# Evaluation and characterization of leaf litter decomposition patterns in ecological succession

（生態遷移におけるリター分解パターンの評価と特徴）

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## Contents

# 要 旨

生態遷移は、地上部環境と地下部環境の相互作用の変化により進行する。リター分解は、その地上部と地下部をつなぐ重要な鍵である。即ち、地上部の植物群集によってもたらされたリターの分解は土壌形成の原動力となり、土壌養分の蓄積が地上部植生に還元され生態遷移が進行する。遷移の進行にともない地上部の植物群集が多様化するにつれ、生産されるリター組成も多様化するが、リター組成の変化とリター分解過程の変化の関係には議論の余地がある。さらに、遷移系列に沿ったリター分解過程の変化に関する研究は少なく、リター分解と遷移の関係には未詳の点が多い。そこで、本研究では北海道における裸地から極相林までいくつかの遷移段階にある植生について、リター分解過程を植生と環境の変化と共に調査した。同時に、リター分解特性を把握するために、同位体元素分析を用い、リター中の炭素量(C)・窒素量(N)と各安定同位体比を測定し、リター分解過程におけるリター特性の変化を調べた。発達した生態系では、リターの生物的分解は、主に菌類および細菌類によってなされる。そこで、各生物群が特異的に生産するリン脂質脂肪酸(PLFA)を分析し、主な分解者とその生体量の時間的変化を特定した。リター分解測定に広範に用いられているリターバッグ法は、攪乱地における測定に不向きであったため、リターバッグ法によらないリター分解量の測定方法を考案した。さらに、その方法を用いて火山遷移上でのリター分解特性を調べた。

遷移しつつある森林におけるリター分解特性を明らかとするために、シラカンバ林およびミズナラ林において調査を行った。植生調査から、シラカンバ林からミズナラ林に移行しつつあることが示された。これらの 2 森林において、リターの混合効果と拠点効果を検証するために、シラカンバ、ミズナラおよび両者を混合したリターの 3 種類を用意し、それらの分解特性を 3 年間にわたり測定した(第 1 章)。リター分解は、リターバッグ法によって測定し、あわせて、各森林の夏期の開空度・林床光量・土壌水分量・温度と、リターの種組成・堆積量を調査した。林床におけるリター組成や光量・温度が 2 つの森林では異なるにも関わらず、リターは最初の 1 年間で大部分は菌類により分解され、リター中の炭素減少と相対的な窒素増加が起こることが明らかとなった。一方、細菌類は、初期リターにはほとんど見られないが、3 年の間に徐々に増加した。PLFA 組成をもとにした正準対応分析は、リター分解量は、細菌量よりも菌類量に規定され、また、リター種は細菌類に生産された PLFA 組成と相関があり細菌組成はリターの質に規定されていることが示唆された。混合リターの分解速度は、単一種リターと異なるが、その応答は森林により異なった。即ち、混合リターの分解は、シラカンバ林では減速したが、ミズナラ林では減速が見られなかった。これらのことから、リターの混合効果は、遷移段階により異なり、また、拠点効果は明瞭ではないことが明らかとなった。

リターバッグ法によるリター分解測定は、攪乱地などにおいては、リターバッグの消失や動物による持ち去りなどによる継続調査の困難性、細粒混入などによる異常値の発

生などの問題点が指摘されている。そこで、火山遷移上でのリター分解を測定するために、リターバッグを用いないリター分解率の推定式を開発した。これまでも、リター分解率と C/N 比や N 含量には高い相関があることが知られていたが、本研究では、複数の測定変量をもとにした重回帰分析によるリター分解量推定を試みた(第 2 章)。調査は、環境の大きく異なる湿原と森林に、リターバッグを敷設し定期的に採取し、リターの重量減少率とリター含有炭素量・窒素量と安定同位体比を分析した。リター重量減少率は、C/N 比と相関が高かったが、さらに C・N とそれらの安定同位体比を加えたモデルによりリター分解率の推定精度が大きく向上した。湿原においては、生息地やリター種を変量に加えると予測精度は、さらに高くなった。したがって、植生やリター種の組成が大きく異なる生態系間の比較研究においては、これらの変量を考慮することで、リター分解推定が可能であることが示された。

本知見を活用し、北海道南西部に位置する有珠山において 3 つの噴火跡地において、クロノシークエンス法によりリター分解を測定した(第 3 章)。1910 年噴火跡にはドロノキ林、1977-78 年噴火跡にはオオイタドリ草地、2000 年噴火跡には裸地が発達しており、裸地、草地、森林という遷移系列に沿ったリター分解特性の変化が観察できる。そこで、各噴火跡地から前年に落葉したオオイタドリとドロノキのリターを採取し、それらの C、N 含有量と PLFA による微生物量の解析を行い、あわせて土壌層発達度を測定した。その結果、遷移が進行した植生ほどリター中の C/N 比は低く、土壌層は厚くなることが明らかとなった。特に、森林において腐植層の発達は著しかった。リター付着微生物量は遷移の進行に伴い増加していたが、いずれの遷移段階においても、リター種を問わずにリターに付着する菌類量は細菌量よりも多かった。

以上のことから、冷温帯において落葉広葉樹が極相を形成する地域では、遷移初期から極相に至るまで、菌類がリター分解の主体をなすこと、遷移の進行にともない菌類量は増加するが細菌類量には大きな変化が見られないが種組成が変化していること、が明らかになった。さらに、遷移に伴う植生やリターの発達などの地上部で見られる現象よりも、地下部の環境変化がリター分解に強く関与していることが示唆された。

## Preface

Understanding the mechanisms of ecosystem replacements, including succession, is prerequisite to conserve and restore ecosystems. Plant succession starts with bare ground after severe disturbances, such as volcanic eruption that develops nutrient-poor habitats. Therefore, interspecific competition through nutrient acquisition (below ground competition) mostly determines the ecosystem replacements in the early stages of succession (Turner 2010). With developing vegetation on the denuded ground surface, interspecific competition through light acquisition (aboveground competition) becomes more intense than competition through nutrient acquisition. The nutrients are primarily supplied by decomposed plant litter (Bardgett et al. 2005), indicating that litter decomposition is a driver of succession.

Litter decomposition becomes fast from a bare ground to a forest in a temperate region, Czech Republic (Urbanová et al. 2014), and from a bare ground to a *Metrosideros polymorpha* fotrest on a Hawaiian lava flow (Crew et al.1995). However, litter decomposition is slowed by biomes where the dominant plants have low carbon-to-nitrogen ratio (C/N ratio) that expresses litter quality (Wadle et al. 2004; Zhang et al. 2008). Therefore, dominant species in ecosystems were focused in this study. Litter is primarily decomposed by three pathways: physical, chemical and biological decomposition (Berg 2000). Of these, biological decomposition conducted mostly by two microbial taxa, bacteria and fungi (De Boer et al. 2005). Fungal biomass in litter increases for the first few months in seral temperate forests (Voříšková and Baldrian 2013) while bacterial biomass is high in nutrient-poor glacial retreats and

boreal forests (Knelman et al.2012). To clarify how litter decomposition is changed across succession, therefore, the temporal changes of bacteria and fungi in litter should be evaluated. Because bacteria and fungi produce their specific phospholipid fatty acids (PLFAs), PLFAs in litter quantify the biomass of bacteria and fungi (Frostegård et al. 2011).

To detect temporal changes in litter decomposition patterns, I firstly examined litter decomposition in two forests (birch and oak forests), of which successional stages were different, for three years, in relation to their plausible determinants, i.e., litter quality, litter species and litter-decomposing microorganisms, by using a litterbag method (Chapter 1). The litterbag method has been widely used to evaluate litter decomposition (Wieder et al. 1982; Bonan et al. 2013). However, the litterbag method is not appropriate when the ground-surface disturbance is severe. Therefore, I proposed a new method to estimate litter decomposition without using litterbags. The non-litterbag method, using changes in chemical properties in litter with the decomposition, was tested in two distinctive ecosystems, a forest and a wetland (Chapter 2). I confirmed that N and/or C/N ratio were effective indicators to estimate litter decomposition rate and microbial activities, based on the comparisons, although there were a few restrictions. Using the indicators, the characteristics of litter decomposition were examined along a successional sere from bare ground to forests on Mount Usu, northern Japan (Chapter 3). The litter decomposition was characterized by fungi throughout the succession. In addition, the succession of bacteria was also observed in the litter. Compared with the references on litter decomposition after disturbances in the cool-temperate regions, I discussed the determinants of the litter decomposition in terrestrial ecosystems.

(a)

(b)

**Fig. 1** Sites for measuring litter decomposition and its related factors in a bare ground on Mount Usu (a) and in an oak forest on Mount Toishi (b).

# Chapter 1

# Litter decomposition patterns are not synchronized with changes in forest structures

## 1.1 Introduction

Litter decomposition determines nutrient and carbon dynamics in terrestrial ecosystems (Wadle et al. 2004). The decomposition is promoted by three processes, physical, chemical and biological degradation. The biological decomposition is conducted mostly by the two microbial taxa, bacteria and fungi (Osono 2007) and, therefore, litter decomposition is often determined by the biomass and composition of microorganisms (Chapman and Newman 2010).

A hypothesis called home field advantage (HFA) has been proposed for the determinants of litter decomposition rate (Ayres et al. 2009); that is: litter decomposes faster in the inside of its own ecosystem than in the outside, because of the predictable and suitable environments for microbial activities (Prescott and Grayston 2013). For example, litter decomposition is 58% faster in the inside of *Betula pendula* forest than in the outside. The slow decomposition in the outside is derived from the environments unsuitable for the litter decomposition (Hobbie 1992), depending on the differences in litter traits, such as nitrogen and carbon/nitrogen ratio (Perez-Harguindeguy et al. 2000). In contrast to HFA, a hypothesis called litter-mixing effect of litter (LME) predicts that the litter decomposition is faster in litter

consisting of multi-species than of mono-species (Wardle et al. 2009), because such mixed litter provides heterogeneous litter quality and structure that improve the chemical and physical environments for litter decomposition (Gartner & Cardon 2004). However, the mixing effects are observed only when the quality and quantity of litter species are different conspicuously, e.g., broad-leaved and needle-leaved litter. Therefore, LME may not occur clearly in seral forests where the leaf litter quality is somehow homogenous. Not only vegetation structures but also its related environments are altered with the progress of succession, suggesting that litter decomposition patterns change with time. Studies on litter decomposition along succession after large-scale disturbances have been a few, even though litter decomposition is a trigger of nutrient cycling (Otaki et al. in press).

Litterbag experiments are often used to investigate litter decomposition patterns (Wieder and Lang 1982; Kazakou et al. 2006). The content of carbon and nitrogen in decomposed litter was also measured to estimate the nutrient fluxes and litter traits (Berg and McClaugherty 2008). Furthermore, the changes in microbial biomass in relation to litter decomposition were evaluated by the quality and quantity of phospholipid fatty acids (PLFAs) (Snajdr et al. 2011). Here, the three hypotheses were proposed: (1) HFA and/or LME were weak in deciduous forests, (2) two microbial taxa, fungi and bacteria, differently contributed the litter decomposition, and (3) the litter decomposition patterns changed with succession.

## 1.2 Materials and methods

### Study sites

The study was conducted on the lowland developed on the foot of Mount Toishi (826 m a.s.l.) in the city of Sapporo, Hokkaido, northern Japan. In the area, oak (*Quercus mongolica* Fisch. ex Ledeb. var. *grosseserrata* Rehd. Wils.) and birch (*Betula platyphylla* var. *japonica* (Miq.) Hara) develop the two distinctive forests (hereafter, i.e., oak and birch forests). These two tree species are widespread throughout the temperate forests in eastern Asia, including Japan (Kitao et al. 2000) and are generally replaced from the birch to the oak forests along the successional sere (Bradshaw et al. 2005). The birch and oak forests were used for investigating the characteristics of litter decomposition in early and late successional forests. The forest soil in the surveyed area was classified into brown forest soil with pH 6.5 ± 0.8 (mean ± SD).

Mean annual temperature was 9.8-9.3°C for the three surveyed years from to 2012 (Japan Meteorological Agency 2012). The maximum temperature was recorded in 33.8°C in August 2011 and the minimum was -12.6°C in February 2010. The annual precipitation ranged from 1254 mm to 1325 mm for the three years, and snow mostly supplied during November and April explained 21% of the precipitation. Therefore, the precipitation and temperature were nearly identical for the three years.

A 15 m × 15 m plot was established in an oak forest and a birch forest, respectively. The elevation was 130-140 m (42°59'N and 141°19'E). The two forests were adjacent to each other. In each plot, stem height and diameter at breast height were measured on each tree of which height was taller than 5 m. The cover and height of each species

in herbaceous layer (0-2 m above the ground surface) was visually measured in each plot. The stem volumes were calculated from the tree height and diameter with an assumption of cylindrical shape.

To detect litterfall quality and quantity in each forest, litter collected from 18 20 cm × 20 cm plots established on each forest in late November soon after the defoliation. The litter was separated into oak, birch and others and was weighed after air-dry for two weeks.

The temperature and light intensity on litter layer were measured at one-hour intervals during snow-free periods every year, by temperature/light data loggers (UA-002-64, Onset computer corporation, Bourne). The snow-free periods were from December to April in all the winter. Three loggers were set up in the oak forest and four were in the birch forest. The light intensity was converted to photosynthetic photon flux density (PPFD) by an equation (Thimijan and Heins 1983). PPFD and temperature were averaged and used for the further analysis. Fisheye photos were taken to upward at 1.2 m above the ground surface on five locations in each plot at two-to four-month intervals in the summer seasons. Canopy openness (%) was measured on each photo by Gap Light Analyzer (GLA) (Frazer et al. 1999), and was averaged in each plot. Soil moisture was measured at nine points in each forest at two-month intervals from April to November by a time domain reflectometry (Hydrosense, Campbell Scientific Australia, Queensland).

## Litterbag

A litterbag method was applied to examine litter decomposition patterns (Wieder and Lang 1982). The litter samples were collected from the ground surface adjacent to the two surveyed forests in late November 2009 when most leaves defoliated. The samples were weighed after air-dry for more than two weeks. The three types of litter composition were made to detect litter-mixing effects on litter decomposition: 5 g of oak litter, 6 g of birch litter and 6 g of litter mixture made by 3 g of birch and 3 g of oak litter.

The litterbag was made of black sheer nets with 2 mm pore, with approximately 20 cm long and 12 cm wide. The litterbags were set up on the ground surface of the two forests in mid-December 2009. The litterbags were collected at two to four month intervals during snow-free periods between April and December 2010 and 2011, and between April and August, 2012. At each sampling, three litterbags were recovered from each forest.

## Measurements of chemical properties in litter

The litter samples recovered from the fields were freeze-dried over seven days soon after the recovery and were weighed to measure the litter decomposition rates. The litter mass remaining was calculated as: (litter remaining weight) divided by (initial litter weight). The litter samples were grinded in a mill with a metal blade. Carbon (C) content and nitrogen (N) content were measured with a stable isotope ratio mass spectrometer (Finnigan MAT252, Thermo Fisher Scientific, Yokohama). Carbon to

nitrogen ratio (C/N ratio) was evaluated to surrogate for the litter decomposition rates, because C/N ratio becomes low with proceeding litter decomposition (Arts and de Caluwe 1997). As the same way, these chemical properties in the initial litter, i.e., zero days after litter decomposition, were measured as control.

**PLFA analysis**

The litter samples were subjected to the quantification of PLFAs. The PLFAs were identified and quantified to measure the bacterial and fungal biomass in the litter (Urbanová et al. 2014). Total lipids were extracted from 1.0 g freeze-dried litter soaked into a mixture of chloroform: methanol: deionized water (1:2:0.8, v/v/v) (White et al. 1979). After the two-phase partition, the low layer containing lipids was extracted. The phospholipids were separated from total lipids by a thin layer chromatography with a silica gel under a developer (91:30:8 = acetone: benzene: water). The phospholipids subjected to a mild alkaline methanolysis and fatty acid methyl esters were detected by a gas chromatography (G-3000 Gas Chromatograph, Hitachi, Tokyo) with a frame ionization detector using a 30-m 5% phenyl silicone capillary column (HP-5) exposed to helium as a carrier gas. The temperatures of the injector and detector were adjusted at 270°C. The temperature in the oven was kept at 160°C for 5 min, and then was raised at 1°C/min up to 180°C and 10°C/min up to 240°C.

PLFAs were identified and quantified on each sample, by the comparisons with the internal standard, nonadecanoate fatty acid (19:0). Fatty acid methyl esters were identified by the standards and literatures by a gas chromatograph-mass spectrometer (Varian Saturn 2200, Agilent Technologies, Santa Clara). The taxon-specific PLFAs

are: i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0 and cy19:0 produced only by bacteria and 18:2 only by fungi (Snajdr et al. 2011). Of these, 10Me-16:0, 10Me-17:0 and 10Me-18:0 are produced only by actinomycete bacteria and 16:1ω7 is produced mostly by bacteria and few by arbuscular mycorrhizal fungi (Graham et. al 1995). Therefore, 16:1ω7 was treated as the production of bacteria. The most of all 18:2 in litter was derived from fungi because 18:2 derived from plants was vanished soon after defoliation (Laczko et al. 2004). The other PLFAs were produced by plants, bacteria and/or fungi, and were treated as PLFAs produced by miscellaneous organisms. B/F ratio was calculated based on the quantification of phosphorus in PLFAs.

**Statistical analysis**

Litter decomposition rate ($k$) was calculated by $\log (X/X_0) = - kt$, where $X$ is litter remaining mass on $t$ days after the decomposition and, $X_0$ is the initial litter mass (Olson 1963).

The temporal changes in litter mass remaining, PLFAs, C, N, and the stable isotopes were compared by a generalized linear model (GLM) with an assumption of Gaussian distribution. The differences in litter mass remaining, C, N, C/N ratios and PLFAs between litter species and between forests were examined by generalized linear mixed-effects model (GLMM) with the day after the litterbag establishment as a random effect. The multiple comparisons confirmed that the differences in litter remaining mass and litter decomposition rates between 0-962 days were performed by Steel-Dwass test. All the significance levels were set at $P < 0.05$.

Canonical component analysis (CCA) investigated the distribution characteristics of PLFAs in the litter. The species matrix was made by PLFAs produced specifically by fungi and bacteria. The environmental matrix consisted of four factors: litter species, forest sites, days after establishing litterbags and litter remaining. All the variables were numerical except litter species that was categorical. Monte Carlo Permutation test was conducted to confirm the significance of CCA. Analysis of similarities (ANOSIM) was performed on years after establishing litterbags to examine the differences between the sampling dates (Anderson and Walsh 2013). All the statistical analyses were performed by a software package R (ver. 3.1.3).

## 1.3 Results

### Forest structures

The stem volume and density totaled 18 $m^3$/plot and 818/ha in the oak forest, respectively. *Q. mongolica* var. *grosserrata* explained 75% of the stem volume, followed by 16%, 7% and 2% of *Kalopanax pictus* (Thunb.) Nakai (16%), *Cornus controversa* Hemsl. ex Prain and *Acer mono* Maxim var. *mono*. No birch trees established in the oak forest. The oak forest had 20 $m^3$ /plot of stem volume and 876/ha of density. *Q. mongolica* var. *grosserrata* explained 80% of the stem volume in the oak forest, followed by 15% of *Q. mongolica* var. *grosserrata* and 5% of *Kalopanax pictus* (Thunb.) Nakai. Therefore, the canopies were monotonic in both the forests with the equivalent stem density and volume.

The species composition in the herbaceous layers of the two forests was common. The cover of *Asperula odorata* (L.) Scop. was 29% and 45% in oak and birch forest, respectively. The other species, *Hydrangea petiolaris* Siebold & Zucc., *Chloranthus japonicus* Siebold and *Amphicarpaea bracteata* (L.) Fernald ssp. *edgeworthii* (Benth.) H. Ohashi (s.l.), sporadically established with low cover less than 9% in both the forests. The seedlings and saplings (less than 2 m in height) of birch covered 6% and 7% of the ground surface in the birch and oak forests, respectively. No birch seedlings and saplings were observed in the oak forest. Therefore, the forests replaced from birch to oak forests, as an ordinal successional sere in the lowland of Hokkaido.

The snow-free periods were 8-9 months in each year, because snow deposits were observed from December to April for the three years with a slight difference. Soil moisture averaged approximately 30% and was not different between the two forests during snow-free period for the three years (GLMM, $P > 0.05$) (Table 1). The ground-surface temperatures averaged 11.9°C in the birch forest and 12.4°C in the oak forest. The mean temperatures were significantly higher in the oak forest than in birch forest (GLMM, $P < 0.05$), although the canopy openness did not differ between the two forests ($P > 0.05$).

**Table 1** Environmental characteristics in the birch and oak forests during snow-free periods from 2010 to 2012. Different letters show significantly different between the two forests at $p < 0.05$ (GLMM).

| Site | Canopy openness (%) | | PPFD (mmol/($m^2$ sec)) | | Temperature ($^oC$) | | Soil moisture (%) | |
|---|---|---|---|---|---|---|---|---|
| Birch | 23 ± 2 | a | 64.6 ± 99.1 | a | 11.9 ± 9.1 | a | 29 ± 10 | a |
| Oak | 22 ± 3 | a | 84.9 ± 136.9 | b | 12.4 ± 10.6 | b | 28 ± 9 | a |

The litter accumulation averaged 192.4 ± 54.2 g/m$^2$ in the oak forest and 190.4± 46.2 g/m$^2$ in the birch forest, and was not significantly different between the two forests (GLM, $P > 0.05$) (Fig. 1). Although the two dominant species, birch and oak, accounted for more than 50% of litter weight, the species dominance in litter were different between the two forests ($P < 0.05$), i.e., 50% explained by birch litter in the birch forest and 60% by oak litter in the oak forest. The litter contained a little *Kalopanax pictus* and *Cornus controversa* litter. These results indicated that the litter was accumulated more in their own forests and both home range effect and the mixing effect of litter were expected in both the forests.

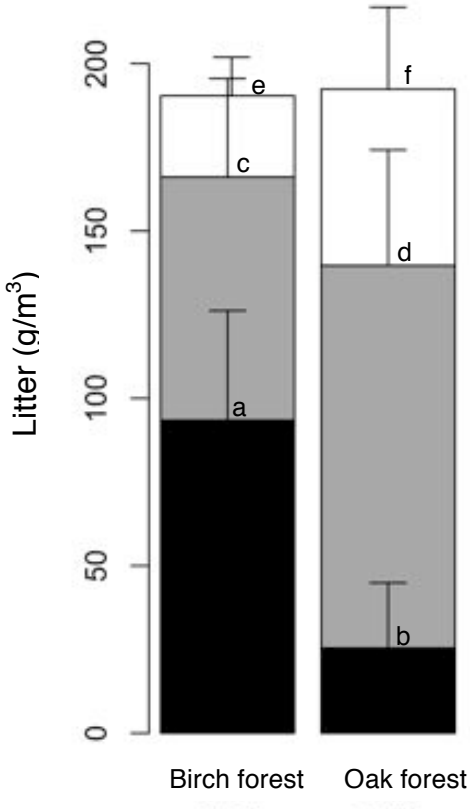



**Fig. 1** Litter amount in the birch and oak forests, measured by nine 25 × 25 cm$^2$ in each forests. The litter samples were collected in November 2013 soon after the defoliation. Litter species: birch (closed columns), oak (shaded) and others (open). Mean (top edges of columns) is shown with standard deviation (bars). Different letters show significantly different at $P < 0.05$ (GLM).

**Litter decomposition**

N in the initial litter prior to the decomposition was 0.81% and 0.68% on birch and oak litter, respectively, and was significantly different between the litter species (GLM, $P < 0.05$). The initial litter showed that C was higher in birch litter (42.7%) than in oak litter (38.7%) (GLM, $P < 0.05$). C/N ratios in the initial litter were 52.8 and 57.3 on birch and oak, respectively, and were not different between the species (GLM, $P > 0.05$). Therefore, the quality of the initial litter between these two species was characterized mostly by N concentration.

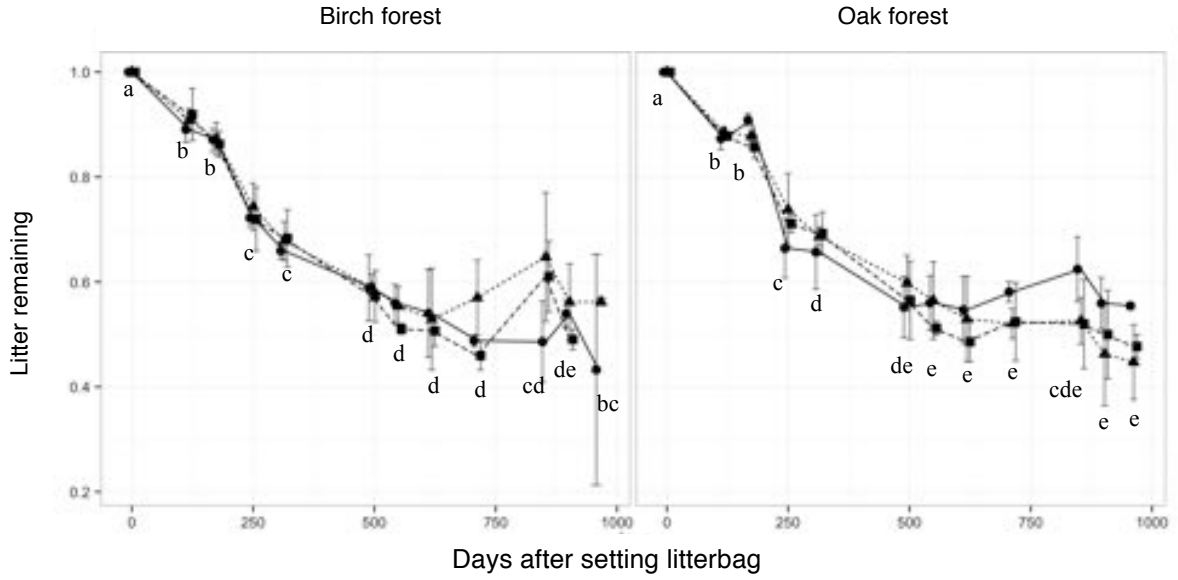



**Fig 2** Temporal changes in the ratios of mass remaining on litter consisting of birch (circles), oak (squares) and mixture (triangles) in the birch and oak forests. Mean (symbols) is shown with standard deviation (error bars). The rates are significantly different between days (GLMM, $P < 0.05$), and between mixture and monoculture litter. The interaction between litter and forest is significant at $P < 0.05$.

The litter decomposition rates ($k$) decreased faster in the early stages than in the later stages on both the forests (Fig. 2). The mixed litter in the birch forest showed slower decomposition than the monoculture litter (GLMM, $P < 0.05$), showing that the mixing effects slowed litter decomposition in the birch forest. However, the mixed litter did not reduce the decomposition in the oak forest after 618 days and therefore the interaction between forests and litter species was significant ($P < 0.05$).

N in litter increased with time and was significantly different between the two forests (GLMM, $P < 0.05$) (Fig. 3a). The litter quality also affected N content, i.e., N in birch litter was higher than N in oak and mixed litter ($P < 0.05$). As well as N, C in litter was different between the forests and among litter quality (Fig. 3b); viz. C was highest for birch litter in the oak forest. C/N ratios decreased with time and were higher in the oak forest than in the birch forest (Fig. 3c). Since C did not change greatly and N increased with time, C/N ratio was mostly reflected by changing N content in the litter. No interactions were detected between the forest and litter quality on C, N and C/N ($P > 0.05$).

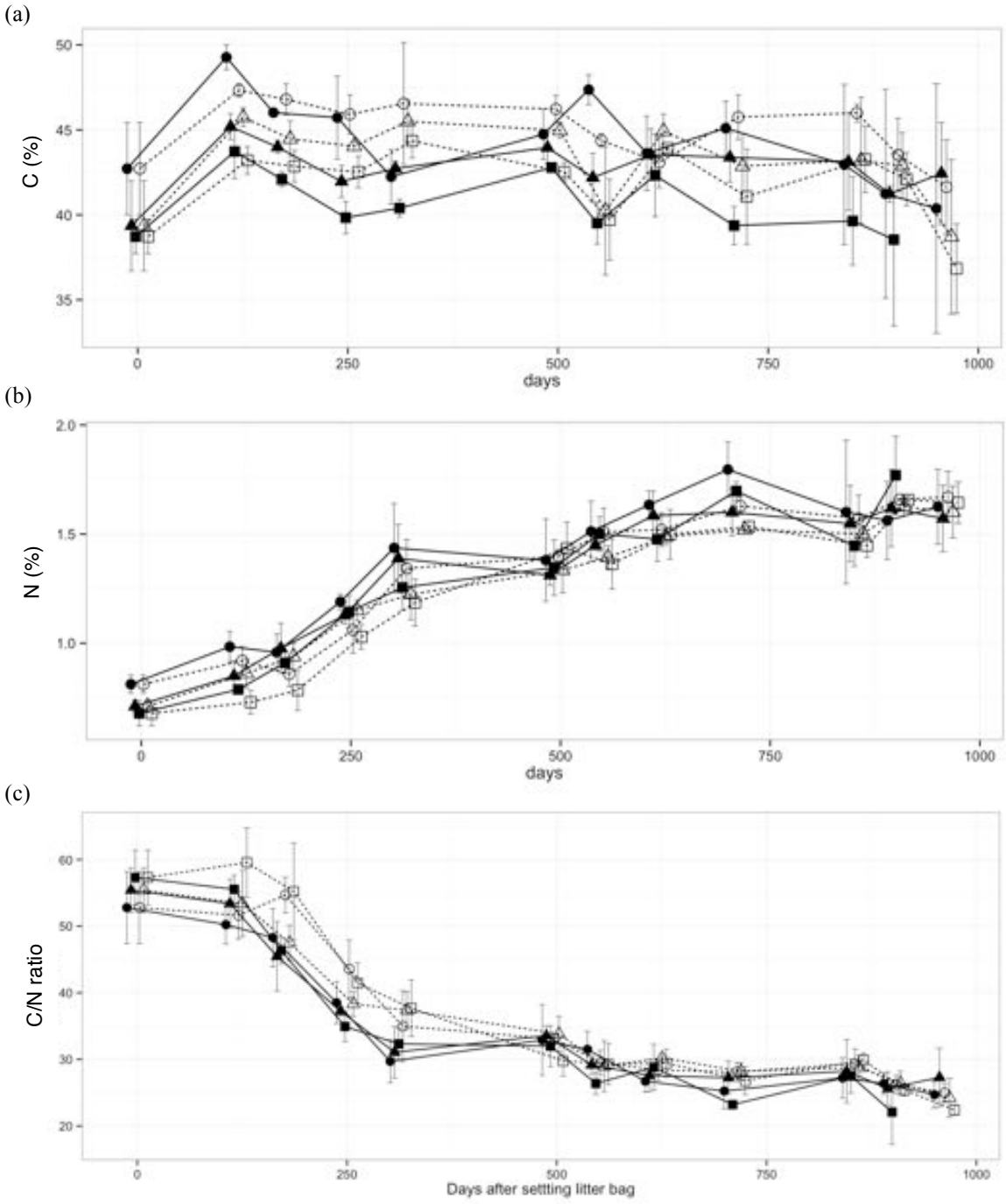



**Fig. 3** Temporal fluctuations of C concentration (a), N concentration (b) and C/N ratio (c) in litter for three years. Litter: birch (circles), oak (squares) and mixture (triangles). Forest: birch (filled) and oak (open). Error bars show standard deviations. The concentrations of C and N and C/N ratio are significantly different between the forests (GLMM, $P < 0.05$). The concentrations of C and N in birch litter are significantly different from oak and mixture litter.

**PLFAs in litter**

Nine bacterial PLFAs, i15:0, a15:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, 10Me-18:0 cy19:0, were detected from the recovered litter samples. A fungal PLFA, 18:2, was also detected. The fungal PLFA showed higher concentration than bacterial PLFAs, in particular, in the early stages of litter decomposition for all the three litter compositions (GLMM, $P < 0.05$) (Fig. 4). The B/F ratios sometimes became zero in the early stages, because of no bacterial PLFAs (Fig. 5). These indicated that the fungi were more dominant than the bacteria for the first three years.

The peaks of fungal PLFA concentrations were observed during 250 and 320 days after setting the litterbags. The days corresponded to summer and autumn in the first year. Therefore, the litter decomposition by fungi should be undertaken mostly in the first year before snow. The concentration of fungal PLFA was lower in the oak forest than in the birch forest (GLMM $P < 0.05$). The interaction was detected between forest and mixed litter (GLMM, $P < 0.05$), implying that the fungal biomass was increased more in mixed litter in oak forest.

In contrast to the fungal PLFA, the bacterial PLFAs gradually and slowly increased with time. The concentrations of bacterial PLFAs were not different between the forests and among the litter (GLMM, $P > 0.05$). Therefore, B/F ratios gradually increased with time (GLM, $P < 0.05$) and were different between the two forests (GLMM, $P < 0.05$) (Fig. 5). Increase in B/F ratio on the oak forest reflected the reduction of fungi biomass without the significant interactions between the forest and litter species ($P > 0.05$).

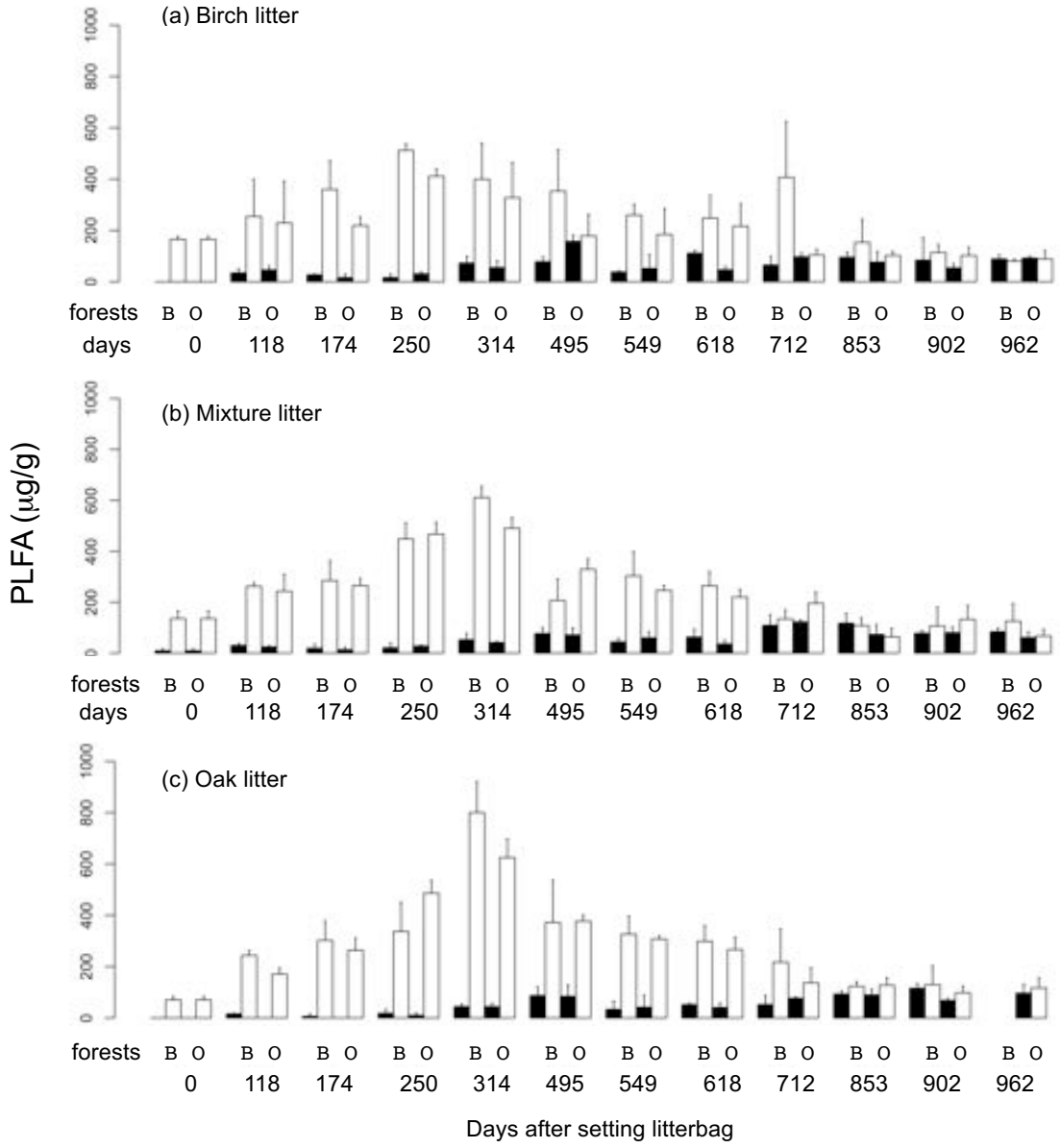



**Fig. 4** Temporal fluctuations of PLFAs produced by bacteria (closed columns) and fungi (open columns). Mean concentration is shown with standard deviation (error bars).

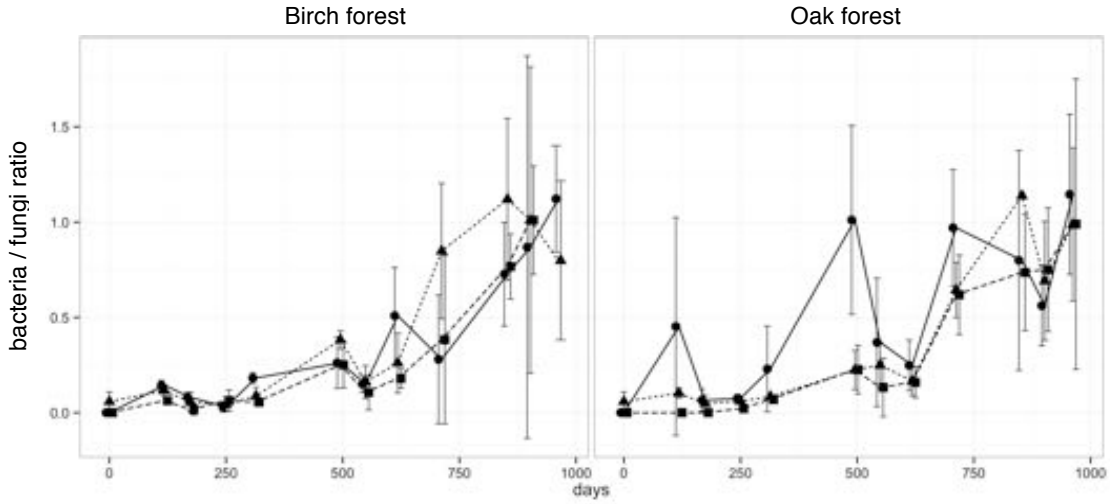



**Fig. 5** Fluctuations of bacterial to fungal ratios (B/F ratios) of phosphorus in the PLFAs of litter recovered from litterbags in the birch and oak forests. The PLFAs were extracted from the litter of birch (circles), oak (squares) and mixture (triangles). Mean B/F ratios are shown with standard deviation (error bars). The ratios are significantly higher in oak forest than in birch forest (GLMM, $p < 0.05$).

**Distribution patterns of PLFAs**

The CCA performed by the composition of PLFAs showed that 72% and 17% of variations were explained by the first and second axes, respectively. The days after the litterbag establishment were negatively related to litter mass remaining and were related more to the first axis (Fig. 6). The C/N ratio and N content in litter were related to the litter mass remaining rates. Therefore, the compositions of PLFAs changed with C/N ratio, N content and litter decomposition. Moreover, C/N ratio and litter mass

remaining showed the comparable impacts on the composition of PLFAs, shown by their scores close to each other (Fig . 6).

The score of fungal PLFA was located to the opposite side of the scores of bacterial PLFAs along the first axis of CCA, indicating that fungal PLFA was not distributed with bacterial PLFAs well (Fig. 6). The fungal PLFA showed the peak in one year after the litterbag establishment (Fig. 4), while the bacterial PLFAs increased slowly during the three years. These suggested that the major litter decomposers were changed from fungi to bacteria with proceeding in the litter decomposition. The scores of PLFAs produced by bacterial decomposers were broadly distributed along the second axes, showing that the bacterial compositions were determined by litter quality more than by litter decomposition ages.

Litter species were correlated to the second axis ($P < 0.05$), showing that litter species influenced little on the litter decomposition. The forest types were not correlated to the first and second axes ($P > 0.05$). Therefore, the habitat differences were not the prime importance on litter decomposition.

The scores of mixed litter on the second axis were located intermediately between birch and oak litter. The litter quality was not related to the first axis determined by the periods of litter decomposition. Therefore, the mixing effects did not enhance and reduce the litter decomposition rates.

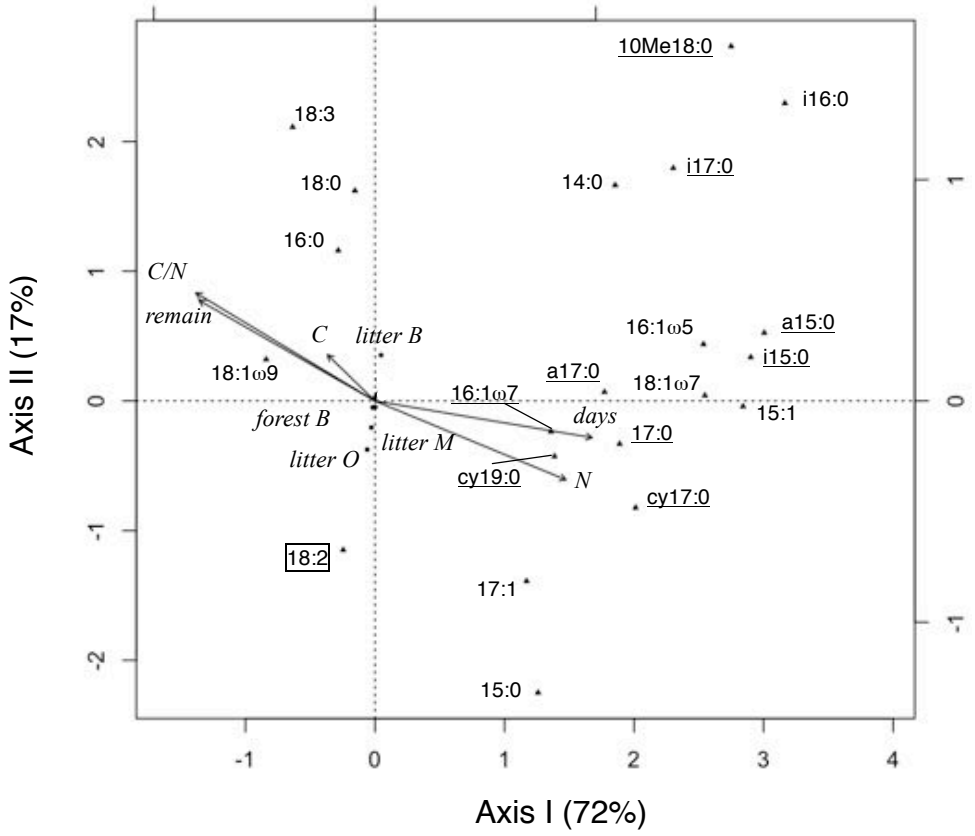



**Fig. 6** CCA scores of PLFAs produced by microorganisms in litter. PLFAs of i15:0, a15:0, i16:0, 16:1 ω 7, i17:0, a17:0, 17:0, cy17:0 10Me-18:0 and cy19:0 (underlined) are produced by bacteria, while PLFA of 18:2 (enclosed) is produced by fungi. Environmental factors: Birch litter = *litter B*, Oak litter = *litter O*, Mixed litter = *litter M*, the amount of remained litter = *remain*, days after incubation = *days*, the forest dominated by birch = *forest B* and the forest dominated by oak = *forest O*.

## 1.4 Discussion

### Forest structures and litter decomposition patterns

The forest succession proceeded from the birch to the oak. The oak forest showed higher PPFD and temperature and showed no differences in canopy openness and soil moisture from the birch forest. Therefore, the environmental differences between the two forests for litter decomposition were derived basically by light and temperature. The quantity of litter supply did not differ between the birch and oak forests, while the quality differed between the two forests, depending on the dominant tree species. These results indicated that the litter-mixed effects occurred, *in situ*, with home range effects.

The decomposition rates were not different between the birch and oak litter. On a forest mixed with birch (*B. platyphylla*) and oak (*Q. mongolica*) in central Hokkaido, litter decomposition rates were not different between the litter of birch and oak (Ono et al. 2013). Therefore, litter-mixing effect is likely to be innately weak when these two litter species are mixed.

However, the mixed litter decomposed somewhat slowly in the birch forest. Litter-mixing effect is positive in N-poor habitats because N is transferred from N-rich to N-poor litter (Setiawan et al. 2016). Litter mixed with *Tilia americana* and *Acer saccharum* slows the decomposition (Madritch and Cardinale 2007). The temporal decrease in C/N ratio was derived mostly by increase in N, suggesting that C release from litter changed the C/N ratio. N accumulation was synchronized with the amount of fungal PLFAs because the N was accumulated in fungal hyphae (Hobara et al. 2014).

Therefore, changes in C/N ratios in the early stages of litter decomposition were tightly related to the fungal activities.

**Temporal changes in microbial composition**

The fluctuations of microbial biomass, estimated by PLFAs, were synchronized with the paces of litter decomposition, in particular, for fungal biomass. Both the birch and oak litter contained more fungal PLFAs than bacterial PLFAs, suggesting that fungal activities had major role on the litter decomposition. The decrease in C/N ratios and increase in fungi biomass were clear in the early stages of decomposition. Fungi have higher role on litter decomposition than bacteria in temperate broad-leaved forests of Czech Republic, because N in litter increases the fungal biomass (Šnajdr et al. 2013). The litter of *Fagus crenata* Blume (Fagaceae), one of the common deciduous trees in Japan, is dominated by fungi for the first one year (Osono and Takeda 2001). Fungi should be major litter decomposers in the early stages of litter decomposition (Schneider et al. 2012). The differences in litter decomposers are likely to be derived from the litter qualities.

C/N ratios in litter decomposed for nine weeks in Argentina and 15 weeks in England are negatively correlated to leaf quality evaluated by the leaf tensile strength (Pérez-Harguindeguy et al. 2000). Microbial succession in litter is promoted by changes in litter nutrients, because the nutrient demands of bacteria are different from those of fungi (Schneider et al. 2012). Therefore, the litter decomposed by fungi in the early stages should facilitate the litter decomposed by bacteria in the late stages.

# Chapter 2

## An estimation technique on litter decomposition rate without litterbag

### 2.1 Introduction

Determining litter decomposition rate is prerequisite for understanding the dynamics of ecosystem development through the belowground dynamics (Hättenschwiler et al. 2005). To detect litter decomposition, litterbag method has been widely applied in various ecosystems. This method is a quantitative study that litter packed into nylon-meshed bags is placed in the field, is regularly recovered and is measured on mass loss and its related factors in the litter (Bonan et al. 2013). However, the litterbag method has a few disadvantages for the measurements: difficulties in persistent monitoring owing to the loss, breach and contamination in litterbags, in particular, on disturbed habitats such as wind-brown and flooded areas. When possible, therefore, the intact estimation of litter decomposition is desirable.

Chemical elements in litter, represented by carbon to nitrogen ratio (C/N ratio) are known as predictors of litter mass remaining (Ågren et al. 2013). C/N ratio is decreased with decrease in litter mass remaining, although the relationship between C/N ratio and litter mass remaining is erratic between habitats (Moor et al. 2006; Parton et al. 2007). In a laboratory experiment, the C/N ratios of decomposers increase with C/N ratio in the litter residue, because of N immobilizations (Nicolardot et al. 2001). In a wetland in Florida, N enrichment in decomposed litter is induced by microbial activities

(Davis et al. 2003). Therefore, litter decomposition should be predicted by C, N and litter species in each habitat.

On C, N and their stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), a large number of samples can be measured by an isotope analyzer for short time without any complex procedures. $\delta^{13}C$ and $\delta^{15}N$ in soil organic matter often increase with increasing soil depth, and therefore, are considered to indicate soil humification (Layese et al. 2002). $\delta^{13}C$ in litter is increased by the metabolism of microbial activities (Adams and Grierson 2001) and $\delta^{15}N$ in litter increases as decay proceeds (Craine et al. 2015). Therefore, these chemical elements may provide new insights on the measurement of litter decomposition. I developed a method to predict litter decomposition by these chemical elements. Multiple regression analysis was applied to investigate what parameters were suitable for predicting litter decomposition. To investigate the effectiveness of method proposed in this paper, the model was compared in two distinctive ecosystems, wetland and forest.

## 2.2 Materials and methods

### Litter mass remaining

Two ecosystems, wetland and forest, were used for exploring non-litterbag method. The wetland is a post-mined peatland located in the Sarobetsu Mire, northern Hokkaido (45°06' N, 141°42' E, 8 m a.s.l.), and the forest is on the foot of Mount Toishi (42°59'N and 141°19'E 135 m a.s.l.) in the city of Sapporo, Hokkaido, northern Japan.

On the wetland, the litter of *R. alba* and *M. japonica* was harvested on 17 April 2008 and on 22 October 2008. The litter samples were dried at 45°C for 4-6 days soon after returning lab. The litterbags were 15 cm ×20 cm with 1 mm mesh. The bags were filled with 5 g of either *R. alba* or *M. japonica* litter. The litterbags were established on bare ground (hereafter, i.e., BG), *Rhynchospora alba* (RA) sedgeland and *Moliniopsis japonica* (MJ) grassland in the post-mined peatland soon after snow-melt in early May 2008 and middle November when just before snow accumulation in 2008. The litterbags were recovered every month during snow-free period for 821 days, including three summer seasons. At each sampling, three litterbags were recovered from each habitat on each species. The bags were kept in a cooling box for transportation from Sarobetsu to Sapporo. The recovered litter samples were freeze-dried immediately after returning, and were weighed.

In the forest, the litter of *Betula platyphylla* and *Quercus mongolica* was corrected from the foot of Mount Toishi (42°59'N and 141°19'E 135 m a.s.l.) in the city of Sapporo, Hokkaido, in late November 2009. The litter samples were air-dried over two weeks. The litterbags were 15 cm × 20 cm made of a polyethylene net with 2-mm mesh and were filled with 6 g of *B. platyphylla*, 5 g of *Q. mongolica*, or mixture (3 g of each of the two species). The litterbags were established in the *B. platyphylla* or *Q. mongolica* forest in late December 2009. Litterbags were recovered every two to four month in April to December until early August 2012. Each sample was freeze-dried over seven days and weighed.

**Chemical measurements**

The litter mass remaining was calculated as: (weight of sampled litter)/(weight of initial litter).

Thereafter, the litter samples were homogenized by an electric grinder for chemical measurements. The contents of nitrogen (N) and carbon (C) were measured by an isotope mass spectrometer (MAT252, Finningan Mat Ltd., Bremen). $^{13}C$ and $^{15}N$ were measured concurrently with C and N. Tyrosine and atmospheric N were used for the external standard of C and N, respectively. $\delta^{13}C$ or $^{15}N$ was calculated as: $\delta^{13}C$ or $^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where $R_{sample}$ was $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and $R_{standard}$ the reference (PeeDee belemnite).

**Prediction of litter decomposition**

To estimate the litter decomposition rate, a generalized linear model (GLM) was used with the assumption of Gasussian distribution. . The response variable was litter mass remaining evaluated by the litterbag method in each ecosystem. The explanatory variables were habitat, litter species, C, N, $\delta^{13}C$, $\delta^{15}N$ and C/N ratio. The analyses were conducted separately on the wetland and forest. The explanatory variables in the best model were selected by Akaike's information criteria (AIC). Each mass remaining was estimated by the predictive model. All of the statistical analyses were performed with the software package R (ver. 3.1.3) (R Core Team 2015).

## 2.3 Results

The litter mass remaining gradually decreased from 100% to 50% for the three years in the forest and to 35% for the two years in the wetland. Therefore, habitat differences should be considered for the estimation of litter decomposition without litterbags. The best GLMs showed significant intercepts and high adjusted $r^2$, 0.689 on the wetland and 0.826 on the forest (Table 1), showing the high predictabilities (Fig. 1). In addition, the $r^2$ was not different between the full and best models. The two models adopted two parameters, habitat and nitrogen. Litter species and carbon were also adopted in the model of wetland, while C/N ratio and $\delta^{15}$N were in the model of forest. $\delta^{13}$C was not selected in both the models.

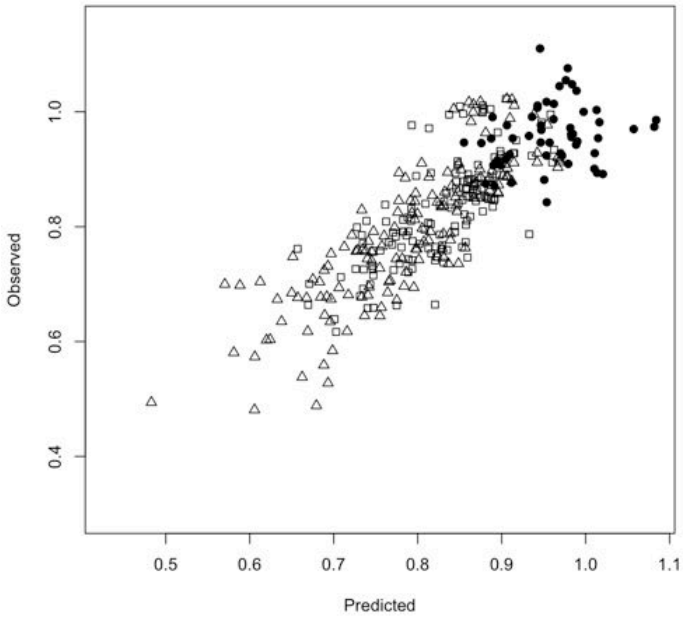
The habitat differences indicated that the litter decomposition was slower in the bare ground of wetland, and was slower in the oak forest (Table 1). In addition, litter species affected the litter decomposition, i.e., *M. japonica* litter showed slower decomposition. These indicated that we should mention that habitats affected litter decomposition patterns.

Because N content remained in both the models, the relationships between litter mass remaining and N content were plotted on the graph (Fig. 2). The $r^2$ of N content was 0.442 in the wetland and was 0.759 in the forest. Therefore, N content and its related parameters were likely to be used for estimating litter decomposition. Of them, $F$ values on C/N ratio were the highest in the wetland and forest (431.3 and 887.3, respectively) and showed 0.421 and 0.781 of adjusted $r^2$ in the wetland and forest, respectively.

**Table 1** Parameters selected by GLM with AIC for predicting litter mass remaining. The estimates and probabilities are shown. n.s.: not selected in the best model.

| Wetland | | | Forest | | |
|---|---|---|---|---|---|
| Coefficients | Estimate | Probability | Coefficients | Estimate | Probability |
| Intercept | 0.643502 | < 0.001 | Intercept | 0.807502 | < 0.001 |
| Habitat | | | Habitat | | |
| *Moliniopsis japonica* grassland | -0.11491 | < 0.001 | *Quercus mongolica* forest | -0.033599 | < 0.001 |
| *Rhynchospora alba* sedgeland | -0.121543 | < 0.001 | | | |
| Litter species | | | Litter species | | |
| *Moliniopsis japonica* | 0.057022 | < 0.001 | mixture | n.s. | n.s. |
| | | | *Quercus mongolica* | n.s. | n.s. |
| Carbon (%) | 0.012543 | < 0.001 | Carbon (%) | n.s. | n.s. |
| Nitrogen (%) | -0.299591 | < 0.001 | Nitrogen (%) | -0.231326 | < 0.001 |
| C/N ratio | n.s. | n.s. | C/N ratio | 0.006404 | < 0.001 |
| $\delta^{13}C$ (‰) | n.s. | n.s. | $\delta^{13}C$ (‰) | n.s. | n.s. |
| $\delta^{15}N$ (‰) | n.s. | n.s. | $\delta^{15}N$ (‰) | 0.044059 | < 0.001 |
| Adjusted $r^2$ = 0.6887 | | | Adjusted $r^2$ = 0.8258 | | |
| AIC = -1850.62 | | | AIC = -1081.45 | | |

(a) Wetland




(b) Forest

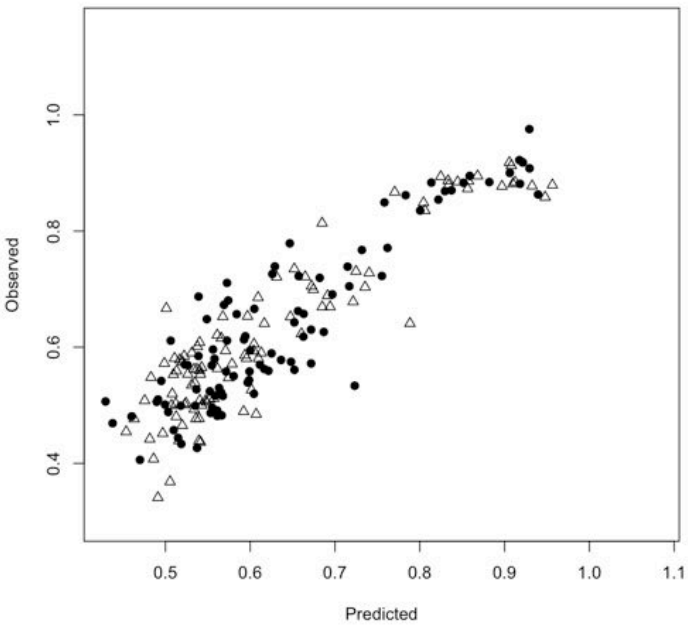
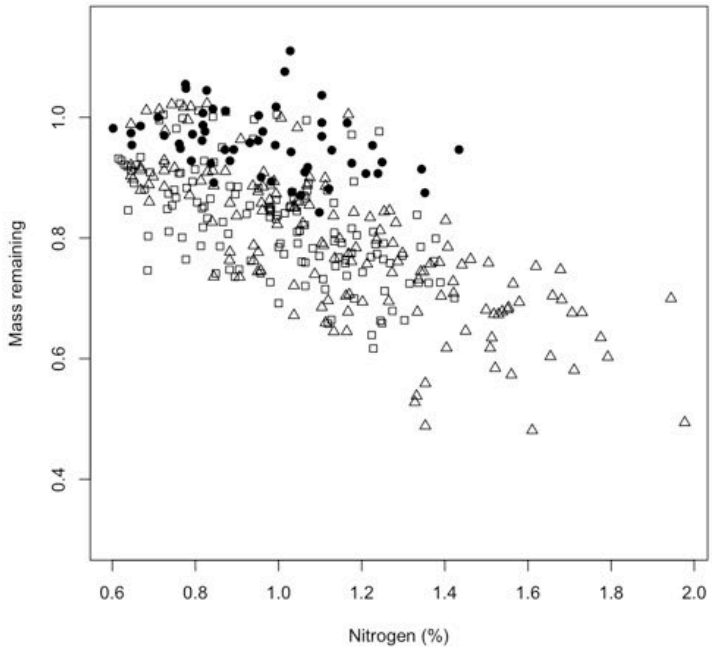



**Fig. 1** Relationships between observed and predicted litter mass remaining in wetland (a) and forest (b). The predicted values are obtained by the best GLM models selected by AIC: (a) Litter mass remaining = +0.0.644 -0.115×grassland -0.122×sedgeland +0.057 ×LitterMJ +0.013 × C -0.300 × N (adjusted $r^2$ = 0.689). The closed circles, open triangles and open squares indicate the habitats of bare ground, grassland and sedge land, respectively. (b) Mass remaining = +0.808 -0.034 ×forestQ +0.006×C/N ratio -0.231×N +0.044×$\delta^{15}$N (selected model). Adjusted $r^2$: 0.826. The closed circles and open triangles indicate the habitats of *B. platyphilla* forest and *Q. mongolica* forest, respectively.

(a) Wetland




(b) Forest

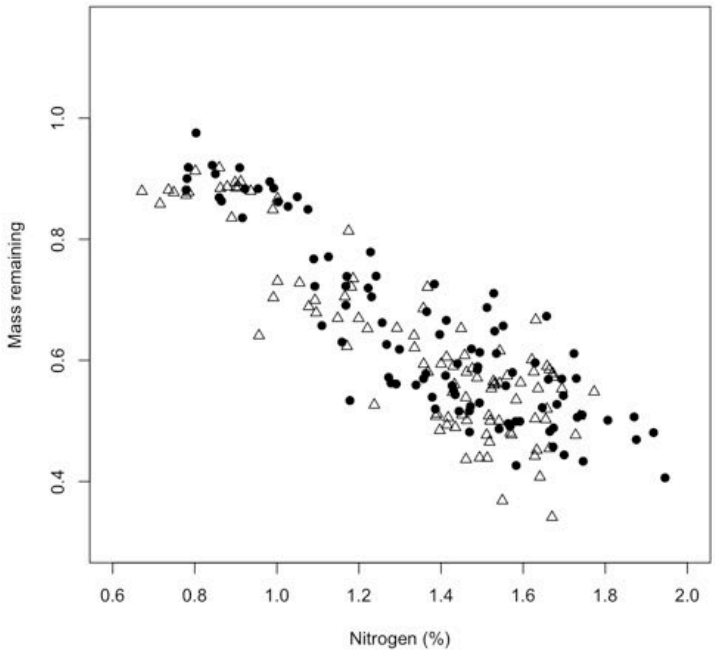



**Fig. 2** Relationships between N content and litter mass remaining in wetland (a) and forest (b). The predicted value calculated with the following regression formula; (a) Mass remaining = +1.136 -0.288×N content; Adjusted $r^2$ is 0.442. The closed circles, open triangles and open squares indicate the habitats of bare ground, grassland and sedge land, respectively. (b) Mass remaining = +1.220 + -0.434×N content; Adjusted $r^2$ is 0.759. The closed circles and open triangles indicate the habitats of *B. platyphilla* forest and *Q. mongolica* forest, respectively.

## 2.4 Discussion

The correlations in GLMs were different between the wetland and forest. The litter decomposition rates were slower on bare ground than on the grassland and sedgeland in the wetland. These differences were caused by the differences of plant species richness, plant cover and/or dominant species (Egawa et al. 2009). The bare ground was characterized by no vegetation and was more harsh (Koyama and Tsuyuzaki 2010), while the environments in the two vegetation types, grassland and sedgeland, were probably more identical. Therefore, the estimates of coefficients were equivalent between the grassland and sedgeland in the wetland. As well as in the wetland, the habitat (forest type) was related to the litter decomposition. The temperature and light intensity on the ground surface were higher in the oak forest (Chapter 1). These indicated that the habitat characteristics should be mentioned when the litter decomposition was estimated by chemical properties of litter.

The wetland showed that the litter decomposition was different between litter species, while the forest did not show it. The decomposition rates are lower for aspen litter than mixed-grass litter, because of differences in N content (Köchy and Wilson 1997). The initial litter prior to starting the decomposition showed higher N content in litter MJ than RA on the wetland, while it showed no differences between birch and oak litter on the forest. These also suggested that N in litter was surrogate for estimating litter decomposition.

The $\delta^{13}C$ was not adopted in any predictive modelswere no significance on the prediction. The litter residues incubated for after three months 119 days of incubation showed few little changes in the $\delta^{13}C$ because the decomposing plant materials were was not related affected by to the isotopic composition in of the easily decomposable

plant litter fractions (Schweizer et al.1999). Moreover, natural $\delta^{13}C$ abundance in plants species depends largely on the interplay among all aspects of plant carbon and water relations (Dawson et al. 2002). Because $\delta^{13}C$ is not changed by decomposition and is largely varied, Therefore, $\delta^{13}C$ was discarded from is not adequate for the parameter to predict the best model on estimating litter decomposition in both the ecosystems.

The humified litter increases in $\delta^{15}N$ but shows no clear trend in $\delta^{13}C$ (Kramer et al.2003). The concentration of $\delta^{15}N$ reflects relationships underlying in N availability by a large isotopic fractionation during a transfer of nitrogen (Hobbie et al. 2000). In a habitat that N metabolism is high, $\delta^{15}N$ shows a high positive correlation to the litter decomposition. Because N retains more in a peat-bottomland than the upland (Moore et al. 2005), $\delta^{15}N$ was not significant in the wetland.

The difference of $r^2$ between of the full model and the selected model was small. The litter decomposition in the wetland was able to predict without isotopic analysis . In the forest, litter mass remaining was predicted without considering difference in the habitats. Furthermore, forest decomposition is predicted well by C/N ratio only. In conclusion, N and its related parameters can be used for the index to predict the litter decomposition rate without the litterbag.

# Chapter 3

# Changes in microbial community composition in the leaf litter of successional communities after volcanic eruptions of Mount Usu, northern Japan

## 3.1 Introduction

Volcanic eruption is one of the triggers of primary succession, which begins with no plants or soils. Two major taxa, fungi and bacteria, contribute to litter decomposition, which promotes soil development. Bacterial composition is determined by vegetation development patterns during early primary succession in glacier forefields (Knelman et al. 2012). Mycorrhizal communities develop on old lava, whereas bacterial communities are stable independently of the lava age (Cutler et al. 2014). The contribution of bacteria to litter decomposition gradually increases with increasing time after the retreat of ice sheets (Pennanen et al. 2001). These previous findings have suggested that in addition to plant communities, the microbial communities of bacteria and fungi change with succession. Subsequently, the formation of soil organic matter is characterized by the patterns and processes of litter decomposition. On volcanoes after eruptions, ecological succession proceeds with soil development via litter decomposition. Therefore, investigating litter decomposition by bacteria and fungi along successional seres is helpful for understanding the mechanisms of ecological succession.

To investigate the contributions of bacteria and fungi to changes in litter decomposition along successional seres, the fungi-to-bacteria ratio (F/B ratio) was used.

This ratio is affected by the chemical and physical properties of litter, such as the pH, moisture, and the C and N contents (Rousk et al. 2010; Brockett et al. 2012). The compositions of fungi and bacteria in litter change with changes in the dominant plant species (Urbanová et al. 2015). Therefore, information regarding the temporal changes in microbial communities is required to understand the mechanisms of succession.

The analysis of stable isotopes is a useful tool for assessing the changes and translocations of chemical components in litter. Light isotopes tend to move faster than heavy isotopes (Glaser 2005; Ehleringer et al. 2000); therefore, the isotope ratio changes when chemical transportation occurs. Because litter decomposition by microbial activity is a chemical process, the ratios of stable C and N isotopes in litter can be used to detect the degrees of organic matter decomposition (Connin et al. 2001). Thus, I investigated the C and N isotopes in the litter.

Because various types of phospholipid fatty acids (PLFAs) are produced by bacteria and fungi, the PLFA compositions are used to estimate the biomass and composition of bacteria and fungi in litter (Helfrich et al. 2015). Chronosequencing is an advantageous method in which a short-term survey is used to characterize the pattern of long-term ecological changes, including plant succession. Therefore, I measured the chemical components related to the microbial biomass described above in three chronosequential vegetation stages (bare ground, grassland and forest) that developed during three different time periods after three different eruptions of a volcano. I addressed two objectives: (1) Could changes in microbial biomass be detected with the chronosequential approach? (2) Does the microbial decomposer community composition change during ecological succession?

## 3.2 Materials and methods

### Study sites

The Mount Usu volcano is located in the southern part of Hokkaido Island, northern Japan (42°32'N-33'N, 140°48'E-50'E, 150-727 m elevation). In 2010, the mean annual temperature was 9°C and the annual precipitation was 900 mm (Japan Meteorological Agency 2012). When litter sampling was conducted in 2010, the maximum monthly temperature was 24°C in August and the minimum was -3.7°C in February. The peak of precipitation occurs in the typhoon season in autumn, and snow between November and April provides 18% of the annual precipitation. Volcanic ash and pumice are the major soil particles in this region. In the early stages of succession (from bare ground to grassland), the pH in the tephra and the soil on Mount Usu is acidic, at approximately 5-6 (Haruki and Tsuyuzaki 2001). The forest soil in the surveyed area is classified as acidic brown forest soil below pH 7.

Three sites damaged by the 1910, 1977-1978 and 2000 eruptions were selected. These three eruptions occurred on the northern slope in 1910 (42°34'N, 140°50'E, 160 m elevation), on the northeastern slope in 1977 (42°33'N, 140°50'E, 470 m elevation), and on the northwestern slope in 2000 (42°33'N, 140°49'E, 150 m elevation). These three sites were surveyed in 2010, at 10, 33, and 100 years after the eruptions, respectively. The distances among the three sites were less than 2.5 km. Because these three sites were closely established and received comparable damage from the respective eruptions, they were suitable for conducting a chronosequential analysis (Garcia-Romero et al. 2015).

**Chronosequence in vegetation and the environment**

Vegetation, soil and overstory openness were measured in each of the three sites. Plant cover for each species was assessed in three randomly established 10 m × 10 m plots at each site in the summer of 2010. Fisheye photos were taken facing straight upward at 1.2 m and 0.5 m above the ground surface at 4-6 different locations in each plot. Overstory openness (%) was measured in each photo with a Gap Light Analyzer (GLA) (Frazer et al. 1999) and was averaged in each plot. The soil profiles were observed in each site by excavation. Three soil layers, i.e., litter, humus and organic layers, were classified on the basis of their colors and textures, and the thickness of each layer was measured at three or six points with a ruler.

In total, the chronosequence showed that broad-leaved forest developed in the grasslands within 100 years. Two species were common throughout the succession: *Polygonum sachalinense* Fr. Schm. (Polygonaceae), which is a deciduous forb that develops in mono-specific grasslands and reaches a height of greater than 2 m, and *Populus maximowiczii* A. Henry (Salicaceae), which is one of the pioneer trees after volcanic disturbances.

**Litter sampling**

The leaf litter of two dominant species, *Polygonum sachalinense* and *Populus maximowiczii*, produced in autumn 2009 was collected from the ground surface in late July 2010. Therefore, the collected litter had remained on the ground for ten months after the leaves had defoliated. When the sampling was conducted, minimal litter had been produced in the current year and could be visually excluded. Additionally, litter

that was collected by shaking the plants was used as initial litter to evaluate the initial properties. In the surveyed period, snow coverage occurred from mid-December 2009 to early April 2010. Each litter sample was randomly collected from each 10 m × 10 m plot. The amount of litter consisted of more than 30 leaves from three locations at each site. The samples were separately packed into paper bags and kept in a cooler box. Soon after the samples were brought to our laboratory, they were kept in a freezer at -70°C in the dark until use. Each sample was separately ground for PLFA analysis.

**Chemical analysis**

Carbon (C), nitrogen (N), and their stable isotopes ($^{13}C$ and $^{15}N$) were measured to investigate the microbial biomass and litter decomposition (Boström et al. 2007; Osono et al. 2008), although the evaluations should be interpreted with caution in chronosequential studies. These four chemical elements were measured with a stable isotope ratio mass spectrometer (Finnigan MAT252, Thermo Fisher Scientific, Yokohama). The standards used were the Vienna Pee Dee Belemnite for carbon and atmospheric N for nitrogen. Before the measurements, the samples were freeze dried at -50°C for 7-14 days.

The ratios of C and N isotopes relative to the standard ($\delta^{15}N$ and $\delta^{13}C$, ‰) in in each sample were expressed by

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3,$$

where $R_{sample}$ and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios in the sample and standard, respectively. $\delta^{13}C$ is correlated with the lignin content in litter (DeBond et al. 2013). $\delta^{15}N$ indicates the nitrogen transfer in litter because microbial immobilization

discriminates against $^{15}N$ in favor of $^{14}N$ (Michener and Lajtha 2007).

Total lipids were extracted from 1.0 g freeze-dried litter soaked in a mixture of chloroform: methanol: deionized water (1:2:0.8, v/v/v) (White et al. 1979). After the two-phase partition, the lower layer containing lipids was extracted. The lipid extracts were digested with 500 ml of 60% perchloric acid, 2300 $\mu$l of 1.78 mM ammonium molybdate solution and 100 $\mu$l of Fiske-Subbarow reagent (Fiske and Subbarow 1925). After the extracts were heated to 90°C, the total lipids were quantified on the basis of the absorbance of $PMo_{12}O_{40}^{7-}$ reacted with phosphate at 815 nm with a spectrophotometer (U-1800, Hitachi High-Tech, Tokyo).

The phospholipids were separated from the total lipids by using thin-layer chromatography with a silica gel under a developer (91:30:8 = acetone: benzene: water). The phospholipids were subjected to mild alkaline methanolysis, and the fatty acid methyl esters were detected with gas chromatography (G-3000 Gas Chromatograph, Hitachi, Tokyo) with a frame ionization detector using a 30-m 5% phenyl silicone capillary column (HP-5) exposed to helium as a carrier gas. The temperatures of the injector and detector were adjusted to 270°C. The temperature in the oven was kept at 160°C for 5 min and then rose at 1°C/min up to 180°C and 10°C/min up to 240°C. PLFAs were identified and quantified in each sample by comparison with the internal standard, nonadecanoate fatty acid (19:0). Fatty acid methyl esters were identified using the standards and/or previous literature with a gas chromatograph-mass spectrometer (JMS-DX303HF, JEOL, Tokyo). The taxon-specific PLFAs were i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0 and cy19:0 produced by bacteria and 18:2 produced by fungi (Šnajdr et al. 2011). Of these, 10Me-16:0, 10Me-17:0 and 10Me-18:0 are produced only by actinomycete bacteria and 16:1ω7 is produced primarily by bacteria and to a

lesser extent by arbuscular mycorrhizal fungi (Graham et. al 1995). Therefore, 16:1ω7 was treated as the production of bacteria. Most of the 18:2 in litter was derived from fungi because plant-derived 18:2 vanishes soon after defoliation (Laczko et al. 2003). The other PLFAs were produced by plants, bacteria and/or fungi and were treated as PLFAs produced by miscellaneous organisms.

**Statistical analysis**

The ratio of carbon to nitrogen (C/N ratio) in the litter was calculated to estimate the litter decomposition. The C/N ratio decreases with increasing litter decomposition because microbial activities promote carbon mineralization and nitrogen accumulation (Šnajdr et al. 2011). The ratio of fungi to bacteria (F/B ratio) was calculated based on the PLFAs in each litter to estimate the dependence of litter decomposition on fungi and bacteria (Schneider et al. 2012). The C/N ratio, F/B ratio, phosphorus content in the lipids, and stable isotopes in the litter were compared between years after the eruptions and between litter species by using a generalized linear model (GLM) with an assumed Gaussian distribution. The interactions between litter species and years after eruptions were also examined. All significance levels were set at $P < 0.05$.

A canonical component analysis (CCA) was used to investigate the characteristics of PLFAs in the litter. The species matrix consisted of PLFAs produced specifically by fungi and bacteria in each litter. The environmental matrix consisted of eleven factors: litter species, overstory openness, the thickness of each of the three soil layers (litter, humus and organic), C and N content, C/N ratio, $\delta^{13}C$, $\delta^{15}N$ content, and the years after the eruptions. The litter species were treated as categorical variables, and the others were treated as numerical variables. A permutation test for CCA was

conducted by using the Bray-Curtis distance matrix to extract the significant axes. An analysis of similarities (ANOSIM) was performed on the years after the eruptions to examine the significant differences between the groups of sampling units (Anderson and Walsh 2013). All of the statistical analyses were performed with the software package R (ver. 3.1.3) (R Core Team 2015). CCA was conducted with the R library vegan (version 2.2.1) (Okasanen et al. 2015).

## 3.3 Results

### Chronosequential changes in vegetation

The chronosequential vegetation changes, based on these surveys, were confirmed as follows.

The youngest site, in which 10 years had passed since the eruptions, was bare ground, had less than 10% plant cover, and had no litter accumulation except in a few concave locations (Fig. 1A). The overstory openness averaged 74 ± 6% (Table 1), although the openness was underestimated because the slope of the crater rim was included in the estimation. The common species consisted of a forb, *Polygonum sachalinense*, and a tree, *Populus maximowiczii*. The tephra lacked humus and organic layers, i.e., the tephra was 0 cm deep.

The grasslands 33 years after the eruptions were dominated by *Polygonum sachalinense* with heights exceeding 2 m (Fig. 1B). The overstory openness was 19 ± 4%, showing that the dense foliage of *Polygonum sachalinense* intercepted the solar radiation in summer. Therefore, the litter consisted primarily of *Polygonum*

*sachalinense*. *Populus maximowiczii* was established sporadically and was less than 2 m in height. The soil showed an organic layer averaging 2 ± 1 cm deep. The litter and humus layers were 3 ± 1 cm and 2 ± 2 cm, respectively. An aggregated soil structure was not observed.

A forest dominated by *Populus maximowiczii* was established in the area damaged by the 1910 eruption (Fig. 1C). The tree height was ca. 20 m with a closed overstory, and the openness averaged 9 ± 1%. The understory of less than 2 m in height was occupied by various herbs, with 70% total plant cover. The herb *Polygonum sachalinense* consisted of 10% of the cover, and the next most abundant cover type was forbs, such as *Asperula odorata* and *Petasites japonicus* in the understory. The litter species was diverse in the forest. The organic layer was less than 15 cm in depth and averaged 12 ± 4 cm. The litter layer was 2 ± 1 cm, and the humus layer was 0.6 ± 0.3 cm. An aggregated soil structure was detected in the organic layer.


**Fig. 1** The landscapes of the three study sites on Mount Usu. (A) Bare ground formed by the 2000 eruption (photo taken on September 8, 2009). (B) Grassland dominated by *Polygonum sachalinense* in an area damaged by the 1977-78 eruption (August 25, 2010). (C) A forest dominated by *Populus maximowiczii*, which developed after the 1910 eruptions (August 10, 2011).

**Table 1** The ages, soil layer depths, and overstory openness of the studied sites.

| | Years from eruption | L layer | H layer | O layer | Overstory openness |
|---|---|---|---|---|---|
| bareground | 10 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 74.3 ± 5.5 |
| grassland | 33 | 2.8 ± 1.3 | 1.7 ± 1.5 | 2.2 ± 0.8 | 19.4 ± 4.0 |
| forest | 100 | 2.4 ± 0.5 | 0.6 ± 0.3 | 11.6 ± 3.9 | 9.2 ± 0.5 |

**Changes in chemical components and isotopes in the litter**

$\delta^{13}C$ was -32 ± 2‰ (mean ± standard deviation) in the initial litter of *Polygonum sachalinense* and -31 ± 0‰ in the initial litter of *Populus maximowiczii*, with no significant differences (Table 2). In the decomposed litter, $\delta^{13}C$ was significantly higher in the bare ground (-29 ± 0‰) and grassland (-29 ± 1‰) than in the forest (-31 ± 1‰). $\delta^{15}N$ in the initial litter of *Polygonum sachalinense* was 0.3 ± 4.0‰ and was significantly higher than in the initial litter of *Populus maximowiczii*, in which $\delta^{15}N$ was -4.8 ± 1.4‰. $\delta^{15}N$ in the decomposed litter did not differ among the sites or between the litter species. The interactions of $\delta^{15}N$ among the litter species and the sites were significant in the forest, showing that with the progression of succession, $\delta^{15}N$ decreased to a greater extent in the *Polygonum sachalinense* litter than in the *Populus maximowiczii* litter.

The P of phospholipids averaged 430 ± 10 $\mu$g/g in the initial litter of *Polygonum sachalinense* and 348 ± 75 $\mu$g/g in the initial litter of *Populus maximowiczii*. The P content in decomposed litter was not different between the species (GLM, $P > 0.05$). The P in the phospholipids was 63 $\mu$g/g litter in the bare ground, 96 $\mu$g/g in the grassland, and 130 $\mu$g/g in the forest (Table 2). The phospholipids in litter increased significantly during succession without interactions between litter species and sites.

The C content in the initial litter was 47 ± 4% in *Populus maximowiczii* and 45 ± 0% in *Polygonum sachalinense* and was not different between the litter species. The N content in the initial litter averaged 3 ± 1% in *Populus maximowiczii* and 3 ± 0% in *Polygonum sachalinense*, and hence was not different between species. The N contents in the decomposed litter were significantly lower in the bare ground and grassland than in the forest. The C/N ratios were significantly lower in the forest than

in the bare ground and grassland and were not significantly different between the litter species.

**Table 2** The C, N, C/N ratio, $\delta^{13}C$, $\delta^{15}N$, and phosphorus (P) in the litter of *Populus maximowiczii* (PM) and *Polygonum sachalinense* (PS), which were collected from three different sites (bare ground, grassland and forest). Each numeral shows the mean and the standard deviation. The total P in the phospholipids is shown. Different letters indicate significant differences between litter species or between years (GLM, $P < 0.05$).

| | C (%) | N (%) | C/N | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | P (μg/g) |
|---|---|---|---|---|---|---|
| PM | | | | | | |
| initial litter | 46.5 ±4.21 | 2.48 ±0.77 | 19.94 ±7.86 | -31.0 ±0.28 | -4.83 ±1.37 | 347.8 ±75.3 |
| bareground | 44.2 ±5.09 a | 1.16 ±0.20 a | 39.6 ±12.1 a | -28.6 ±0.25 a | 0.77 ±0.13 a | 75.3 ±13.8 a |
| grassland | 41.0 ±1.13 a | 1.44 ±0.19 a | 28.9 ±3.83 a | -28.9 ±0.73 a | 1.44 ±0.19 a | 113.0 ± 24.6 b |
| forest | 40.0 ±1.83 a | 1.80 ±0.20 b | 22.4 ±1.62 b | -30.3 ±0.53 b | 1.68 ±0.04 a | 118.4 ±1.24 c |
| PS | | | | | | |
| initial litter | 45.1 ±0.28 | 2.93 ±0.34 | 15.49 ±1.69 | -31.8 ±2.33 | 0.31 ±4.04 | 429.8 ±10.4 |
| bareground | 47.5 ±16.3 a | 1.20 ±0.64 a | 46.1 ±18.1 a | -28.8 ±0.28 a | 0.11 ±1.62 a | 51.6 ±20.4 a |
| grassland | 43.5 ±0.54 a | 1.08 ±0.26 a | 42.2 ±11.5 a | -28.2 ±0.93 a | 1.08 ±0.26 a | 78.9 ±15.0 b |
| forest | 43.0 ±0.90 a | 2.06 ±0.14 b | 21.0 ±1.56 b | -31.7 ±0.25 b | -2.01 ±0.90 b | 142.5 ±5.70 d |

**Microbial biomass**

In total, 19 PLFAs were detected and identified in all the litter samples. Of these, 18:2 was from fungi, and i15:0, a15:0, 16:1ω7, a17:0, 17:0 and 10Me-18:0 were from bacteria in both the initial and decomposed litter. The initial litter contained 908 ± 303 $\mu$g/g PLFAs. Bacterial and fungal PLFAs averaged 10 ± 2 $\mu$g/g and 216 ± 64 $\mu$g/g, respectively. The average concentration of 16:1ω7 ranged from 10 ± 3 μg/g in the bare ground to 44 ± 12 μg/g in the forest. Because 16:1ω7 was produced by bacteria, the contribution of bacteria to the microbial biomass was reduced (Fig. 2). The total PLFA content increased significantly with sites independent of the litter species. The PLFAs in the bare ground were 1/2 and 1/4 of those in the grasslands and forests, respectively. These results indicated that the biomass of microbial organisms increased across the chronosequential succession. The PLFA marker of fungi, 18:2, increased five-fold from 112 $\mu$g/g at 10 years after the eruptions to 497 $\mu$g/g at 100 years after the eruptions, and the bacterial PLFA contents increased four-fold from 24 $\mu$g/g in the bare ground to 91 $\mu$m/g in the forest. The miscellaneous PLFAs were 3.7 times higher in the forest than in the bare ground (967 μg/g vs. 263 μg/g, respectively). Because the plant leaves did not produce any PLFAs after defoliation, the detected PLFAs were produced by microbial activities. The fungi-to-bacteria ratios averaged 5.3 ± 1.4 and did not differ significantly among the three sites and between the two litter species.

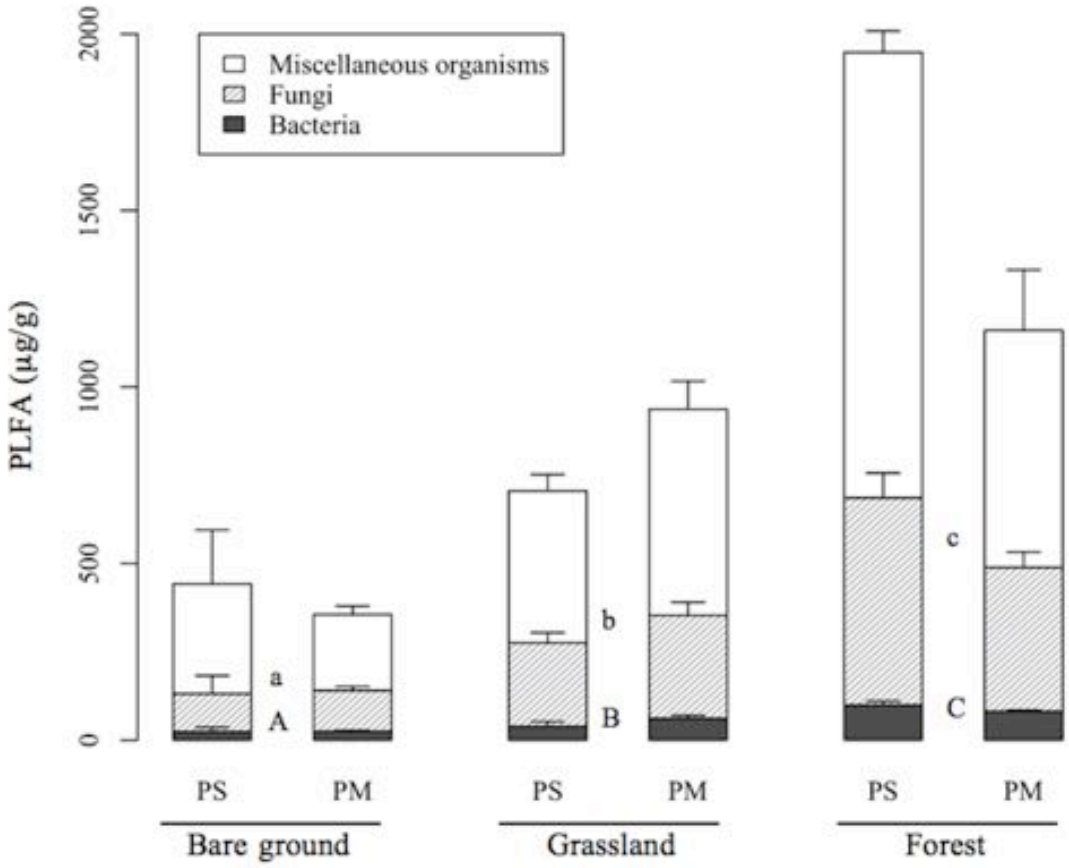



**Fig. 2** PLFAs produced by bacteria that were evaluated by the sum of i15:0, a15:0, 16:1ω7, a17:0, 17:0 and 10Me-18:0 (closed columns); fungi evaluated by the sum of 18:2 (hatched columns); and miscellaneous organisms evaluated by the sum of 14:0, 15:0, i16:0, 16:0, br17:0, 17:1, 18:1ω9, 18:1ω7 18:0, 19:1, 20:0 and 22:0 (open columns). PLFAs produced by plants and/or microbes (bacteria and/or fungi) were classified into miscellaneous organisms. PS = *Polygonum sachalinense* litter, and PM = *Populus maximowiczii* litter. Different lowercase and uppercase letters indicate significant between-year differences in fungal and bacterial PLFAs, respectively (GLM, $P < 0.05$). The PLFAs were not significantly different between litter species ($P > 0.05$).

**Canonical correspondence analysis of PLFAs**

The CCA performed by using the composition of PLFAs showed that the variations were 46% for axis I and 37% for axis II (Fig. 3). The cumulative variations of the two axes reached 83%. The permutation test of the CCA showed that the first two axes were significant. ANOSIM indicated that the years after the eruptions, canopy openness and soil layers were significant variables in each of the separate groups. The scores of the bare ground were much different from the scores of the grassland and the forest over time after the eruptions, and the scores of the grassland and the forest roughly overlapped. These trends indicated that the compositions of PLFAs in the litter differed between the nonvegetated (open-canopy) and vegetated (closed-canopy) habitats. In addition, the scores of the forest exhibited a wide range, which indicated that the compositions of PLFAs in the litter became diverse in the forest.

The overstory openness was negatively correlated with the number of years after the eruptions (Fig. 3). The thicknesses of the organic and litter layers in the soils were positively correlated with the number of years after the eruptions, showing that the soil developed chronologically. However, the thickness of the humus layer was not greatly related to the successional ages of the eruptions. The angles of the litter species, *Populus maximowiczii* and *Polygonum sachalinense*, were approximately orthogonal to the ages, and their positions close to the origin indicated that the litter species was not strongly related to the composition of PLFAs.

The PLFA produced by fungi, i.e., 18:2, was positioned near the origin of the CCA plot (Fig. 3), indicating that most of the litter contained 18:2 produced by fungi. Of the PLFAs produced by bacteria, the scores of 10Me-18:0 represented the PLFAs detected from the grassland and the scores of a17:0 represented the PLFAs

compositions in the bare ground. These results indicated that bacterial flora changed along the chronosequence. The other bacterial PLFAs compositions were common in the litter, showing that certain bacteria were present in these three habitats.

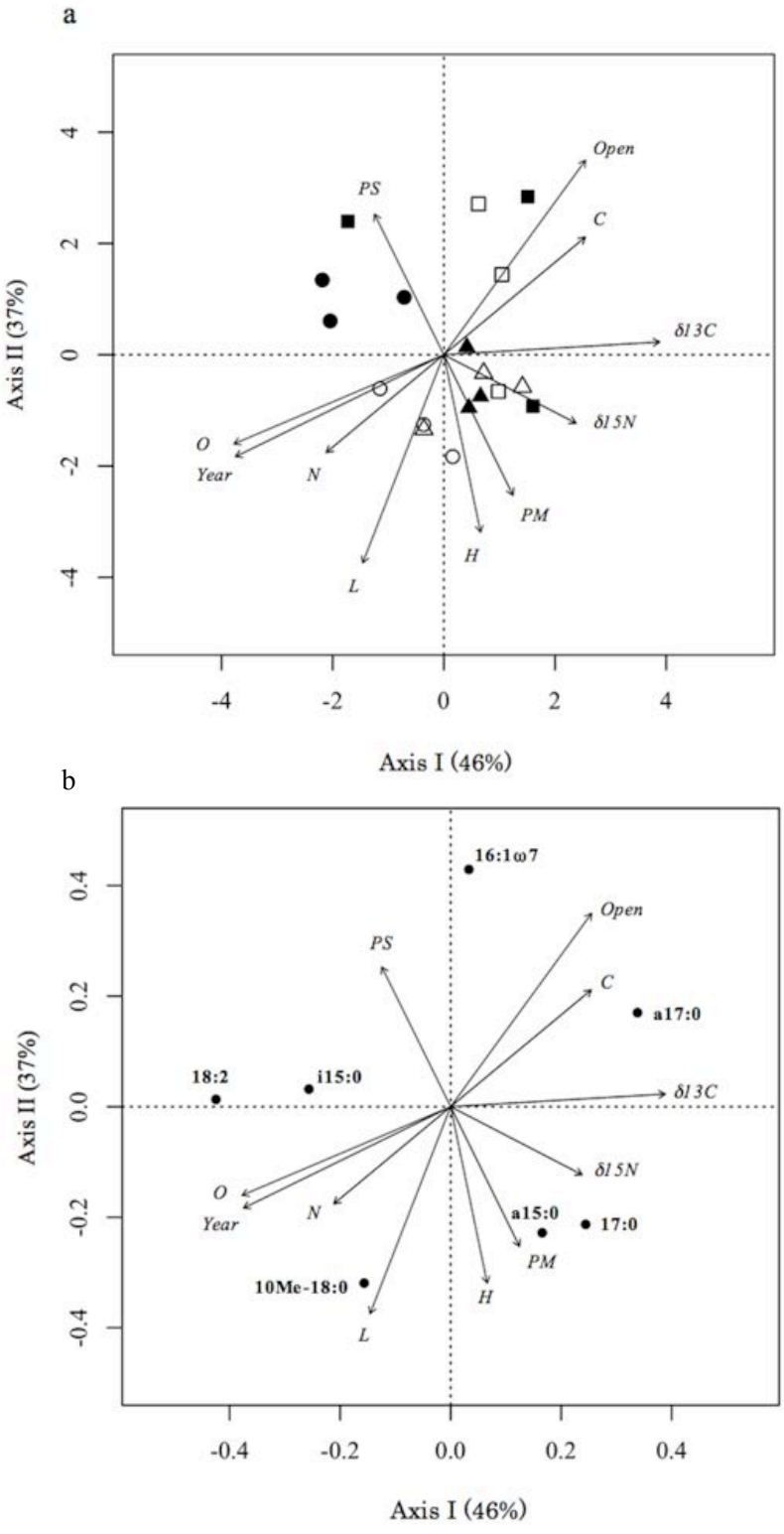


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**Fig. 3** CCA conducted by using PLFAs produced by bacteria and fungi in litter. The litter was collected from the three sites with different ages. The contribution rates on the first two axes are shown in parentheses to the right of the axis label. (a) The scores of litter of the two examined species collected from the three sites. The open and closed symbols show the litter of *Populus maximowiczii* and *Polygonum sachalinense*, respectively. The years after the eruptions are indicated as follows: Circles = forest; triangles = grassland; and squares = bare ground. Environmental factors: soil organic layer = *O*; humus layer = *H*; litter layer = *L*; canopy openness = *Open*; years after the eruptions = *Year*; C content (%) = *C*; N content (%) = N; C/N ratio = C/N; $\delta^{13}$C content = $\delta^{13}$C; $\delta^{15}$N content = $\delta^{15}$N; *Polygonum sachalinense* litter = *PS*; and *Populus maximowiczii* litter = *PM*. (b) The PLFA scores of i15:0, a15:0, 16:1ω7, a17:0, 17:0 and 10Me-18:0 are produced by bacteria, and 18:2 is produced by fungi. Note that the scales of figures (a) and (b) are different.

## 3.4 Discussion

### Succession of microbial communities investigated through PLFAs

The chronosequential approach, which has been widely used in vegetation science, demonstrated that the PLFA contents produced by fungi and bacteria increased with increasing successional ages after the eruptions of Mount Usu. PLFAs that were produced by both bacteria and fungi were classified into miscellaneous PLFAs; thus, their origins were not determined. However, the miscellaneous PLFAs produced by microbial activities also increased over time. The phosphorous in lipids gradually increased with succession on Mount Usu. This increase was most probably derived primarily from an increase in the PLFA content. Phosphorus fluctuations in litter have been shown to be synchronized with fluctuations in the soil with volcanic succession on the Hawaiian Islands, i.e., the phosphorus content increases from the early to middle stages and decreases from middle to late stages (Crews et al. 1995). The phosphorus in the lipids of the litter still increased with succession on Mount Usu. Because the *Populus maximowiczii* forest was in the early and middle successional stages, the phosphorus in the litter increased and the litter decomposition accelerated toward the climax vegetation (Crews et al. 1995).

The composition of PLFAs and CCA based on PLFAs showed that the biomass of fungi and bacteria was higher in the grassland and forest than the bare ground. The PLFA contents, C/N ratio, $\delta^{13}C$ and $\delta^{15}N$ in the forests were significantly different from those in the bare ground and grassland, i.e., the forest showed the highest PLFA content and lowest C/N ratio, $\delta^{13}C$ and $\delta^{15}N$. Therefore, not only the soil properties but also the aboveground vegetation structure probably influences the

microbial biomass and composition.

**The effects of C and N contents of litter microorganisms**

The $\delta^{15}N$ in the litter that had remained for 10 months after the defoliation did not differ between the two examined species, *Polygonum sachalinense* and *Populus maximowiczii*, whereas the $\delta^{15}N$ in the initial litter was higher in *Polygonum sachalinense* than in *Populus maximowiczii.* The leakage of dissolved organic matter from litter occurs rapidly after defoliation (Bourbonniere and Creed 2006), and soluble organic matter content differs among plant species (Taylor and Barlocher 1996). The leakage of N may have been faster from the *Polygonum sachalinense* litter than from the *Populus maximowiczii* litter. The results of GLM for $\delta^{15}N$ and $\delta^{13}C$ showed that the interactions between the litter species and the years were statistically significant. These results indicated that decreases in $\delta^{15}N$ and $\delta^{13}C$ became more pronounced with succession.

However, the CCA and GLM analyses showed that the litter species had a small effect on the compositions of the taxon-specific composition of PLFAs. The chemical properties in the initial litter were likely to be different between the two litter species, although the contents of C and N in the initial litter were not. These results suggested that the translocation of nutrients from leaves formed litter of similar quality. In general, tree litter decomposes more slowly than herb litter due to its low nitrogen content, which in particular is derived from slowly dissolving lignin (Enriquez et al. 1993). $C_3$ and $C_4$ plants generally show $\delta^{13}C$ values $-36 \pm 3‰$ and $-21 \pm 2‰$, respectively (Chikaraishi and Nagaoka 2003), thus indicating that *Populus maximowiczii* and *Polygonum sachalinense* are $C_3$ plants. $\delta^{13}C$, an indicator of lignin

content (Wedin et al. 1995), did not differ between the two litter species. Therefore, both the *Populus maximowiczii* and *Polygonum sachalinense* litter decomposed rapidly. Litter decomposability has been consistently related to the ecological strategy of plant species (Cornwell et al. 2008). Because *Populus maximowiczii* and *Polygonum sachalinense* are often established sympatrically soon after volcanic eruptions (Tsuyuzaki 1987), their ecological strategies are likely convergent; thus, the effects of litter species on the litter decomposition were not clearly detected in the early and middle stages of succession. Because the C content in the litter does not change greatly among most vascular plant species, the N content and its related variables, such as the C/N ratio, in plant substrates should be included in descriptions of litter decomposition patterns.

**Successional changes in fungi and bacteria**

The PLFAs showed that the biomass of fungi and bacteria increased with succession on Mount Usu. In addition, the F/B ratio did not differ among the three sites and was constant along the successional seres. The biomass of fungi and bacteria is positively correlated to litter decomposition rate (Neely et al. 1991). These results suggested that the litter decomposition occurred more rapidly with succession and with constant contribution rates, shown by F/B ratios. The fungi-to-bacteria ratio is influenced by soil pH (Bååth and Anderson 2003). The soils and volcanic deposits on Mount Usu are acidic, although the soil chemical properties in each of the three sites were not measured in this study. The phosphorus and nitrogen contents in the soil were likely to increase with time and in synchrony with the changes in PLFAs. However, these changes did not affect the F/B ratio. Further studies are required to detect why fungal

PLFAs were dominant throughout succession.

In nutrient-poor soil, mycorrhizal fungi contribute more to litter decomposition than bacteria in soil-pore water (Unestam and Sun 1991) because fungi transport nutrients through elongated hyphae (Brunner 2001). Fungi are more advantageous to litter decomposition than bacteria when the soil is dry, acidic and/or depleted (Blagodatskaya and Anderson 1998). Fungal communities play a dominant role in early stages of litter breakdowns in black alder forests, whereas bacteria complete the mineralization of C (Dilly et al. 2001). Laboratory experiments have shown that the fungi-to-bacteria ratio increases with decreasing pH over a range of 9 to 3 (Bååth and Anderson 2003); in contrast, the fungi-to-bacteria ratio increases when the soil in cool, temperate forests undergoes a dry/wet cycle (Scheu and Parkinson 1994). The fungi-to-bacteria ratio has been shown to increase gradually in primary succession on a glacier forefront during primary succession from bare ground to needle-leaved boreal forests in Scandinavia (Pennanen et al. 2001). Microbial activities are regulated by the habitat characteristics determined by the litter quality (Dilly and Munch 2004). The differences in the activities of fungi and bacteria are likely to be derived from litter quality, i.e., forbs or trees (Cornwell et al.2008). The volcanic deposits on Mount Usu are acidic and often become dry, even in the forests. Therefore, the characteristics of volcanic deposits should benefit the presence of fungi.

In conclusion, the biomass of fungi and bacteria in litter increased with the successional stages with constant F/B ratios throughout the succession. This pattern probably occurred because the characteristics of volcanic ejecta, i.e., the porosity and permeability of volcanic deposits, persist for several decades or up to a century.

## General conclusion

Litter decomposition process is a key to determine nutrient dynamics on terrestrial ecosystems, including succession, and is spatio-temporally changed. Therefore, leaf litter decomposition patterns were evaluated and characterized in various successional stages and were compared between the successional stages with changes in chemical properties, i.e., nitrogen (N), carbon (C) and their isotopes. On biological decomposition of litter, fungi and bacteria are major components. To detect the microbial biomass, therefore, the composition of fatty acids (PLFAs) in litter was also quantified.

Litter decomposition in two forests, of which successional stages were different, was investigates by using a litterbag method, to examine a few hypotheses proposed previously on litter decomposition (Chapter 1). The first hypothesis is home field advantage (HFA) that is: litter decomposes fast in the inside of its own ecosystem because of suitable environments for the microbial activities (Gholz et al. 2000; Ayres et al. 2009). The second hypothesis is litter-mixing effect of litter (LME) that is: the litter decomposition is faster in litter consisting of multi-species than of mono-species because such mixed litter provides heterogeneous litter quality and structure improving the chemical and physical environments (Wardle et al. 1997). Although these hypotheses have been examined mostly in mono-specific forest for examining HFA and between the distinctive forests for LME (Laganière et al. 2010), forests at seral successional stages consist of species mixed with early and late colonizers. Therefore, these effects on mixed forests may act differently from the effects on mono-specific

forests, and are likely to occur more frequently in the field. Therefore, two forests, both of which were in the seral stages of succession, were selected for this study: birch (*Betula platyphylla*) forest that was in earlier successional stage and oak (*Quercus mongolica*) forest. Litter decomposition rates were not different between the two forests and between the litter species for three examined years. In addition, strong HFA and LME were not detected. The amount of fungal PLFAs and C/N ratios were lower in the oak forest, showing that the fungal biomass increased with the progress of litter decomposition. This study also suggested that the litterbag method had a few disadvantages on long-term monitoring.

The alternative parameters, without litterbags, estimating litter decomposition were searched by using two characteristic ecosystems, wetlands and forests (Chapter 2). Litter decomposition estimated by C/N ratio was highly correlated to decomposition measured by litterbags in both the ecosystems. C/N ratio was related more to N than to C, because C did not fluctuate greatly with litter decomposition in the forest. Therefore, C/N ratio and N in litter of the examined variables were used for indicators of litter decomposition, although there were a few conditional assumptions for use. This non-litterbag method is likely to be utilized in various ecosystems.

Differences in litter decomposition between the successional stages were examined on Mount Usu, without litterbags that were not applicable because of ground-surface instability (Chapter 3). Two litter species, *Polygonum sachalinense* (herb) and *Populus maximowiczii* (tree), were selected for the study, based on the dominance. Litter decomposed for one year was collected from three sites, a bare ground damaged by the 2000 eruptions, a grassland by the 1977-78 eruptions and a forest by 1910 eruptions. Along the successional sere, the organic layers in soil increased. The litter decomposition evaluated by C/N ratio increased with the

development of vegetation. The biomass of both bacteria and fungi increased along the successional sere, independent of litter species, showing that the litter decomposition was influenced mostly by the biomass of microbial decomposers. PLFAs indicated that the bacterial composition changed with the succession rather than litter species, suggesting that the bacterial succession occurred with the plant succession.

The temporal changes in bacterial composition (Chapters 1 and 3) suggested that the succession of microbial communicates occurred, although the succession did not affect the litter decomposition rates. N was related to litter decomposition more to C (Chapter 2), showing that N determined litter decomposition. N is often related to the quality of the initial litter, i.e., high N in the litter before defoliation induces slow decomposition owing to low litter quality (Enríquez et al.1993; Berg and Matzner 1997). However, the litter decomposition did not differ greatly between litter species. Microbes, i.e., fungi and bacteria, import N into litter during litter decomposition (Frey et al. 2000) and therefore the effects of litter species are sometimes masked (Trogisch et al. 2016). Based on these, I concluded that the changes in litter decomposition rates occurred slowly across the succession and were affected by microbial biomass and the litter N residues, but not affected greatly by litter quality.

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