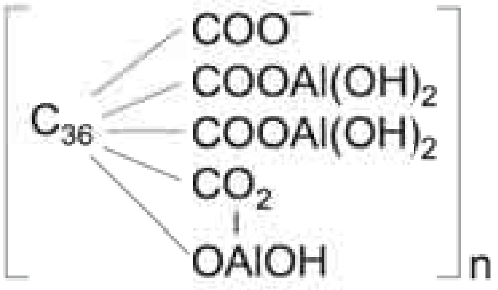

Fig. 9. An estimated rational formula for Al-humus in Andosols.

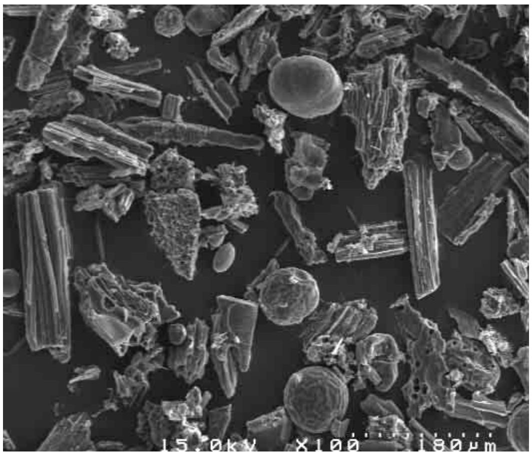

Fig. 10. SEM image of light fraction (d<2) including charred materials (mainly rectangular particles, prepared from the A2 horizon of Yunodai, Towada, Japan).

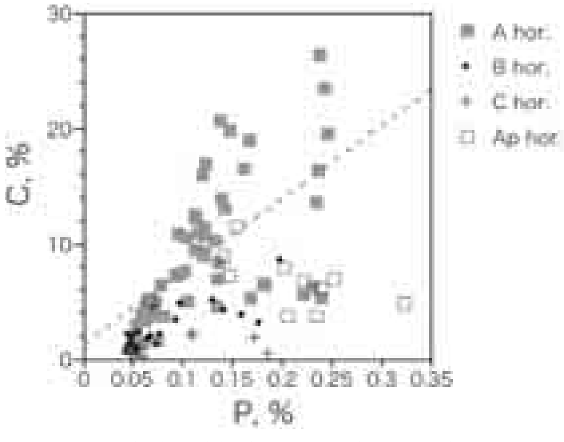

Fig. 11. Relationship between the P and C contents in Andosols (Regarding the A-horizon, r=0.60***, n=53).

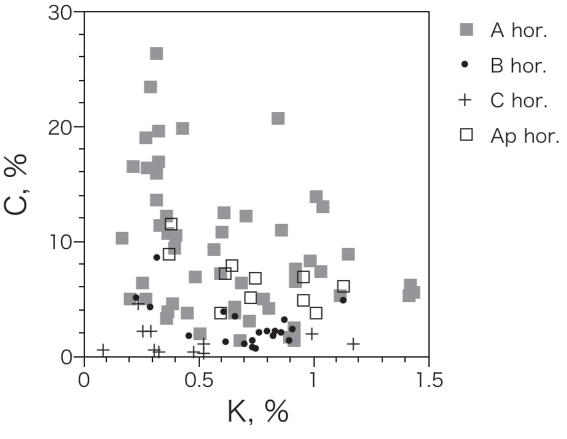

Fig. 12. Relationship between the K and C contents in Andosols.

can be used by organisms. Potassium is commonly one of the major essential nutrients for plants. However, the C content in the A-horizon soils of Andosols tends to decrease with the total K content (r= -0.36**, n=53, Fig. 12) with very high scattering. It is difficult to obtain evidence that K contributes to C accumulation from Fig. 12. Possible reasons may be (i) K^+ in the plant residue is easily lost under a humid climate, (ii) K^+ does not show preference for humus compared with divalent exchangeable cations, (iii) the K content of tephra depends on the rock type, etc.

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7th International Symposium on Integrated Field Science

Biological Interactions in Arable land-Grassland-Forest Continuums and their Impact on the Ecosystem Functions

October 10-12, 2009, Sendai, JAPAN

Organized by
Field Science Center, Tohoku University
Ecosystem adaptability Global COE, Tohoku University

Program

October 10 (Saturday)

10:00	Y. Nakai M. Saito		Opening address Scope of the workshop
10:15	P. M. Kotanen	Tronto Univ Canada	Habitat-specific effects of soil pathogens on seed germination.
10:55	K. Seiwa	Tohoku Univ	Is Janzen-Connell hypothesis valid in temperate forests?
11:15	M. Yamazaki	Tohoku Univ	Distance- and density-dependent seedling mortality and host specific differentiation of the fungal pathogen near the conspecific adult trees in a temperate forest.
11:30	A. Imaji	Tohoku Univ	Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species
11:45	T. Shimada	FFPRI	Effects of seed size and chemical variation on seed fates in a deciduous oak species *Quercus serrata*.
12:05	LUNCH		
13:20	M. K. Lau	North Arizona Univ	Addressing complexity of biological interactions in natural systems with community genetics
14:00	K. Saito	Shinshu Univ	Arbuscular mycorrhizal communities in semi-natural grasslands
14:20	M. Saito	Tohoku Univ	Significance of AM fungi in revegetation process in a N-limited degraded ecosystem
14:40	POSTER SESSION		
15:40	X. L. Li	China Agric. Univ China	Arbuscular mycorrhizal fungi diversity and contribution to crop growth in agricultural fields
16:20	T. Ito	Tohoku Univ	Impact of Tubifex on dynamics of rice field ecosystem with empahsis on organic farming
16:40	M. Utomo	Lampung Univ Indonesia	Earthworm and Soil Carbon after Twenty One Years of Continuous No-tillage Corn-Legume Rotation in Sumatra, Indonesia
17:20			
19:00	Welcome Reception (Hotel JAL City, Sendai)		

October 11 (Sunday）

9:30	POSTER SESSION		
10:00	W. T. Baisden	GNS Science, New Zealand	Evidence for contrasting soil carbon dynamics in volcanic and non-volcanic soils: towards implications for climate change mitigation through land-use and management
10:45	H. Kanno	Tohoku Univ	Organic Carbon Accumulation in Andosols: (1)Unaccounted Andosols in Japanese forest soil
11:00	T. Takahashi	Tohoku Univ	Organic Carbon Accumulation in Andosols: (2) Contribution of aluminum-humus complexes to carbon accumulation in non-allophanic Andosols
11:15	M. Nanzyo	Tohoku Univ	Organic Carbon Accumulation in Andosols: (3) Occurrence of apatite and biotite in young and matured volcanic ash soils, and discussions on nutrient supply for ecosystems
11:30	General discussion Closing remarks		
12:00			

October 12 (Monday）

Field excursion to Field Science Center (Naruko-Onsen, Osaki city)

POSTER SESSION

1	K. Akita	Tohoku Univ	Effects of winter-flooding on soil nutrients and rice yield in paddy field with organic farming
2	M. Ichihara	Sizuoka Univ	Increased agricultural landscape diversity enhances post-dispersal weed seed predation
3	K. Ono	FFPRI	Comparisons of early humification process between coniferous and hardwood forest: Effects of litter species on the compositional quality of soil organic matter in temperate forests in Japan.
4	K. Hirai	FFPRI	Importance of asymbiotic nitrogen fixation to nitrogen immobilization in decomposing green needles of Japanese cedar (Cryptomeria japonica)
5	A. Matsuo	Tohoku Univ	The relationship between reproductive success and clonal structure in a simultaneous flowering population of the monocarpic dwarf bamboo, Sasa veitchii var. hirsuta.
6	Y. Sato	Tohoku Univ	Evaluation of PorySilicate-Iron Sludge from Water Purification Plants as Paddy Soil Amendments
7	J. Shinagawa	Tohoku Univ	Effect of allophonic water treatment residual on paddy rice cultivation
8	K. Sugawara	NILGS	Clavicipitaceous endophytes in grass species; guardians against herbivores evolved from plant pathogen.
9	C. Tada	Tohoku Univ	Chemical and microbial characteristics of forest soil by the difference of forest management
10	R. Tajima	Tohoku Univ	Nitrogen cycle of agricultural system in Field Science Center, Tohoku University
11	T. Tateishi	Iwate Univ	Effects of periodic application of cattle slurry on soil microbial biomass in an Andisol grassland
12	M. Ueno	Tohoku Univ	Higher mortality of conspecific compared to heterospecific seedling beneath the adult for three hardwood species (仮)
13	R. Wagai	NIAES	Comparison of allophanic soils of contrasting carbon contents induced by agricultural management: organo-mineral interaction, carbon mineralization, and it's temperature sensitivity
14	H.Yaginuma	Tohoku Univ	Influence of pH on soil washing and phytoextraction of Cd from soil, a case study using the paddy-field soil with a high pH
15	K.Yamada	Tohoku Univ	Aluminum bioavailability of aluminum-humus complexes in Andosols
16	T. Yamanaka	FFPRI	Actinorhizal plants and Frankia in Japan

Habitat-specific effects of soil pathogens on seed germination

P. M. KOTANEN

University of Toronto Mississauga

For most plants, the majority of mortality occurs at the seed or seedling stage. Evidence suggests that soil fungi play a major role in this mortality. Populations of such fungi are expected to vary among habitats, in response to factors including soil moisture and the availability of hosts. This variation has the potential to affect recruitment by susceptible species, perhaps ultimately determining their abundance and spatial distribution.

Our lab has investigated spatial variation in patterns of seed mortality for a wide range of temperate woody and herbaceous species. In our experiments, we have compared germination of buried seeds protected with fungicide against germination of seeds in water controls; this allows us to isolate the effects of fungal pathogens from other sources of mortality. Our results usually indicate detectable effects of fungal pathogens, and often indicate these patterns are spatially variable.

For instance, the risk of attack may be increased by the proximity of conspecifics, as predicted by the Janzen-Connell Hypothesis. Losses of seeds of the forest tree *Tsuga canadensis* (eastern hemlock) to fungi were high in conspecific-dominated sites, but not in sites dominated by the co-occurring tree, *Acer saccharum* (sugar maple). Interestingly, *Tsuga* often recruits on fallen logs rather than the forest floor. Comparing the effects of fungicide on seeds planted in logs vs. the forest floor suggests logs provide refuges from pathogenic soil fungi.

Attack also can be affected by the abiotic environment. Canopy gaps are likely to be drier than the surrounding forest understory, and therefore less hospitable to fungal pathogens. When we compared losses of seeds of the early successional tree *Betula papyrifera* (paper birch) in old fields, treefall gaps, and forest understory sites, we found that the benefit of fungicide was greater in understory than in openings. This suggests *B. papyrifera* is prevented from establishing in understory environments in part by its susceptibility to pathogen attack.

Such patterns are not restricted to woody plants. For instance, we have found that many herbaceous species suffer higher seed mortality in wetter habitats, but that fungicide additions reduce this difference, suggesting fungi represent a greater hazard in wetter soils. As another example, germination of two *Solidago* (goldenrod) species was lower in habitats dominated by conspecifics than in grass-dominated habitats, but was improved by fungicide treatment. Similarly, seeds of the serious agricultural weed *Ambrosia artemisiifolia* (common ragweed) buried in ragweed populations were less likely to germinate than seeds buried in ragweedfree sites, though this difference was not reduced by fungicide; consequently, the mechanism is unclear.

We also have found many cases of both woody and herbaceous plants whose seed mortality does not follow such predictable patterns. Nonetheless, these examples indicate that for many ecologically and economically important species, spatial patterns of recruitment and perhaps even habitat occupancy may be determined in part by spatial variation in pathogen populations.

Is Janzen-Connell hypothesis valid in temperate forests

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For forest managers, the maintenance of biodiversity has become an increasingly important management goal, to provide a broad array of ecosystem services that directly or indirectly benefit human endeavors. In forest ecosystems, however, little information is available in the mechanisms of maintaining the species diversity particularly in temperate forests. In this study, we examine whether the Janzen-Connell hypothesis is valid in temperate forests, we investigated the density, growth, mortality, and agents of mortality of seedlings, and the density, size, and age of saplings of *Prunus grayana* at three distances (0–3, 6–10, and 16–26 m) from conspecific adults in a temperate forest in Japan. An inoculation experiment was also conducted to test the host range of a leaf pathogen. The probability of mortality was highest at 0–3 m during the first two years of growth. Mortality mainly resulted from distancedependent attack by two types of pathogen that caused damping-off epidemics and spot symptoms on leaves. The leaf pathogen was identified as *Phaeoisariopsis pruni-grayanae* SAWADA, which infected many more seedlings of *P. grayana* than of the two other tree species tested in an inoculation experiment. The vertical and diameter growth was lowest at 0–3 m and highest at 16–26 m in both seedlings and saplings. As a result, the greatest number of large and older saplings was observed at 16–26 m. Our results demonstrate that the Janzen-Connell mechanism operates in a beech-dominated forest in the temperate region of Japan.

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Distance- and density-dependent seedling mortality and host specific differentiation of the fungal pathogen near the conspecific adult trees in a temperate forest

M. YAMAZAKI[1] and K. SEIWA[2]

[1]Miyagi Prefectural forestry technology Institute; [2]Tohoku University.

Janzen-Connell (J-C) hypothesis proposed that not only abiotic factors (e.g., water, light and nutrient limitations) but also host-specific, distance- and/or density dependent natural enemies (e.g., pathogens, insects and small mammals) maintain high tree diversity in tropical forests. High mortality caused by natural enemies might in turn liberate area for recruitment of other tree species and thereby contribute to maintenance of high local diversity. This hypothesis has been widely tested in tropical forests but rarely in temperate forests. Furthermore, little is known about host specificity of natural enemies especially pathogens through tropical forests and temperate forests, although host specificity of natural enemies is crucially important to apply to this hypothesis.

To examine whether J-C hypothesis is valid for common tree species (i.e., *Prunus grayana*, *Cornus controversa*, *Magnolia obovata*, *Fraxinus lanuginose*, *Acer mono*, *Castanea crenata*, *Fagus crenata* and *Quercus serrate*) in temperate forests of Japan, seedling survival and causes of the mortality were investigated at two densities (high, low) at each of two distances (beneath, far) from conspecific adult trees. Furthermore, for seedlings of *P. grayana* and *F. lanuginose*, we conducted the inoculation tests using strains (*Colletotrichum* sp.) isolated from dead seedlings of four tree species. For each tree species, the degrees of damages by damping-off disease were compared among the strains that isolated from different host species.

Higher seedling mortality was observed beneath- compared to far from- conspecific adults for most of the study species. One exception was *Q. serrate*. For most of the study species, disease accounted for the large proportion of the seedling death. In seedlings of *P. grayana* and *F. lanuginose*, the damage by fungal strains from conspecific dead seedlings under conspecific adults was more severe than those from heterospecific dead seedlings under heterospecific adults, suggesting species-specific attack by the pathogens. The result of this study clearly revealed that pathogenic fungi strongly influence the spatial distribution of tree species and consequent species diversity in temperate forests.

Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species.

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Optimal carbon allocation to growth, defense, or storage is a critical trait in determining the shade tolerance of tree species. Thus, examining interspecific differences in carbon allocation patterns is useful when evaluating niche partitioning in forest communities. However, very few studies have examined carbon allocation to all three fundamental traits (i.e., growth, defense, storage) simultaneously. In gaps and forest understory, we measured relative growth rates (RGR), carbon-based defensive compounds (condensed tannin, total phenolics), and storage compounds (total non-structural carbohydrate, TNC) in seedlings of two tree species differing in shade tolerance. We found that RGR was greater in the shade-intolerant species, *Castanea crenata*, than in the shade-tolerant species, *Quercus mongolica* var. *grosseserrata*, in gaps, whereas concentrations of condensed tannin and total phenolics were greater in *Quercus* than in *Castanea* at both sites and TNC pool sizes did not differ between the species. We found also condensed tannin concentrations increased with increasing growth rate of structural biomass (GRstr) in *Quercus* but not in *Castanea*, and TNC pool sizes increased with increasing GRstr in both species, but the rate of increase did not differ between the species. These results suggest that *Quercus* preferentially invested more carbon in defense than in storage. Such a great carbon allocation to defense would be advantageous for a shadetolerant species, allowing *Quercus* to persist in the forest understory where damage from herbivores and pathogens is costly. In contrast, the shade-intolerant *Castanea* preferentially invested more carbon in growth rather than defense (and similar amounts in storage as *Quercus*), ensuring establishment success in gaps, where severe competition occurs for light among neighboring plants. In conclusion, these contrasting carbon allocation patterns are closely associated with strategies for persistence in their respective habitats.

Effects of seed size and chemical variation on seed fates in a deciduous oak species *Quercus serrata*

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Plants in stages from seed dispersal to seedling establishment are subject to be damaged by many factors: seed consumers, decomposers, and unfavorable conditions, such as shortage of light and soil drought (Moles & Westoby 2004). These factors may affect seed fates and, subsequently, the recruitment of plant populations. Various seed traits, such as seed size and chemical compositions, are thought to have considerable impacts on seed fates via interrelations with the biotic and abiotic environmental factors.

Seed traits are not uniform even within a species. Interestingly large intraspecific variations have been reported in some seed traits. A number of previous studies documented those in seed size and examined effects of seed size on seed fates in individual seed scale based on intraspecific variation (*e.g.* Gómez, 2004). Intraspecific variations in the content of seed chemical constituents have also been reported (*e.g.* Sork et al., 1983). However, effects of seed chemical content on seed fates have not been examined yet in individual seed scale, though chemical contents may affect their seed fates like seed size.

Tannins are one of the most popular plant secondary metabolites, which act as a defense chemical against herbivory and fungal attack. Some of oak species include high level of tannins in their seeds, *i.e.* acorns (Shimada & Saitoh 2006). We found a large intraspecific variation in acorn tannin content in a deciduous oak species *Quercus serrata* within a local population and, further, within individual mother trees (mean ± SD, 6.6% ± 3.5; range, 0.7-27.1%). Such large differences in tannin content may likely have substantial influences on seed fates even in the individual seed scale.

In this talk, I will introduce our recent experimental studies examining the effects of acorn tannin content on their seed fate in the individual seed scale. We examined 1) behavioral responses of the Japanese wood mouse *Apodemus speciosus* (an effective seed consumer and disperser) and 2) infection success of *Ciboria batschiana* (an acorn specific decomposing fungus) according to individual acorn tannin content. As a result, it has been demonstrated that acorns with higher tannin content tended to escape more successfully from attacks of both organisms. It indicated that intraspecific differences in seed traits, including chemical content, should not be passed over when studying on interactions between seeds and their consumers.

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Addressing complexity of biological interactions in natural systems with community genetics

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Ecological communities play an integral role in determining ecosystem functions. However, communities, their dynamics and functional consequences, are complex because they are typically comprised of many interacting components and influenced by stochastic processes, such as weather and fire. Because of this, pairwise reductionist investigations of interactions among species will not yield reliable information for building answers to higher-level questions. Here, I present results from studies looking at the structure and functioning of whole communities associated with foundation species, which are species that modulate resources and create stabile conditions for a large number of other community members.

The results of these studies suggest that a large proportion of the variation in communities can be explained by genetic variation in foundation species; and, therefore, we can potentially address community complexity and increase our power for predicting community dynamics and function by putting communities in an evolutionary context. The integration of this kind of information from intensive ecological and genetics studies is the focus of the new field of community genetics.

I will present current research into the mechanisms behind community genetics, showing evidence for self-organization of associated communities beyond the influence of the foundation species, and discuss future research directions.

Arbuscular mycorrhizal communities in semi-natural grasslands

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Grassland vegetation in Japan has been maintained artificially such as by mowing, grazing and firing because grasslands in temperate rainy climate tend to change to forest without the grassland managements. Many of grasslands in Japan locate on volcanic ash soil which is short of available phosphate. Under such conditions, arbuscular mycorrhiza (AM), a symbiotic association between fungi and plant roots, would play an important role in phosphate uptake of plants. The AM fungi are associated with the vast majority of vascular plants and promote nutrient uptake of the host plant, especially phosphate acquisition from soil. The AM fungi could be one of the most significant microbes for nutrient cycling in grasslands. In this presentation, I will provide some findings on dynamics of AM fungal communities in relation to grassland managements and plant succession, and discuss how we can link AM fungal diversity and ecological functions of AM symbiosis in grassland ecosystems.

Plants in grasslands frequently lose their photosynthetic parts by mowing and cattle grazing. Under these conditions where the photosynthate available for AM fungi is reduced, colonization of some AM fungal group is affected by defoliation of host plants (Saito et al., 2004). In grazing-intolerant tall-grass species, *Miscanthus sinensis*, AM fungal phylotypes of *Glomus*-Ab, *Glomus*-Ac, and *Glomus*-Ad were detected in roots. The colonization of *Glomus*- Ac and *Glomus*-Ad groups was significantly reduced by artificial defoliation. The decreased colonization of *Glomus*-Ac and *Glomus*-Ad coincided with decreased non-structural carbohydrate (NSC) levels in a host plant. On the other hand, *Glomus*-Ab group was not affected by defoliation, and the colonization of *Glomus*-Ab did not correlate with plant NSC levels. AM fungal groups may have different carbohydrate requirements on host plants. It is well known that a community of a *Miscanthus*-type grassland changes to that of a *Zoysia*-type grassland when the *Miscanthus*-type grassland is subjected to intensive mowing or cattle grazing (Numata 1969). Interestingly, grazing-tolerant turf-grass, *Zoysia japonicus*, was colonized by *Glomus*-Ab group dominantly which was also dominant in *M. sinensis* root after defolization. When the *Miscanthus*-type grassland subjected to such disturbances is succeeded by the *Zoysia*-type grassland, the community structure of AM fungi will change with this succession.

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Significance of AM fungi in revegetation process in N-limited degraded ecosystems

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Arbuscular mycorrhiza (AM) is a sophisticated symbiotic system composed of AM fungi and plant roots. It is well documented that AM fungi absorb P from soil and supply P to host plant in exchange for C from plants. Currently it is recognized that AM fungi also absorb N from soil and supply it to plants. In contrast to P, mineral N in soil is not adsorbed with soil particles and moves with mass flow of water. Therefore, N supply through AM fungi may not much increase plant growth under usual arable conditions.

First, we examine if AM fungi can enhance plant growth through their N supply to plant using a model system. Secondly, the significance of AM fungi in N-limited degraded soils is discussed with emphasis on revegetation process.

We used a split compartment system composed of root and hyphal compartments (Tanaka and Yano 2005). In the system, the compartments were separated with fine nylon mesh and air gap so that neither mineral nitrogen moved nor roots elongated across the mesh. A seedling of Welsh onion (*Allium fistulos*) was transplanted and inoculated with *Glomus* sp. R10 in the root compartment (RC). After transplanting, ^{15}N labeled ammonium nitrate was added to either hyphal compartment (HC) or RC. “N addition to HC” treatment increased plant dry weight, shoot N and root ^{15}N concentrations, and P uptake. These indicate that, when movement of soil mineral N to plant roots is limited, AM fungi can enhance plant growth by supplying N to plant.

Secondly, we investigated how AM fungi were functioning through primary development of vegetation occurring in the lahar (mud flow of volcanic deposit) area of Mt. Pinatubo, Philippines (Oba et al. 2004). Because the lahar is comprised of newly erupted volcanic materials, it contains little organic matter and nutrients available for plants. Most of the area was sparsely vegetated with only a few gramineous plants, especially *Saccharum spontaneum*. However, some densely vegetated areas could be found in patch. These patches were characterized by coexistence of the gramineous plants and leguminous plants such as *Calopogonium muconoides* and *Centrosema pubescens*, which were well nodulated. Growth of the gramineous plants in these patches was greatly favored. In both less and densely vegetated areas, high density of arbuscular mycorrhizal (AM) fungal spores were found. *S. spontaneum* was slightly colonized with AM fungi while the leguminous plants were highly colonized. AM inoculation experiment carried out in the laboratory involving these gramineous plants showed that these plants were not highly mycorrhiza-dependent, and that some of them responded to added N only when these were mycorrhizal. These suggest that AM fungi might help their host plants to acquire N efficiently in N-limited soil environments.

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Selection of dark septate endophytes from *Ericaceae* plants to enhance blueberry (*Vaccinium corymbosum* L.) seedling growth

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An experiment was conducted to find fungal root endophytes in order to enhance blueberry seedling growth as well as to confirm the role of dark septate endophyte (DSE) under axenic condition. The plant material for DSE isolation was selected from the *Ericaceae* family, from which the blueberry belongs. A total of 91 isolates was obtained from 300 root sections of *Rhododendron pulchrum* Sweet, *Rhododendron obtusum* and *Pieris japonica* L. Inoculation test was conducted to select isolates to enhance the growth of blueberry seedlings. Among the 91 endophytes, 9 had no visible pathogenic symptoms on the appearance of blueberry seedlings at 10 weeks after the inoculation. The remaining 82 isolates, mostly fast growing and rapidly sporulating fungals, were excluded from the subsequent steps of the experiment, due to their inhibitory effect on the growth of blueberry seedlings. There was no significant differences observed after 10 weeks of inoculation on shoot length, root length and fresh weight of the seedlings, however there was a tendency of having longer root in the seedlings with some isolates especially with Pj029 (78.6 mm) and Pj022 (68 mm) compared with the control (51 mm) The 10 putatively benefical endophytes were subjected to phylogenetic analysis, according to their rDNA ITS-RFLP banding patterns, which derived from the digestion of each isolates with restriction endonucleases *Afa*I, *Hinf*I and *Hae*III, respectively. The ITS2 regions of each RFLP types were selected for direct sequencing, in order to find a matching sequences in the GenBank database. The Rp005 isolate from *Rhododendron pulchrum* host was identified as *Heteroconium chaetospira* that means the first report from an *Ericaceae* plant in Japan. The Rp022 and Rp011 isolates both from *Rhododendron pulchrum* host and Pj023 isolate from *Pieris japonica* host were the first *Leptodontidium orchidicola* strain from *Ericaceae*. All of the isolates have the ability to form intracellular structure within the host epidermal cell and assume to have bidirectional nutrient flow between the host and endophyte.

Key words: blueberry, colonization, Dark Septate Endophyte, *Ericaceae*, intracellular structure

Impact of tubificid worms on ecosystem in ricefield with organic farming

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Tubificid worms (aquatic oligochaetes) are one of the major benthos in lake bottom sediments and are well known to influence lake ecosystems through increasing nutrient release to water from the bottom sediments (Fukuhara and Sakamoto, 1987). It is also known that tubificids present at high density in the ricefield with organic farming and increase phytoplankton and zooplankton in the overlaying water of ricefields due to increased release of nitrogen and phosphate from soils (Kikuchi and Kurihara, 1982).

In this study, we investigated the impact of tubificids on ricefield ecosystem on the viewpoint of nutrient change in the soil and surface water of the organically managed ricefield.

Materials and methods

We measured the population density of tubificids and the soil mass perturbed by tubificids in the winter-flooded and organically managed ricefield of Miyagi, Japan. The incubation experiment were conducted under continuous dark and dark/light (12/12 hours) at 30 ˚C for 4 weeks using 300 mL vials with 7 cm depth of alluvial soil and 5 cm depth of overlaying water. Ammonium and phosphate concentrations were measured in the soil and the overlaying water with and without a kind of tubificid (*Branchiura sowerbyi*) (0-78 g m^{-2} on the basis of wet weight).

Results and discussion

The major species of tubificids were *Limnodrilus socialis* and *Branchiura sowerbyi* in the ricefields surveyed. The population densities of tubificids were higher in the four ricefields with organic farming (maximum densities: 15,000-40,000 ind. m^{-2}) than those of the control ricefields with application of agrichemicals. Soil mass perturbed by feeding and excretion action of tubificids were estimated to be about 6 cm of thickness from the results of two no-tilled organic ricefields. Concentrations of ammonium nitrogen and available phosphate in the soils significantly increased with the increasing of tubificid densities under continuous dark and dark/light. Amounts of ammonium nitrogen and dissolved inorganic phosphate released into the overlaying water from the soils increased in proportion to the tubificid densities under continuous dark. Tubififids increased bioavailable nutrients in the submerged soil and the overlaying water probably due to accelerating soil organic matter decomposition and nutrient diffusion in the soilwater interface. From the results, tubificids probably increase organism production in the overlaying water of ricefield with organic farming.

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Earthworm and Soil Carbon after Twenty One Years of Continuous Notillage Corn-Legume Rotation in Sumatra, Indonesia

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Keywords: Conservation tillage, biomass of microorganism, and crop residues

Earthworm and soil carbon are important components in sustainable tropical agro ecosystem. Long-term experiment was initially conducted in 1987, at the experiment farm of Politeknik Negeri Lampung, Sumatra, Indonesia. The soil is a Typic Fragiudult with slope ranging from 6 to 9 percent. The site of experiment was previously a *ladang* (a local land rotation with period of fallow), which was abandoned and covered by *Imperata cylindrica* grass with dry matter of 15 Mg ha^{-1}. The experiment was a factorial, randomized complete block design, with 4 replications. Tillage treatments were conservation tillage (no-tillage, NT and minimum tillage, MT), and intensive tillage (IT); while nitrogen fertilization rates were 0 and 200 kg N ha^{-1}. Cropping pattern of the long-term experiment was cereal-legume-fallow rotation. Nitrogen fertilizer treatment was not applied when legume was planted. Due to soil compaction in 1997 and 2002, all plots of conservation tillage were plowed. After eleven years of cropping, the soil became acid therefore; in 2004 all plots were limed with 4 Mg ha^{-1} of $CaCO_3$. To determine the effects of long-term no-tillage and N fertilization on earthworm and soil carbon, the soil samples were collected at depth of 0-10 Cm in 2008, after twenty one years of long-term continuous experiment.

The only treatment that affected earthworm population was tillage treatment. After twenty one years, NT had averaged 1.0 Million ha^{-1} earthworm population, 245 percent higher than IT, but 67 percent lower than MT. Refer to previous experiment after nine years (1996), the response of earthworm to conservation tillage after twenty one years was similar, but weaker than after nine years. After nine years of cropping, NT had averaged 4.7 Million ha^{-1} earthworm population, 158 percent higher than IT, but 40 percent lower than MT. Different from earthworm response, the biomass of microorganism after twenty one years of cropping was affected by either tillage or N fertilization treatments, even though did not affected by their interaction. No-tillage had 56.1 mg C-CO_2 kg^{-1} day^{-1}, 15 percent higher than IT, but 33 percent lower than MT; while 200 kg N ha^{-1} N treatment had 42 mg C-CO_2 $kg^{-1}day^{-1}$, 34 percent lower than 0 kg N ha-1 treatment. The superiority of conservation tillage over IT was mainly due to additions of previous weed and crop residues on the soil surface. The additions of averaged 13 Mg residues have created a favorable micro-climate for earthworm, and have increased soil organic C of conservation tillage. After twenty one years of cropping, soil organic carbon of NT at 0-10 Cm soil depth was 13.9 Mg ha^{-1} and MT was 15.8 Mg ha^{-1}, or 17.8 percent and 34 percent higher than IT, respectively

Evidence for contrasting soil carbon dynamics in volcanic and non-volcanic soils: towards implications for climate change mitigation through land-use and management.

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In 1959, Athol Rafter began a substantial programme of monitoring the flow of ^{14}C produced by atmospheric thermonuclear tests through New Zealand's atmosphere, biosphere and soil.

The programme produced important publications (e.g. O'Brien and Stout, 1978) and leaves an legacy of unpublished data critical for understanding soil C dynamics. A database of over ~400 soil radiocarbon measurements spanning 50 years has now been compiled, and can be extended to ~600 measurements including systematic measurements made elsewhere (e.g., Baisden et al., 2002; Torn et al., 1997). Key aspects of the dataset are described here, with an emphasis on quantifying the differences in soil C dynamics between volcanic and non-volcanic soils, as well as the response of different soils to land-use change and management.

Among Rafter and Stout's most compelling data is a comparison of soil carbon dynamics in deforested dairy pastures under similar climate in the Tokomaru silt loam (non-allophanic) versus the Egmont black loam (allophanic), originally sampled in 1962, 1965 and 1969. After adding surface soils sampled to a similar depth in 2008, we can use a relatively simple 2-box model to calculate that the residence time of soil C in the Tokomaru soil is ~9 years compared to ~15 years for the Egmont soil. This difference represents nearly a doubling of soil C residence time, and roughly explains the doubling of the soil C stock. With three measurements in the 1960s, the data is of sufficient resolution to estimate the parameters for an "inert" or "passive pool" comprising approximately 15% of soil C, and having a residence time of 600 years in the Tokomaru soil versus 3000 years in the Egmont surface soil. While these differences are large, they are nevertheless smaller than those calculated using the single-pool model of Torn et al. (1997) to imply residence times of many millennia for allophanic soils in Hawai'i. Data examined versus soil depth adds additional knowledge.

The Tokomaru/Egmont comparison is necessarily illustrative since neither site was replicated extensively, but provides globally unique data. Moreover, the Tokomaru/Egmont comparison supports evidence that C dynamics does differ in volcanic versus non-volcanic soils. Additional lines of evidence include emerging theories of soil organic matter stabilisation processes, rates of soil organic matter change following land-use change, and chemistry data. The contrasting soil C dynamics in these different soils appear to have implications for land-use change and management schemes that could be eligible for "C credits". The residence time data enhances opportunities for robust exploration of options, including the net benefit of biochar incorporation and afforestation/deforestation scenarios.

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Organic Carbon Accumulation in Andosols: (1) Unaccounted Andosols in Japanese forest soil

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The soils are major absorbers, depositories, and releasers of organic carbon (OC) on the earth surface. The amount of OC in soils is more than four times that of carbon in terrestrial biota and three times of that in the atmosphere. The content of OC in soils varies greatly, from less than 1% by mass in some arid-zone soils to 50% or more in waterlogged organic soils (Hillel and Rosenzweig, 2009).

Andosols, which occur in volcanic regions all over the world, show the highest amount of OC density aside from the organic soils. Rapid weathering of porous volcanic ejecta or glasses results in accumulation of stable organo-mineral complexes and/or short-range-order minerals such as allophane, imogolite and ferrihydrite. Because of the stability of organomineral complexes, Andosols largely accumulate OC.

Andosols, whose area is estimated about 975,000 km^2 or 0.8% of the world soils, contain approximately 1.8% of global soil carbon. Even though Andosols' contribution of OC stock is not so large at a global level, it is relatively important in Japan. Andosols cover 17% of national land in Japan and therefore they would play an important role in the domestic carbon storage.

An Andosol with a thick dark-brown horizon called the fulvic horizon is classified into Fulvic Andosol. Fulvic Andosols have been identified in Chile, Indonesia, Japan and New Zealand. In Japan, Fulvic Andosols commonly form under forest ecosystems and often show a biosequential relationship with Melanic Andosols which have a thick black horizon called the melanic horizon. Fulvic horizons are rich in OC but colors lighter than the melanic ones, and have andic properties and 30 cm or more cumulative thickness, therefore Fulvic Andosols also largely store OC alike other Andosols.

Unfortunately, Fulvic Andosols in Japanese forest soil remain in a state of unaccounted for. The forest soil covering 60% or more of national land in Japan has been classified into 15 soil units by the Classification of Forest Soil in Japan (1975). In this classification, about 70% of forest soils are occupied by Brown forest soils correlated with mostly Cambisols and some Andosols, while Black soils (Andosols) are the second largest group at 13%. Because of the criteria used to classify soils are mainly morphology-oriented characteristics or properties, it is difficult to distinguish the area of Andosols from that of Cambisols within the area of Brown forest soils (Morisada et al., 2004). Since most of Andosols included in Brown forest soils will be potentially classified as Fulvic Andosols, they should be counted as superior OC accumulators.

Brown Kuroboku soils, which were correlated with Fulvic Andosols (WRB) or Fulvudands (Soil Taxonomy), were newly defined in the Unified Soil Classification System of Japan –2nd Approximation (2002)–. However, the distributional area of Brown Kuroboku soils is almost unaccounted for in Japanese forest soil yet. For an accurate estimate of the OC stock in Japanese soil, further study is necessary to distinguish the area of them from that of Brown forest soils and assign map units in the soil map of Japan.

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Organic Carbon Accumulation in Andosols : (2) Contribution of aluminumhumus complexes to carbon accumulation in non-allophanic Andosols

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Non-allophanic Andosols largely accumulate organic carbon (OC) due to stabilization of OC by formation of aluminum-humus (Al-humus) complexes, low soil pH and high Al toxicity. These factors can be easily changed by soil managements, and this may lead to a considerable decrease of OC.

Factors for high OC accumulation in Andosols

Andosols accumulate large amounts of soil organic matter (SOM). Andosols cover only 0.8% of earth's surface, but they contain approximately 1.8% of global soil carbon. This is because not only of vigorous growth of vegetation due to high fertility of the soils, but of high stability of SOM against decomposition. The SOC stabilization is generally related to 1) formation of SOM in organo-mineral and/or organo-metallic (Al-humus) complexes, 2) low activity of soil microorganisms due to the low soil pH and the high level of toxic Al, 3) physical protection of SOM by high porosity of the soils, and 4) presence of charcoals (especially in melanic Andosols).

High SOM accumulation in non-allophanic Andosols

Non-allophanic Andosols are characterized by dominance of Al-humus complexes in their active Al. They usually show low soil pH values (pH (H_2O) of about 5 or less) and high Al toxicity. Therefore, as compared with allophanic Andosols, non-allophanic Andosols tend to accumulate the larger amounts of SOM. Even in allophanic Andosols, when soils are strongly acidified (such as observed in tea gardens in Japan), SOM largely increases in comparison to inorganic noncrystalline materials (Takahashi et al. 2008). This is due to the increase of Alhumus complexes, namely non-allophanic-andosolization of allophanic soils.

Stability of Al-humus complexes – is all of them highly stable?

Results of Al release rate and soil equilibrium studies showed that Al solubility of nonallophanic Andosols is controlled by Al-humus complexes through the exchange reaction of Al ions and H^+ on the negative charge of humus. Accordingly, the amounts of easily exchangeable Al from soils (an indicator of Al toxicity) are closely related to soil pH and organically complexed Al concentration (Takahashi et al. 2003). Thus, a part of organically complexed Al is rather unstable. This is supported by the fact that even liming significantly decreases organically complexed Al (pyorophosphate- and $CuCl_2$-extractable Al fractions) (Takahashi et al. 2006).

Changes in land-use of non-allophanic Andosols will affect the ability of carbon accumulation. For example, liming increases soil pH and decreases Al toxicity, and so activity of microorganisms will increase. Liming may accelerate decomposition of highly humified SOM because of the liberation of a part of Al-humus complexes.

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Organic carbon accumulation in Andosols: (3) Occurrence of apatite and biotite in young and matured volcanic ash soils, and discussions on nutrient supply for ecosystems

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Volcanic ash soils locate near the volcanic areas in the world under various environmental conditions. Volcanic ash significantly affects soils of the volcanic areas as an almost pure parent material or a mixture with other soil materials. Although apatite and biotite are minor components in volcanic ash, these minerals contain important nutrients, P and Ca in the former, and K, Mg, Fe etc. in the latter. Weathering of these minerals is relatively rapid under humid and warm climatic conditions and these nutrients are considered to play an important role in the development of natural ecosystems at least at the initial stage of volcanic ash soils formation.

Fresh tephra contain 1 – 3 g kg^{-1} or more of P_2O_5 in some cases. These P_2O_5 content values are greater than those for sedimentary rocks. The major P bearing mineral in the tephra is apatite (Nanzyo et al., 1997). It is single crystalline and much less soluble than those of sedimentary origin. In spite of being hardly soluble, apatite in the tephra must be an important P source for plants and other living organisms in the tephra-affected areas and in the tephra where P-containing fertilizers are not applied (Nanzyo et al., 2003). In the northern part Japan, the Ca-P/Total P ratio decreased to less than 0.2 in the Andosols with $Al_o + Fe_o/2$ of 20 g kg^{-1} or more, where Al_o and Fe_o show oxalate-extractable Al and Fe, respectively.

Biotite in the new lahar deposit from Mt. Pinatubo, Philippines was easily converted to vermiculite during lowland rice-cultivation in pots. The K_2O content gradually decreased, and the peak intensity of the X-ray diffraction at 1.4 nm increased during seven cultivations of the paddy rice (Nanzyo et al., 2001). The vermiculite found in the clay fractions of the soils derived from the old lahar deposits in this area was tri-octahedral type and it appears biotiteorigin.

Under humid and temperate climates, volcanic ash soils sustain plentiful ecosystems. In the matured volcanic ash soils, especially in the A horizon of Andosols, cycling of some nutrient elements may depends more on biological cycling and formation of A horizon (carbon accumulation) than weathering of the parent volcanic ash (Nanzyo et al., 2007). In the agricultural or artificial ecosystems, crops or plants depend strongly on various kinds of fertilizers.

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Effects of winter-flooding on soil nutrients and rice yield in paddy field with organic farming

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In Japan, more than half of natural wetlands have been lost in the last century primarily through draining for agriculture (Geographical Survey Institute, 2000). On the other hands, waterfowl, for example white-fronted goose (*Anser albifrons* Scopoli) migrating to Japan are increasing. Waterfowl habitat environments are getting worse and it may cause risks of food shortage or disease spread.

Flooding rice fields during winter might function as alternative wetlands for waterfowl. Winter flooding is conducted worldwide, in California in the United States, Ebro delta in Spain, Cheonsu bay in South Korea, Miyagi, Sado and Toyooka in Japan and so on.

We researched the effects of winter-flooding on soil nutrient availability and rice yield in the organically managed ricefields.

Material and Methods

The field experiment was conducted in 2008 in the paddy field of the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Miyagi prefecture. Treatments were three; organic rice farming with and without winter flooding (WF and NWF) and conventional farming (CF), with no replication. Each plot area is 280 m^2 to 380 m^2. Winterflooding was begun in December 11th, 2007. Inorganic fertilizer and agrochemicals were used in the CF plot and were not used in the WF and NWF plots. Organic or inorganic nitrogen fertilizer were incorporated into plow layer with the application rate of 7g N m^{-2} in WF and NWF plots, or CF plot, respectively. Transplanting and harvest were conducted at May 27 and October 10 in all plots.

Concentrations of NH_4^+-N, available P_2O_5 (modified Bray 2 method) and Fe^{2+} in the plow layer soil, and number of tiller and SPAD value were measured periodically during the growing season. Rice yield and yield components were determined at the harvest time.

Results and Discussion

WF plot showed greater concentration of NH_4^+-N in the plow layer soils than NWF and CF plots throughout the growing season. Available phosphate contents did not show significant difference among three treatments. Reduction of ferric iron to ferrous iron is index of oxidation-reduction condition of submerged soils. Ferrous iron contents of soils were larger in WF than NWF and CF. It showed that reductive condition of soils developed more rapidly and strongly in WF plot than NWF and CF plots probably due to longer flooding period and organic fertilizer application in WF plot.

Number of tillers of rice in CF plot was the highest among three treatments because rice plants can uptake more rapidly nitrogen from chemical fertilizer than organic fertilizer. Brown rice yields were 535, 440 and 600 g m^{-2} in WF, NWF and CF plots, respectively. The yield and nitrogen uptake of rice plants was significantly higher ($p<0.05$) in the WF plot than the NWF plot. Panicle number and grain number per head of WF plot showed greater tendency than those of NWF plot. The total grain number of rice was significantly different between WF and NWF.

From the preliminary results of one year research obtained, the winter-flooding management (longtime flooding) probably accelerates the mineralization of soil organic nitrogen and added organic fertilizer and increases rice yields compared to common water management.

Increased agricultural landscape diversity enhances post-dispersal weed seed predation

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Agricultural biodiversity provides many ecological services, including pest control, and supports sustainable crop production. Post-dispersal seed predation is one of the main causes of weed seed mortality and could contribute to biological weed control (Westerman *et al*. 2003, 2005). However, the extent of post-dispersal weed seed predation in monsoon Asia, including Japan, must be quantified before it can be implemented as a form of weed control. Furthermore, it is necessary to understand the relationship between seed predation and landscape structure. Landscape features may influence the degree of seed predation because the components of non-crop areas (such as field margins, grassland, set-aside areas, and forest) in agricultural landscape are important habitats for seed predators and provide population sources for field colonization (Menalled *et al*. 2000). In this study, we investigated the rate of post-dispersal weed seed predation and the seed predators in contrasting agricultural landscapes.

The rate of post-dispersal seed predation of Italian ryegrass (*Lolium multiflorum*), an exotic winter annual weed that has invaded both experiment sites, was compared between simple (a wheat-soybean double-cropped field in a large-scale farming area) and complex (a traditional terraced paddy) agricultural landscapes in Shizuoka Japan. The cumulative seed predation rate during three months after Italian ryegrass seed shed (from August to October) was 46 % (average predation rate per two weeks = 9 %) in the field interior and 82 % (23 %) in the field margin in the large scale farm, and 99 % (47 %) in the field margin in the terraced paddy. The main seed predators were invertebrates (crickets and carabid beetles) and vertebrates (rodents or birds) in the large scale farm, and invertebrates (crickets and carabid beetles) in the terraced paddy. The results of this study indicate that the rate of post-dispersal seed predation in the complex agricultural landscape is higher than the simple landscape.

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Comparisons of early humification process between coniferous and hardwood forest: Effects of litter species on the compositional quality of soil organic matter in temperate forests in Japan

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The chemical nature of plant litter, which soil organic matter (SOM) originates, can affect the quality of SOM accumulated in the soils. To quantitatively clarify the effect of the forest type on SOM accumulation, it is necessary to differentiate the SOM accumulation processes on the forest floors between coniferous and hardwood forests in Japan. Authors have already evaluated SOM accumulation processes on the forest floor during an early humification stage in hardwood forest by using a solid-state ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (CPMAS NMR) technique (Ono et al., 2009). In the present study, we conducted a litterbag experiment at coniferous plantations and determined the mass loss rates of the organic carbon components in phased-humified coniferous litters (Japanese cedar and cypress) by applying solid-state ^{13}C NMR technique. Organic carbons in both coniferous litter remained almost constant during the early humification process. Therefore, the composition ratios of aliphatic to *O*-alkyl carbons, that are indicators of the degree of litter humification, were quite stable at 0.5 throughout humification although litter masses decreased during the 3-year incubation. This result indicated that the coniferous litters incubated for 3 years were not well humified. And/or the coniferous litter mass loss might be susceptible to physical fragmentation by raindrop impact, freeze-thaw, and drenching-drying as well as biochemical degradation. The mass loss rate of carbon components during the humification for both coniferous litters was in the following order: aliphatic ≥ *O*-alkyl > aromatic > carbonyl carbons. Decomposability of each carbon component differed between coniferous and hardwood litters. Especially, mass loss rate of aliphatic carbon in coniferous litters with high initial contents of resins and waxes were higher than in hardwood litters because of their quick leaching. A_1 horizon soils in coniferous forests have higher aliphatic and lower *O*-alkyl carbon contents than that of a hardwood forest. The compositional trend of A_1 horizon soils in the coniferous forests would be caused by high aliphatic supply rate due to high initial contents of resins and waxes in fresh coniferous needle litters and their rapid leaching. In conclusion, the difference of litter origins can strongly affect the processes of organic carbon supply into the soil and SOM accumulation.

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Importance of asymbiotic nitrogen fixation to nitrogen immobilization in decomposing green needles of Japanese cedar (*Cryptomeria japonica*)

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Japanese cedar (*Cryptomeria japonica*) is one of the most important conifers planted in Japan, and its litter has high asymbiotical nitrogen fixation activity(Nioh and Haruta; 1988). It was also well known that increased nitrogen content resulting from nitrogen immobilization during the early decomposing stage(Berg; 1988). To determine the effrcts of nitrogen fixation activity to nitorgen immobilization in decomposing green needdles of Japanese cedar and the factors concering nitrogen fixation activity, we investigated decomosing rate, nitrogen activity and nitrogen and carbon contents of green neddles of Japanese cedar.

The study was conducted at the Katsura experimental forest in central Japan and the Nagasaka experimantal forest in northeastern Japan. There were snow at the Nagasaka bur not snow at the Katsura in winter, and srand age of both forests were nearly 40 years old. Fresh green needles of Japanese ceder were collected from each forest after thinning, and litter bags containning green neddles were installed both of on the ground and in the air. Bags were collected periodically. The remaining weight, acetylene-reducing activity and nitrogen and carbon contents of the remaining needles were determined.

Weight loss began in the third months after installation and continued, but the decomposition rate among the treatments was equivalent until 12 months after. Nitrogen fixation activity also appeared at 3 months after and increased until 6 months after, then decreased until 12 months after. Higher nitrogen fixation activity was observed with a high water contents in the needles. For each collection time, the activity and nitrogen contents of the samples on the ground were higher than those in the air. It was clear that putting needles on the ground surface accelerate increments of nitrogen contents in the remaining needles. It considered that contribution of asymbiotical nitrogen fixation activity to nitrogen immobilization of remaining needles was high in the samples on the ground. These facts ware observed at the both of Katsura and Nagasaka experimental forest.

Decomposition rate during 12 month was higher in the Nagasaka than those of Katsura. There were considered that physical compaction and high humidity with snow coverage during winter accelerate decomposition at Nagasaka. At the Nagasaka, effects of thinning was also examined. Higher decomposition rate was observed in the non-thinng than thinning site.

It suggest that returnning green neddles to the soil surface is important for maintain the nitrogen in the thinning site. Thinng also has a mechanism to retain a nitrogen in the green neddles with delaying the decomosition in the early decomoposition stage.We will try to identification of nitrogen fixation bacteria to clear the mechanisms and quantitative effects of nitrogen fixation activity to nitrogen immobilization in the litter of Japanese cedar in the near future.

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The relationship between reproductive success and clonal structure in a simultaneous flowering population of the monocarpic dwarf bamboo, *Sasa veitchii* var. *hirsuta*.

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Dwarf bamboos (*Sasa* spp.), which often dominate in the understory vegetation of Japanese forests, have a peculiar life-history: they flower synchronously over an extensive area and then die once in a hundred years or more. Before the simultaneous flowering they grow vegetatively by extending rhizomes, so they can be considered as having “initial seedling recruitment” (ISR) strategy. In this study, we investigated the spatial distribution and fecundity of each ramet and genet in a simultaneous flowering population of *Sasa veitchii* var. *hirsta*, and asked how clonal structure affects reproductive success. Using microsatellite markers, we genotyped all flowering ramets (4516 culms) in the 10 _ 10 m study plot and 1736 seeds (representing 10% of the resulting seeds in the plot) for which pollen donors were determined through paternity analysis. Our results showed that a number of genets with different sizes intermingled in the population (on average, 12.5 genets / m^2), and that larger genets had higher male and female reproductive success. Although selfing rate was not related with genet size (on average, 22%), larger genets produced more seeds that were sired by a larger number of pollen parents. These results suggest that vegetative growth significantly enhanced reproductive success both quantitatively and qualitatively.

Evaluation of PorySilicate-Iron Sludge from Water Purification Plants as Paddy Soil Amendments

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PolySilicate-Iron (PSI) is flocculant for water purification and is composed of $FeCl_3$ and polysilicic acid. The sludge from water purification plant using PSI (PSI sludge) contains iron (Fe), silicon (Si) and organic nitrogen (N). In Japan, Fe amendments have been applied to the degraded (akiochi) paddy fields and some papers showed that its application decreased the methane emission from submerged soils (e.g., Furukawa and Inubushi, 2002). Silicon and N are very important nutrients for rice plant. PSI sludge has the possibility of effective amendment for paddy soils (Horikawa et al., 2007) but there is little information on the properties of PSI sludge. The purpose of this study is to evaluate the effectiveness of the PSI sludge as paddy soil amendment based on the N, Si and Fe availabilities of the several samples.

Seven samples of PSI sludge (3 from the same plant, and others from different plants) were collected. The chemical properties; total-N, C, Si and Fe, and amorphous Fe (acid oxalate extractable Fe) were determined. Amounts of mineralized N, soluble Si and microbial reducible Fe of PSI sludge were determined by the incubation experiments at 30°C for 4-weeks using soilsludge mixture under submerged condition. The availabilities of Si and Fe in PSI sludge was compared with commercial Si and Fe amendments derived from basic slag, by-products of the steel industry. The form of soluble Si was estimated by the selective dissolution method.

PSI sludge had total-N, Si and Fe contents of 5.8, 115, 315 g kg^{-1} on average, respectively. The range of mineralization rates of organic N after 4-weeks incubation and amounts of available N in the PSI sludge were 15-31% and 0.4-4.2gN kg^{-1}, respectively. Relative values of soluble Si content in the PSI sludge were 9-29% of the basic-slag silicate fertilizer compared on the weight basis. The main fraction of soluble Si was estimated to be Si combined with Fe. Acid oxalate Fe (low-crystalline Fe) contents of PSI sludge samples were almost equal to total Fe and 34-63% of total Fe was reduced to ferrous Fe after submerged incubation for 4 weeks. The averaged reducible Fe contents of the PSI sludge were 2.6 times greater than the slag Fe amendments. These results indicate that PSI sludge is very effective Fe amendments containing bioavailable N and Si.

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Effect of allophonic water treatment residual on paddy rice cultivation

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Water Treatment Residual (WTR) is a by-product of municipal drinking water treatment plants. WTR is generated in large quantities every year and most of WTR is disposed as an industrial waste. It is desirable to develop the effective way to use WTR from the viewpoint of the resources recycling.

In the present study, we applied WTR to the paddy field soil and examined the growth improvement of rice plants in 2006, 2007. In those experiments, we found promotive growth at the medium WTR rate and defective growth at the high WTR rate. In 2008, we examined the cause of defective growth when the WTR rate was high.

MATERIALS AND METHODS

The WTR sample was taken from Asaka filtration plant and soil was taken from the plow layer of the paddy rice field at Furukawa Agricultural Experiment Station.

The soil and WTR were mixed at various rates (0, 6, 12, 24, 100% in 2006, 0, 12, 24, 90, 95, 100% in 2007, 0, 90, 100% in 2008) and were packed in pots to become 3.3kg/pot in the total weight. During the growing season in 2006 and 2007, we measured plant height, number of leaves and tillers, leaf color index, and dry weight and the amount of nitrogen uptake after harvest. Further, in 2008, EC of the flooded water and soil solution were determined during the growing season, and concentrations of P and Mn in plant body, Mn concentration in soil were determined after harvest.

RESULT

Growth improvement was observed in plant height, number of tillers, and dry weight of rice plants up to the WTR rates of 24%. Dry weight of ear in the 24% WTR treatment increased twice that of control. However, when the WTR rate was more than 90%, rice growth became defective.

The amount of nitrogen uptake gradually increased to the WTR rate of 24%. Although an increase in nitrogen uptake was significant at the WTR rate was 90%, it decreased at the WTR rate was 100%.

The EC value of the flooded water and soil solution showed very high values compared with control in the 90 and 100% WTR treatments. The P concentrations of plant body were very low, and the Mn concentration in plant body and soil were very high in these treatments. These were considered to be the causes for the defective rice growth.

Clavicipitaceous endophytes in grass species; guardians against herbivores evolved from plant pathogen.

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Endophytic fungi belong to the clavicipitaceae family (clavicipitaceous endophytes), are very common among grass species and known to have variouls influences for host plants (Rodriguez et al. 2009; Clay and Schardl, 2002). Their effects are not limited within the hosts, but can be extended to the entire ecosystem, trough the modification of food chain and flow of elements in which the plants are involved (Omacini et al. 2001, 2004). Among the endophytes, *Neotyphodium* endophytes, asexual fungi derived (/evolved) from pathogen causes disease on plant inflorescences (*Epichloë* species), are famous for their ability to form permanent association with their hosts through seed transmission, and provide protection to insect herbivory by producing anti-insect compounds (Clay and Schardl, 2002). Since the endophytes often associated with grass species used as forage and turf (Sugawara et al., 2006), we are tring to use a species of them for forage grass breeding, rivaling with other research groups in the world, help prevent propergation of insect pests in meadows and reduce their population, hence damage caused by them, in the entire agro-ecosystem including other commercial crops such as rice (Shiba and Sugawara, 2008). The endophytes also getting attention as they look abondant and can be found from diverse grass species, not only in continents but also here in Japanese islands (Sugawara et al., 2009), and likely to be protecting seeds of host grasses from insect predetors (Maruyama et al., 2009). International symposium about the endophytes will be held hosted by the University of Kentuky, USA, in the summer of 2010 (http://www.ca.uky.edu/msaisefg/).

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Chemical and microbial characteristics of forest soil by the difference of forest management

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Artificial forest in Japan occupied more than quarter of the country, where cedar and cypress were planted. After 1955, importation of cheap woods from abroad to Japan increased, and depopulation of mountain village continues, there were many abandoned artificial forests. Then, surface soil runoff, windfall tree and pest arise became problems.

To decrease global warming, utilization of forest is important. Forest management is changing for making biodiversity and healthy forest. However, effects on chemical and microbial characteristics of forest soil by the change of forest management need to be fully elucidated.

In this study, chemical and microbial characteristics of forest soil by the difference of forest management were investigated. Quality of water extracted from different management soils was compared by ICP analysis. Concentrations of potassium, iron and manganese in water from 67% thinning forest soil were higher than that from no thinning forest soil. By analyzing with electrochemical analysis, cyclic voltammetry, reduction wave peak in extracted water from 67% thinning forest soil were clearer than that in water from no thinning forest soil. The peak potentials of the reduction wave between water from 67% thinning forest soil and from no thinning soil were different. These results suggested that characteristics of organic complex were different between 67% thinning forest soil and no thinning forest soil. Analysis of bacterial community in soil using PCR-DGGE, the community of 67% forest soil and that of no thinning forest soil were almost same.

Nitrogen cycle of agricultural system in Field Science Center, Tohoku University

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In Japan, large losses of nitrogen from arable and livestock farming threat the environment. Therefore, nitrogen should be used more efficiently by collaboration between arable and livestock farming systems. To reduce the nitrogen loss, nitrogen balance in agricultural systems should be more accurately estimated. In this study, we assessed nitrogen balance in Field Science Center, Tohoku University, using the data of bulletin and some literatures. The Field Science Center is composed of the integrated system of arable and livestock farms. In the arable farm (60 ha), rice, potato, some vegetables and forage crops are grown. In the livestock farm, about 200 head of cattle are raised for dairy and beef production. Compost from livestock is used in the arable farm.

We estimated the nitrogen cycle in Field Science Center in 2007. The nitrogen loss was estimated to be 12.7 tN/yr. The output as products was 2.3 tN/yr, while the input from chemical fertilizer, feed and litter straw were 5.9, 7.4 and 0.3 tN/yr, respectively. Forage crop and litter straw (6.2 tN/yr) were supplied from arable land to livestock. The amount of animal waste was about 10.3 tN/yr. It was partially composted, and the compost (3.5 tN/yr) was supplied to arable land. Consequently, the input-output ratio was calculated as about 17 %. To improve the environmental performance of the Field Science Center, future direction will be discussed.

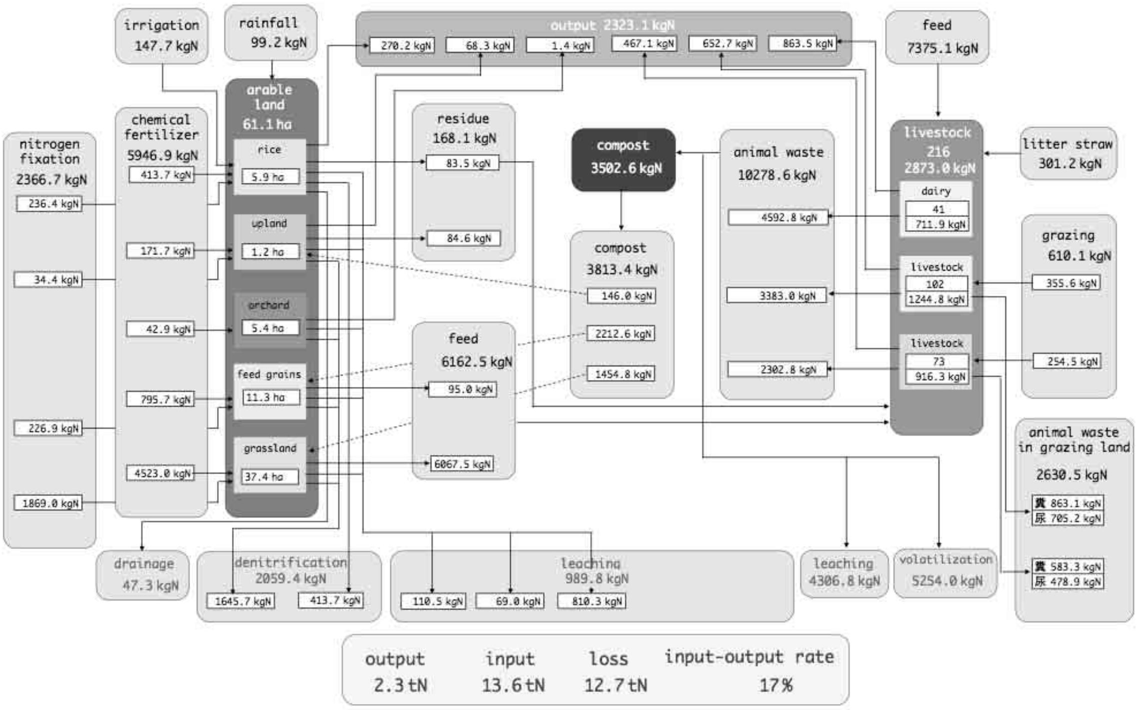

Fig. 1. Nitrogen cycle of agricultural system in the Field Science Center in 2007.

Effects of periodic application of cattle slurry on soil microbial biomass in an Andisol grassland

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Dairy farming is an important agricultural industry in northeastern of Japan. Large amounts of cattle wastes are produced in dairy farms. Most of the wastes are used in the production of compost. In some cases, slurry originating from cattle waste is periodically applied to farmlands as a fertilizer. Mizota *et al*. (2006) investigated the fate of nitrogen compounds in the soil after the application of cattle slurry to an Andisol grassland with a focus on temporal changes in chemical composition and the natural abundance of nitrogen isotopes. Although they mentioned that microbial action might contribute to the transformation of nitrogen in the soil, the microbial characteristics of the study site have not been investigated. The purpose of this study is to investigate the microbial response of grassland soils after the application of cattle slurry.

The study was performed on an Andisol grassland located in the middle of Iwate Prefecture, northeastern Japan that was not grazed by cattle. In the study site, the dominant herbs were *Pharalis arundinacea* L. Cattle slurry from dairy farms was applied to the soil surface of the whole area in the study site in June and October 2005. In addition, 36 kg of cattle slurry was applied to the soil surface in September 2005 in small quadrats (2 x 2 m^2) set up at the middle and lower slope positions. Soil samples were collected from June to November 2005 from the upper 0-5cm soil layer in each slope position. The chemical characteristics and microbial properties of the samples were analyzed.

Application of cattle slurry caused a flush of NH_4-N release in the soil. A slight increase in the NO_3-N content followed a sharp decrease in the NH_4-N content in the soil. This showed that rapid nitrification occurred by the action of nitrifying bacteria in the soil. The colony forming units of heterotrophic, urea-decomposing and protein-decomposing bacteria in the soil fluctuated during the investigation period. The CO_2 evolution rate from the soil surface was strongly affected by the application of slurry. In contrast, soil microbial biomass C and N contents remained constant, regardless of the application of slurry. As a consequence of this, the metabolic quotient qCO_2 (microbial respiration per unit biomass) changed in parallel with the fluctuation of the CO_2 evolution rate. These results indicate that the application of slurry with high concentrations of nitrogen compounds influences the microbial quality in the soil.

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Higher seedling mortality of conspecific seedling compared to heterospecific ones beneath the adults for three hardwood species

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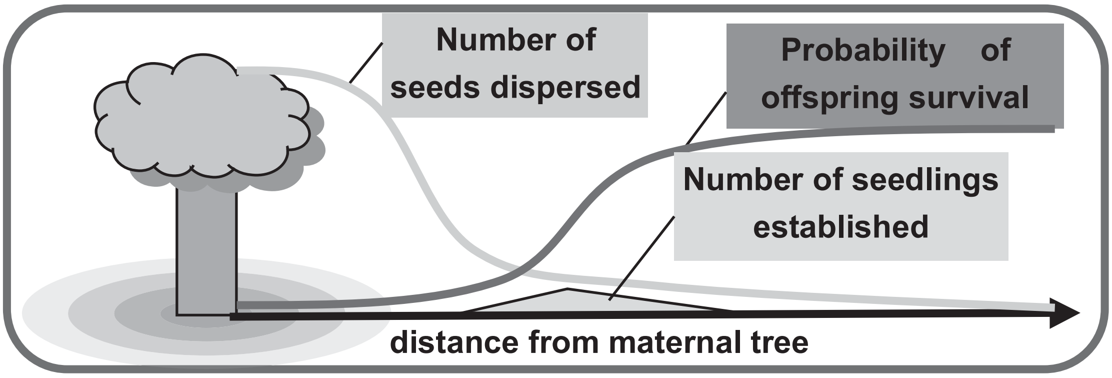
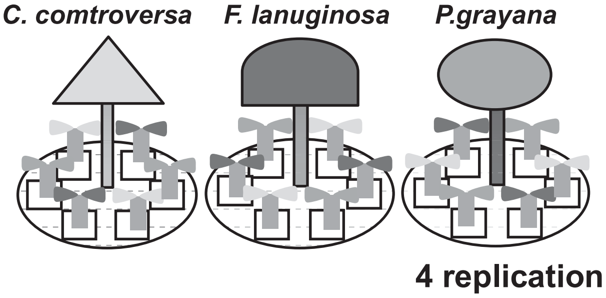


Janzen-Connell model

Janzen-Connell model is one of the most important models explaining the species diversity in forests. The model suggests that conspecific progeny which dispersed beneath the adults can’t establish because species specific natural enemies (e.g., pathogens, herbivores) attack the progeny in negative-density and/or positive-distant dependent manner. Thereby, the mechanism freeing space for other species, resulting in species diversity. There are increasing evidences in the Janzen-Connell model even in temperate forests. However, little is known in the mechanism of the replacement from conspecific to heterospecific juveniles beneath the adults. Particularly, little is known whether conspecific seedlings show higher mortality compare to heterospecific ones beneath the adults.



Seeds of three hardwood species were sown beneath each of the adults of the three hardwood species. We investigated the seedling survival and the causes of the mortality during three years after germination.

Conspecific seedlings suffered most severe damage by pathogenic fungi compare to the two heterospecific species beneath the adults. As a result, seedling mortality was higher for the conspecific compare to the two heterospecific species. This species-specific attack would lead to promote the species replacement nearby the adult generating species diversity in forest communities.

Comparison of allophanic soils of contrasting carbon contents induced by agricultural management: organo-mineral interaction, carbon mineralization, and it's temperature sensitivity

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Volcanic-ash soils hold significant amounts of organic matter (OM) largely because inorganic constituents unique to such soil (e.g., poorly-crystalline minerals and dissolved aluminum) have high capacity to stabilize OM. Better understanding of stabilization and destabilization processes is critical to effectively manage volcanic-ash soils for C sequestration and fertility.

Here we focused on an allophanic Andisol from a long-term experimental field (Tsukuba, Japan) and compared three soil samples (Ap horizon) of contrasting OM contents that resulted from agricultural practices. NT soil was from the plot under no-tillage plus leaf-manure addition for two decades and had 10% total C. NT soil was separately sampled for 0-5 and 5-20 cm due to clear change in soil structure and color. CT soil (5% C) was from conventional tillage with no manure. BA soil (4% C) was from a bare plot which had almost no OM input in recent years.

One-month lab incubation showed that the amount of respired C as well as the percent of total C respired increased in the following order: BA < CT < NT 5-20cm < NT 0-5cm, showing that the pool size of labile C positively correlated with that of soil total C. Density separation revealed that samples with lower total C contents had greater proportions of total C in higher-density fractions (presumably stabilized via organo-mineral interaction).

Based on simple enzyme kinetic theory, we expected that temperature sensitivity (Q_{10}) of soil C mineralization progressively increase from NT to CT to BA based on the OM distribution of labile low-density fraction and more stable high-density fraction among the soils. Estimated Q_{10} from 25 vs. 35˚C incubation showed the highest Q_{10} for BA soil. The rest of soils, however, had similar Q_{10} values despite 3-fold difference in total C and 9-fold difference in respired C among them. These results suggest non-linear response of Q_{10} to soil C pool size and warrant more detail assessment of the temperature control on soil C dynamics.

Influence of pH on soil washing and phytoextraction of Cd from soil, a case study using the paddy-field soil with a high pH

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Applications of alkaline materials and continuous flooding are practical methods to suppress uptake and accumulation of Cd into rice grains in moderately low cadmium contaminated paddy fields. However, flooding is not applicable to upland fields. When alkaline materials are not enough to suppress Cd uptake, additional managements are needed. Among various remediation methods, soil washing (Hayashi 2007) and phytoextraction are promising methods to reduce Cd uptake.

The objectives of this study were to evaluate the influence of pH on soil washing and phytoextraction using Cyoukoukoku when these restorations are conducted in the paddy-field soil with a high pH.

As chemicals for soil washing, $FeCl_3$ was used. The paddy-field soil was previously limed to reduce Cd uptake by rice plants. A 40 kg portion of field-moist soil was placed into a 75 L plastic container with 30 L tap water and 540 g $FeCl_3 \cdot 6H_2O$, the soil suspension was thoroughly stirred for 1 h and the mixture was allowed to stand for 1 day. After sedimentation of soil, the supernatant solution was removed. The soil was rinsed repeatedly with tap water to eliminate excessive salts. After $FeCl_3$ washing, the content of Cd extractable in 0.1 mol L^{-1} HCl decreased from 5.6 to 3.0 mg kg^{-1}, soil pH changed from 7.7 to 5.3. Subsequently, we conducted a pot experiment to evaluate the effect of soil washing with $FeCl_3$ on Cd adsorption by soybean. We designed four kinds of treatments using washed and unwashed soil. Cd concentration of soybeans which were grown in washed soil was more than 1 mg kg^{-1} and evident reduction by soil washing was not found. High pH of the used soil may be a major reason for the results. Cadmium in soil was transformed between exchangenable and inorganic fractions with pH change. Because $FeCl_3$ extracted mainly the acid-soluble form of Cd, exchangenable fraction increased with decreasing soil pH caused by the washing treatment.

Phytoextraction using Cyoukoukoku, a rice variety, was conducted to evaluate the reduction of soil Cd. We carried out rice cultivation experiments twice for a year using washed and unwashed soil in 5 L plastic pots. Water treatment used for the present study was a mimic midseason drainage with intermittent irrigation. After 2 croppings, the content of Cd extractable in 0.1 mol L^{-1} HCl was approximately 10% lower than those before the rice cultivation. Cadmium uptake in the shoot was affected by the water treatment and soil pH.

Judging from the results of this study, we confirmed the Cd extraction with $FeCl_3$ was more effective than the present phytoextraction using Cyoukoukoku although cultivation was only twice, but it is necessary to pay attention to chemical forms of Cd affected by pH.

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Aluminum bioavailability of aluminum-humus complexes in Andosols

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Non-allophanic Andosols often show aluminum (Al) toxicity to Al-sensitive plant roots. The origin of the toxic Al has been considered to be primarily Al^{3+} adsorbed on permanently charged sites of 2:1 type minerals. However, it was suggested that Al-humus complexes are one of the pools of toxic Al (Takahashi et al. 2007). In contrast, natural allophanic Andosols rarely show Al toxicity to plant roots although allophanic soils also contain Al-humus complexes. With strong acidification, allophanic Andosols then come to possess toxic Al which injure to plant roots (Takahashi et al. 2008). The origin of the toxic Al in allophanic Andosols is not still clear. The aim of this study is to clarify the origin of bioavailable Al in Andosols using cultivation of Al-sensitive plants and Al-tolerant plants.

Nine A horizon soil samples were used to this study; two typical non-allophanic soils (pH (H_2O) 4.4-4.7), their limed soils (pH (H_2O) 6.0), two typical allophanic soils (pH (H_2O) 5.7-7.0), three acidified allophanic soils (pH (H_2O) 4.6-5.4). We cultivated burdock (*Arctium lappa*) and barley (*Hordeum vulgare*) as Al-sensitive plants and buckwheat (*Fagopyrum esclentum*) as an Al-tolerant plant. We measured the root lengths of burdock and barley after 4-day culture, and determined Al concentrations of buckwheat plants after a month culture.

Typical non-allophanic soils showed strong toxicity of Al to roots of burdock and barley. Although Al toxicity was not observed in the typical allophanic soils in the Al-sensitive plants, acidified allophanic soils did show the toxicity as observed in the non-allophanic soils.

Reflecting the toxicity (bioavailability) of these soils, Al concentrations in buckwheat plants grown in non-allophanic soils were much higher (2.6-4.3 mg kg^{-1}) than those in typical allophanic soils (0.4-1.4 mg kg^{-1}). However, those concentrations of buckwheat in acidified allophanic soils were comparable (2.7-4.0 mg kg^{-1}) to those in the non-allophanic soils. Because these allophanic Andosols contained few 2:1 type minerals, it is assumed that Al^{3+} adsorbed on permanently charged sites of the minerals is not abundant. Therefore, we considered that Al-humus complexes play important roles of Al toxicity (availability) in the acidified allophanic Andosols as well as non-allophanic Andosols.

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Actinorhizal plants and *Frankia* in Japan

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The actinomycetal genus *Frankia* forms root nodules in some woody plants that fix atmospheric nitrogen. Such plants are called *actinorhizal plants*. Actinorhizal plants indigenous to Japan are the genus *Alnus*, *Myrica*, *Elaeagnus* and *Coriaria* as well as the introduced species *Casuarina* and *Hippophaë*. Among these plants, 12 species from six genera of actinorhizal plants were examined in terms of the morphological characteristics of root nodules and of *Frankia* strains isolated.

Actinorhizal root nodules are generally perennial, with coralloid structures consisting of multiple nodule lobes. The tissue of the nodule lobe consists of a nodule meristem at the tip of the nodule, vascular tissue, cortical tissue and a superficial periderm. In contrast to legume root nodules in which microsymbiont-infected cells are surrounded by vascular tissue, *Frankia*-infected cells are distributed in the cortical tissue around vesicular tissue. Root nodules of *Alnus* are dark orange to brown, with the color becoming lighter towards the apex of the nodule. The size and shape of the nodule vary; nodule lobes of *A. japonica* are densely packed, whereas those of *A. sieboldiana* and *A. firma* are discrete. *Alnus serrulatoides* has tiny and discrete nodule lobes (Fig. 1). *Myrica* nodules are khaki to sandy-brown with nodule roots from the apices of the nodule. *Elaeagnus* nodules are light brown with dark-brown scales. *Coriaria japonica* has pale golden nodules in which vascular tissue is not central. *Casuarina* nodules are light yellow with nodule roots.

Frankia strains have been isolated from actinorhizal plants from four genera. The color of these isolates varied: *Alnus* strains were white to light gray/light purple. *Casuarina* strains were light gray. Strains from *Myrica*, *Elaeagnus* and *Hippophaë* were light pink to pink. Under a microscope, these strains have round or highly irregular sporangia filled with spores and vesicles, the sites of nitrogen fixation (Fig. 2), both of which are characteristics of *Frankia*.

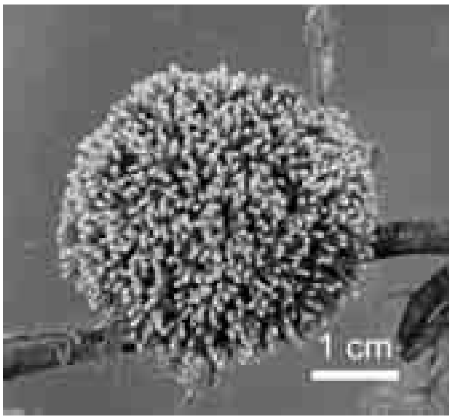

Fig. 1. Root nodule of *Alnus serrulatoides*

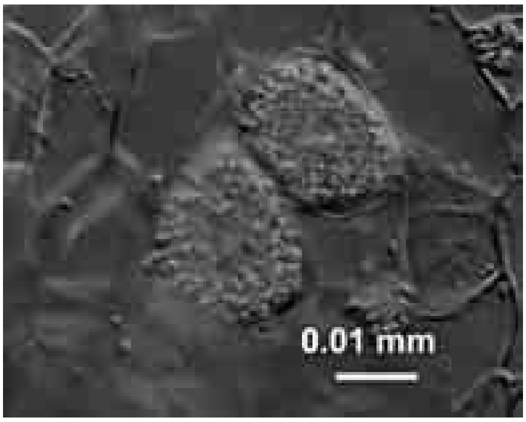

Fig. 2. Vesicules in cortical cells in a root nodule of *Alnus sieboldiana*

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