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Abstract

There is little knowledge about microbial functional community structures and the relationships between microbial communities and nitrogen transformation processes. Here, we investigated the relationships between soil microbial communities and nitrogen mineralisation potentials in a cool temperate forest throughout the growing season. Microbial communities were assessed by quantification of the total bacterial, archaeal, and fungal gene abundances and the bacterial and archaeal amoA gene abundances, functional predictions of bacteria and fungi, and analysis of the bacterial-fungal co-occurrence network. In mid-summer, ectomycorrhizal fungal abundance was significantly higher, whereas the total bacterial abundance was significantly lower. Bacterial and archaeal amoA gene abundances were also significantly higher in mid-summer. However, regardless of the seasonal fluctuation of microbial gene abundances, the net nitrification and nitrogen mineralisation potential did not show clear seasonality. In the network analysis, the microbial community was divided into 13 modules, which were subgroups assumed to have similar niches. Furthermore, two modules that mainly consisted of microbial species of Proteobacteria and Bacteroidetes were significantly and positively correlated with the net nitrification and mineralisation potentials. Our results indicated that microbial subgroups sharing similar niches, instead of total microbial abundances and functional gene abundances, could be important factors affecting the net nitrogen mineralisation potential.

Keywords

Soil microbial community, co-occurrence network, modules, nitrogen cycle

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' contribution

MN, SI and RT conceived and designed the experiment; MN, SI and RT performed the experiment; MN, SI, CT and TT performed data analysis; MN prepared figures and tables; MN and RT took the lead in writing the manuscript with input from all authors.

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Introduction

The availability of nitrogen (N) often limits the primary production of temperate forests (Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). Nitrogen mineralisation and nitrification, wherein organic N is converted into plant-available N forms, are the key processes regulating aboveground and belowground net primary production and N cycling in forest ecosystems (Aber *et al.*, 1985; Reich *et al.*, 1997; Tateno *et al.*, 2004). Soil microbial communities, including fungi and bacteria, are thought to be the main drivers of soil N transformation processes (Schimel and Bennett, 2004; Isobe and Ohte, 2014; Tatsumi *et al.*, 2019; Isobe *et al.*, 2020). Therefore, to understand the forest soil N cycle, it is essential to understand soil microbial communities.

In the last two decades, studies have revealed that microbial abundances, communities, and functional gene abundances are affected by environmental conditions including pH, carbon/nitrogen (C/N) ratio, and availability of nutrients and substrates (Högberg *et al.*, 2007; Lauber *et al.*, 2008; Ushio *et al.*, 2010; Wan *et al.*, 2015). Therefore, soil microbial communities can respond to changes in environmental conditions caused by seasons and/or tree species composition of the forests (Moore-Kucera and Dick, 2008; Ushio *et al.*, 2010; Prevost-Boure *et al.*, 2011). Individual tree species also affect soil microbial communities directly by organic carbon supply from their roots (Kaiser *et al.*, 2010, 2011; Urbanová *et al.*, 2015), which can change between seasons and/or with the tree species composition of forests. In particular, mycorrhizal fungi rely on the carbon supply from their symbiotic trees (Gessler *et al.*, 1998; Högberg *et al.*, 2010; Ekblad *et al.*, 2013), which can affect the free-living microbial community that is involved in soil N transformation processes (Tatsumi *et al.*, 2020).

Furthermore, relationships between microbial communities and soil N transformation processes have been reported in earlier studies (Boyle *et al.*, 2008; Gubry-Rangin *et al.*, 2010; Isobe

106 *et al.*, 2015, 2020; Ribbons *et al.*, 2016; Tatsumi *et al.*, 2020). For example, Ribbons *et al.* (2016)
107 found that the total bacterial abundance had a positive and significant correlation with N
108 mineralisation rates. In addition, rare bacterial and archaeal groups sometimes have significant roles
109 in specific processes (Caffrey *et al.*, 2007; Isobe and Ohte, 2014; Isobe *et al.*, 2020). For example,
110 the abundance of ammonia-oxidising bacteria and archaea (AOB and AOA, respectively) affected
111 the nitrification rate (Isobe *et al.*, 2015, 2020; Ribbons *et al.*, 2016). However, since microbial
112 functions and metabolisms have high diversities (Torsvik and Øvreås, 2002; Strickland *et al.*, 2009;
113 Mendes *et al.*, 2014; Lladó *et al.*, 2016; Wilhelm *et al.*, 2019), the underlying mechanisms by which
114 microbial communities drive N transformation processes are still largely unknown.

115 Recently, the microbial co-occurrence network analysis has provided new insights for
116 understanding microbial communities (Toju *et al.*, 2016; Sun *et al.*, 2017; Nakayama *et al.*, 2019). It
117 has detected densely co-occurring microbial subgroups, called modules (Newman, 2006; Langfelder
118 and Horvath, 2008; Deng et al., 2012), and the analyses of these modules could be used to uncover
119 ecologically relevant interactions that could not be detected by the traditional analyses of community
120 compositions and abundances. Although it is difficult to detect which co-occurring relationships
121 could be a direct physical interaction or an apparent co-occurrence, the microbial subgroups detected
122 as modules would have an individual preference for substrates and environmental conditions (Deng
123 *et al.*, 2012; de Menezes *et al.*, 2015; Jones and Hallin, 2019). Highlighting the microbial modular
124 subgroups would allow for a detailed investigation of the microbial communities that could not be
125 achieved by analysing the whole microbial community (de Menezes *et al.*, 2015). For example,
126 Purahong *et al.* (2016) reported that individual fungal sub-communities detected as modules had
127 different preferences for the chemical compositions of leaf litter, and only a part of the detected
128 modules had significant roles in litter degradation. Thus, investigating the relationships between
129 modular microbial subgroups and N mineralisation processes would reveal the detailed mechanisms

of microbial regulation for soil N transformation.

Recently, we investigated the impact of conversion of a forest to a monoculture plantation on the characteristics of microbial communities, such as the diversities and co-occurrence network structures of the communities. We found that forest conversion reduced the robustness of the co-occurrence network in mineral soil, while microbial diversities were maintained (Nakayama *et al.*, 2019). We also reported that chemical properties, including pH and the C/N ratio, significantly varied among seasons but not among forest types (Nakayama *et al.*, 2019). However, the details of the interactions between each functional group of the microbial communities and the N mineralisation processes are still unknown. Therefore, in the current study, we aimed to reveal the relationships between the roles of each group of microbial communities and the N transformation processes in the soil of a cool temperate forest. We measured the net nitrification and N mineralisation potentials, total gene abundances of bacteria, archaea, and fungi, and the bacterial and archaeal *amoA* gene abundances among forest types (monoculture larch and fir plantations, and natural deciduous broad-leaved forests) and months (May, July, August, September, and November). Furthermore, we predicted the bacterial functional gene abundances, fungal guilds, and trophic modes by using the sequencing results reported previously (Nakayama *et al.*, 2019). Moreover, a microbial co-occurrence network was constructed and the microbial community was divided into modules. We then analysed the relationships between the microbial modules and the net N transformation processes. We hypothesised that: (1) microbial abundance and functional communities change due to the differences in seasons and/or forest types, (2) differences in microbial abundance and functional communities regulate changes in the N transformation processes, (3) individual modules in the co-occurrence network each have relationships with N mineralisation and nitrification potentials, and (4) modules that fluctuate with differences in seasons and forest types are particularly important for the N mineralisation and nitrification potentials.

Materials and Methods

Study site and soil sampling

We conducted this study in cool-temperate deciduous broad-leaved natural forests (hereafter referred to as “Natural”) and two kinds of plantations (“Larch” and “Fir”) that were converted from natural forests in the Shibecha Branch of the Hokkaido Forest Research Station at the Field Science Education and Research Center, Kyoto University, in north-east Japan (43°24.2′N, 144°38.5′E). The mean annual precipitation and air temperature were 1,189 mm and 6.3℃ (1986–2015), respectively. The highest average air and soil temperatures were usually observed in August, while leaves fell from deciduous trees in October (Nakayama and Tateno, 2018). The soils at this site were characterised as andosols (IUSS Working Group WRB, 2015).

Natural forests were typically dominated by *Quercus crispula* Blume, *Ulmus davidiana* var. *japonica* Nakai, *Phellodendron amurense* Rupr., *Acer pictum* Thunb. subsp. mono (Maxim.) H. Ohashi, and *Fraxinus mandshurica* Rupr. var. *japonica*, and the forest floor was densely covered with *Sasa nipponica* Makino et Shibata. The Larch and Fir plantations were plantations of *Larix kaempferi* (Lamb.) Carr. and *Abies sachalinensis* (Shmidt) Masters, respectively. We established four plots in each of the three forest types (Natural, Larch, and Fir) for soil sampling. The dominant trees of all the forest types were ectomycorrhizal trees (Matsuoka *et al.*, 2020). On May 29, July 15, August 26, September 17, and November 9, 2012, five surface mineral soil cores (0–10 cm depth) were collected using a cylindrical soil core sampler (20 cm^2 in surface area) from each plot and composited. The details of sampling plots and soil sampling have been reported previously (Nakayama *et al.*, 2019).

178 The samples were sieved with a 4 mm mesh sieve and divided into subsamples for wet,
179 fumigation, oven dry, and frozen treatments. The chemical properties (pH, moisture content,
180 microbial biomass C and N, total C and N, and the concentrations of dissolved organic C, NO_3^--N,
181 NH_4^+-N, dissolved inorganic N, amino acid, and dissolved organic N) of the wet and oven-dry
182 samples were previously reported (Nakayama *et al.*, 2019) and summarised in Supplementary Table
183 1. Briefly, the investigated chemical properties except for the C/N ratio significantly differed
184 between sampling months. For example, average pH ranged from 4.5 to 5.6 and was significantly
185 lower in August (Supplementary Table 1; means ± standard deviations; 4.5 ± 0.2, 4.8 ± 0.2, and 4.5 ±
186 0.4 for Larch, Fir, and Natural, respectively). There were no significant differences in other chemical
187 properties except for the microbial biomass of C and N among the forest types, which were
188 significantly higher in Natural than Fir (Supplementary Table 1).
189 DNA was also extracted from frozen subsamples (stored at −20°C) using a DNA extraction
190 kit (DNeasy Power Soil Kit, QIAGEN, Hilden, Germany), sequenced by the Ion Personal Genome
191 Machine™ (PGM™) with the Ion 318™ Chip V2 (Thermo Fisher Scientific, Waltham, MA, USA)
192 and the sequence data were processed. Detailed methods and results of the chemical properties, DNA
193 extraction and sequencing are shown in a previous report (Nakayama *et al.*, 2019), and the sequence
194 data were deposited in the DDBJ Sequence Read Archive under accession number DRA007965.
195
196 *Net nitrification and mineralisation potentials*
197
198 Wet subsamples (30 g) were put in plastic bottles and aerobically incubated for one month at 25°C.
199 To maintain the soil moisture at initial values, ion-exchanged water was added every ten days. After
200 the incubation period, ten grams of each subsample were extracted in 100 ml of 0.5 M K_2SO_4
201 solution. The net nitrification and mineralisation potentials were calculated as the net increase in the

amount of NO_3^--N and the total inorganic N over the incubation period, respectively. The initial values of NH_4^+-N and NO_3^--N were reported by Nakayama et al. (2019).

Microbial gene quantifications and functional predictions

Microbial gene abundance was quantified by the real-time quantitative polymerase chain reaction (qPCR) performed by the LightCycler 96 System (Roche Diagnostics K.K., Mannheim, Germany), with an intercalating dye, SYBR Green I (FastStart Essential DNA Green Master, Roche Diagnostics K.K., Mannheim, Germany). The bacterial and archaeal 16S rRNA genes, fungal ITS regions of the rRNA genes, and the bacterial and archaeal ammonia monooxygenase genes (*amoA*) were quantified to estimate total bacteria, total archaea, total fungi, AOB and AOA, respectively. The bacterial 16S, archaeal 16S, fungal ITS, bacterial *amoA*, and archaeal *amoA* were determined using universal primer sets 338f (Amann *et al.*, 1990)/518r (Muyzer *et al.*, 1993), 109f (Großkopf *et al.*, 1998)/344r (Raskin *et al.*, 1994), ITS1F_KYO2/ITS2_KYO2 (Toju *et al.*, 2012), amoA 1F/amoA 2R (Rotthauwe *et al.*, 1997), and CrenamoA 23F/CrenamoA 616R (Tourna *et al.*, 2008), respectively. The amplifications of bacterial and archaeal 16S and fungal ITS were performed under the following conditions: an initial denaturation at 95℃ for 10 min, followed by 45 cycles at 95℃ for 1 min, 53℃ for 30 s and 72℃ for 1 min (Fierer *et al.*, 2005). The amplification of bacterial and archaeal *amoA* was performed under the following conditions: an initial denaturation at 95℃ for 10 min, followed by 45 cycles of 95℃ for 1 min, 55℃ (for bacterial *amoA*) or 52℃ (for archaeal *amoA*) for 30 s, and 72℃ for 30 s (Okano *et al.*, 2004). More details are described in previous reports (Iwaoka *et al.*, 2018; Tatsumi *et al.*, 2019).

The bacterial functional genes and the fungal trophic modes and guilds were predicted by using PICRUSt pipeline (Langille *et al.*, 2013) and FUNGuild database (Nguyen *et al.*, 2016) based

on previously published rarefied data of mineral soil 16S rRNA gene sequences and the fungal taxonomic data of the ITS sequence of the samples (Nakayama *et al.*, 2019), respectively. The number of rarefied sequence reads per sample was 1441 for the 16S rRNA gene and 5817 for the ITS region (Nakayama *et al.*, 2019). This corresponded to 1170 bacterial OTUs (186 ± 18 per sample) and 2288 fungal OTUs (99 ± 26 per sample). The estimated counts of genes by PICRUSt were tagged with the KEGG Orthology (Kanehisa *et al.*, 2016). The occurrence of the genes encoding enzymes for N degradation and mineralisation, chitinase (EC 3.2.1.14), N-acetylglucosaminidase (NAGase, EC3.2.1.52), leucine aminopeptidase (EC 3.4.11.1), arginase (EC 3.5.3.1), and urease (EC 3.5.1.5) were predicted from the counts of K01183, K01207, K01255, K01476, and K01428, respectively (Isobe *et al.*, 2018; Tatsumi *et al.*, 2020). The activities and genes abundance of these enzymes are commonly measured to evaluate forest soil N cycling (e.g. Saiya-Cork *et al.*, 2002; Sinsabaugh *et al.*, 2008; Isobe *et al.*, 2018; Tatsumi *et al.*, 2020). Furthermore, to test the enzymatic interactions in the network, the occurrences of genes encoding the enzymes involved in nitrification, ammonia monooxygenase (K10944, K10945, K10946), hydroxylamine dehydrogenase (K10535), and nitrite oxidoreductase (K00370, K00371) were predicted by PICRUSt for each OTU occurring in the network. The fungal trophic modes (e.g. saprotroph, symbiotroph) and the guilds within the symbiotrophic fungi (e.g. ectomycorrhiza, arbuscular mycorrhiza, endophyte, lichenised) were predicted by using the online application FUNGuild (Nguyen *et al.*, 2016). FUNGuild frequently predicts multiple trophic modes for a single OTU, such as Pathotroph-Saprotroph, which we then designated to the “Others” category.

The microbial co-occurrence network and module level correlation analysis

The soil microbial co-occurrence network was constructed by using the same rarefied soil bacterial

and fungal sequencing data as functional prediction (1441 and 5817 reads per sample, respectively) that was previously published in Nakayama *et al.* (2019). Because the numbers of bacterial and fungal sequence reads were not enough to analyse the rare OTUs, we mainly focused on the core and abundant microbial community in the network analysis. Our previous study aimed to reveal the difference in network structures among forest types (Nakayama *et al.*, 2019); however, this study tried to explore the relationships between the modules of the microbial co-occurrence network and N cycling processes. Therefore, we reconstructed the mineral soil co-occurrence network using all of the microbial sequencing data of the mineral soil instead of dividing it by forest types. Before the construction of the network, the abundance data for rarefied soil bacteria and fungi were mixed into one dataset to analyse bacterial-bacterial, fungal-fungal, and bacterial-fungal relationships. In total, there were 12 plots (4 plots for each 3 forest types) and 5 sampling dates (n = 60). The network construction and module detection followed our previous paper (Nakayama *et al.*, 2019). Briefly, we removed very rare OTUs occurring in less than 1/3 of samples (i.e. less than 20 samples) before the network construction to avoid the network being too complex, enhancing the determination of the core microbial community (Barberán *et al.,* 2012). After this elimination, 190 and 33 OTUs remained for bacteria and fungi, respectively. Spearman's rank correlations were calculated with all remaining OTU pairs, and *p*-values were processed based on the false discovery rate. When the correlation coefficient ρ and *q*-value of the OTU pairs were > 0.6 and < 0.001, respectively, we considered that there was a significant positive co-occurrence relationship between the OTU pair (Chao *et al.*, 2016). The OTUs that had at least one significant co-occurrence relationship with other OTUs occurred in the network as nodes. The network was visualised using the software package Gephi (ver. 0.9.2) (Bastian *et al.*, 2009). Modules in the microbial co-occurrence network were detected based on the fast greedy modularity optimisation algorithm (Clauset *et al.*, 2004).

The relationships between modules and factors (chemical properties, N mineralisation

potentials, sampling month, and forest type) were assessed by correlation analysis (Deng *et al.*, 2012). Briefly, the relative abundance matrix was divided per module and the relative abundances of each OTU was standardised to mean 0 and variance 1. The module eigenvectors were calculated by singular value decomposition following a previous study (Langfelder and Horvath, 2007). The sign of each module eigenvector was fixed by assigning a positive correlation with the average relative abundances across OTUs within modules (Langfelder and Horvath, 2007). We then analysed the Pearson correlation coefficients between the module eigenvalues and factors as the correlations between modules and factors. Categorical factors (sampling month and forest type) were converted as 0 or 1 dummy vectors before analysis. We chose a significance level of $p < 0.05$. We also tested the relationships between the estimated absolute abundances of modules and factors after multiplying each relative abundance by the total bacterial 16S rRNA and total fungal ITS gene abundance.

Statistical analysis

We used a two-way split-plot factorial analysis of variance (ANOVA) followed by Tukey's honestly significant difference test to examine the differences in the net nitrification and mineralisation potentials, the total bacterial, archaeal and fungal gene abundance, the bacterial and archaeal *amoA* gene abundance, the predicted bacterial functional gene abundance, and the fungal trophic modes and guilds among forest types (Larch, Fir, and Natural), and sampling months (May, July, August, September, and November). The correlations between microbial gene abundances and chemical properties were assessed using Pearson’s correlation analysis. We chose a significance level of $p < 0.05$ for all statistical analyses. All statistical analyses were conducted using R software (version 3.5.0).

298

299 **Results**

300

301 *Changes in net N transformation potentials*

302

303 Net N mineralisation and nitrification were temporally stable (Fig. 1; two-way ANOVA: $F = 0.69$
304 and $p = 0.607$, and $F = 0.63$ and $p = 0.643$, respectively). Net nitrification did not significantly differ
305 among forest type (Fig. 1a; two-way ANOVA: $F = 4.01$ and $p = 0.057$), while natural forest had
306 significantly higher values of net mineralisation potentials throughout all sampling months (Fig. 1b;
307 two-way ANOVA: $F = 12.98$ and $p < 0.002$). The interaction effects of sampling month and forest
308 type on net nitrification and mineralisation were not significant (Fig. 1; two-way ANOVA: $F = 0.76$
309 and $p = 0.644$, and $F = 1.22$ and $p = 0.317$, respectively).

310

311 *Changes in microbial gene abundance*

312

313 There were significant effects of the sampling month on total bacterial and archaeal 16S rRNA gene
314 abundances (two-way ANOVA: $F = 25.58$ and $p < 0.001$, and $F = 22.41$ and $p < 0.001$, respectively).
315 According to Tukey's honestly significant difference test, bacterial abundance was significantly
316 lower in August, while archaeal abundance was higher in August for all three forest types (Fig. 2a, b).
317 Total fungal ITS gene abundance did not differ significantly among sampling months (Fig. 2c;
318 two-way ANOVA: $F = 1.02$ and $p = 0.409$). The total bacterial, archaeal, and fungal gene
319 abundances were similar among the forest types regardless of sampling months (Fig. 2a, b, c;
320 two-way ANOVA: $F = 1.75$. and $p = 0.228$, $F = 2.80$ and $p = 0.114$, and $F = 0.75$ and $p = 0.500$,
321 respectively).

Bacterial and archaeal *amoA* gene abundances differed significantly among sampling months (two-way ANOVA: $F = 8.84$ and $p < 0.001$, and $F = 15.04$ and $p < 0.001$, respectively). Archaeal *amoA* gene abundance had a similar trend with archaeal 16S rRNA gene abundance, while bacterial *amoA* gene abundance had the opposite trend of bacterial 16S rRNA gene abundance, i.e. the abundances of bacterial and archaeal *amoA* gene were significantly higher in August than in other months for all the forest types (Fig. 2d, e). Bacterial and archaeal *amoA* genes did not significantly vary among the forest types (Fig. 2d, e; two-way ANOVA: $F = 0.17$ and $p = 0.844$, and $F = 1.02$ and $p = 0.400$, respectively).

There were some significant positive and negative correlations between total bacterial, archaeal, and fungal gene abundances and chemical properties such as pH, moisture content, and the C/N ratio (Supplementary Table 2). Among these relationships, only the pH was positively correlated with bacterial 16S gene abundance (Supplementary Table 2; $R^2 = 0.08$ and $p < 0.05$). In contrast to the bacterial abundance, pH was negatively correlated with archaeal and fungal gene abundances (Supplementary Table 2; $R^2 = 0.31$ and $p < 0.001$, and $R^2 = 0.11$ and $p < 0.01$, respectively). The C/N ratio had a positive and the strongest relationship with fungal ITS gene abundance (Supplementary Table 2; $R^2 = 0.35$ and $p < 0.001$). There were no significant relationships between the total microbial gene abundances and N mineralisation potentials (Supplementary Table 2). Bacterial and archaeal *amoA* genes were also significantly correlated with chemical properties such as pH and dissolved organic N content (Supplementary Table 2). Bacterial and archaeal gene abundances were not correlated with the nitrification and mineralisation potentials (Supplementary Table 2).

Changes in bacterial predicted functional gene and fungal trophic modes and guilds

Among the predicted functional genes (the genes encoding N degrading and mineralising enzymes:

chitinase, NAGase, leucine aminopeptidase, arginase, and urease), there were no significant effects of forest type (Fig. 3; two-way ANOVA: $F = 0.05$ and $p = 0.949$, $F = 0.54$ and $p = 0.601$, $F = 0.15$ and $p = 0.867$, $F = 0.02$ and $p = 0.985$, and $F = 0.37$ and $p = 0.700$, respectively) and sampling month, except for urease (Fig. 3; two-way ANOVA: $F = 2.36$ and $p = 0.072$, $F = 0.42$ and $p = 0.795$, $F = 1.19$ and $p = 0.332$, $F = 1.80$ and $p = 0.150$, and $F = 5.03$ and $p < 0.01$, respectively) on their relative abundance. The relative abundance of urease in August was significantly higher than that in May, September, and November (Fig. 3e). The estimated absolute abundances of the predicted functional genes were temporarily changed, reflecting the changes of bacterial 16S rRNA gene abundance, and were significantly lower in August than in the other months for all three forest types (Supplementary Fig. 1).

The symbiotrophic fungi (Supplementary Fig. 2; 2.0%–81.1% of total fungi) tended to have a higher relative abundance throughout all sampling months and forest types than the saprotrophic fungi (Supplementary Fig. 2; 0.3%–30.5% of total fungi), and most of the symbiotrophic fungi belonged to the ectomycorrhizal guilds (Supplementary Fig. 3; 61.2%–100% of symbiotrophic fungi). The relative abundance of the ectomycorrhizal fungi differed significantly among sampling months (two-way ANOVA: $F = 13.65$ and $p < 0.001$), and the abundance was significantly higher in August than in the other sampling months (Fig. 4). The relative abundance of the saprotrophic fungi also differed significantly among sampling months and forest types (two-way ANOVA: $F = 4.65$, $p < 0.05$ and $F = 3.88$, $p < 0.05$ for months and forest types, respectively), and the abundance was significantly lower in August (2.7% ± 1.6% of total fungi) than in November (9.1% ± 9.1% of total fungi) and in Fir (2.1% ± 1.2% of total fungi) than in Larch (10.8% ± 7.4% of total fungi). The estimated absolute abundances of symbiotrophic and ectomycorrhizal fungi differed among sampling months (Supplementary Fig. 4; two-way ANOVA: $F = 3.74$ and $p < 0.01$, and $F = 3.87$ and $p < 0.01$, respectively), reflecting the results of the relative abundance of trophic modes and

the guild (Supplementary Fig. 2 and 3) rather than the total abundance of fungi (Fig. 2c).

The relative abundances of bacterial functional genes had significant correlations with chemical properties (Supplementary Table 3). In particular, the C/N ratio was positively and strongly correlated with chitinase, NAGase, leucine aminopeptidase, and arginase, and negatively correlated with urease (Supplementary Table 3; $R^2 = 0.37$ and $p < 0.001$, $R^2 = 0.32$ and $p < 0.001$, $R^2 = 0.48$ and $p < 0.001$, $R^2 = 0.43$ and $p < 0.001$, and $R^2 = 0.25$ and $p < 0.001$, respectively). Only weak correlations were found between fungal groups of higher relative abundances (symbiotrophic, saprotrophic, and ectomycorrhizal fungi) and chemical properties, such as pH and the C/N ratio (Supplementary Table 3). The relative abundance of saprotrophic fungi positively correlated with moisture content (Supplementary Table 3; $R^2 = 0.09$ and $p < 0.05$) and the relative abundance of ectomycorrhiza negatively correlated with pH (Supplementary Table 3; $R^2 = 0.16$ and $p < 0.01$). The relative abundances of chitinase, NAGase, leucine aminopeptidase, and arginase were significantly and negatively correlated with net nitrification potential (Supplementary Table 3; $R^2 = 0.16$ and $p < 0.01$, $R^2 = 0.29$ and $p < 0.001$, $R^2 = 0.17$ and $p < 0.01$, and $R^2 = 0.14$ and $p < 0.01$, respectively), and urease had a significant and positive relationship with net nitrification (Supplementary Table 3; $R^2 = 0.27$ and $p < 0.001$). The net mineralisation potential had a significant negative relationship with NAGase only (Supplementary Table 3; $R^2 = 0.08$ and $p < 0.05$). The relative abundances of the fungal groups did not have significant relationships with N mineralisation potentials (Supplementary Table 3).

The relationships between the estimated absolute abundances of the bacterial functional genes and fungi groups, and the net N mineralisation potentials are shown in Supplementary Fig. 5. The bacterial functional genes, except urease, were significantly and negatively correlated with net nitrification potentials (Supplementary Fig. 5; $R^2 = 0.10$ and $p < 0.05$ for chitinase, $R^2 = 0.09$ and $p < 0.05$ for NAGase, $R^2 = 0.07$ and $p < 0.05$ for leucine aminopeptidase, and $R^2 = 0.08$ and $p < 0.05$).

394 The estimated absolute abundances of the fungal groups had no significant correlations with net N
395 transformation processes (Supplementary Fig. 5).
396
397 *Modules and microbial functions in the co-occurrence network and their relationships with net N*
398 *transformation potentials*
399
400 The microbial co-occurrence network was divided into 13 modules (Fig. 5a). Modules 1 to 6 had a
401 higher number of OTUs belonging to the modules (12–18 OTUs) and higher relative abundances
402 representing these larger modules, while modules 7 to 13 had only 2 to 4 OTUs and lower relative
403 abundances, except for module 9, which occupied 9.76% of the total fungal community (Fig. 5b, c);
404 further analyses focused on modules 1 to 6. The relatively higher numbers of OTUs within modules
405 2 to 4 belonged to the phylum Acidobacteria (Fig. 5b; 10, 5, and 14 OTUs for each module,
406 respectively), and most of the Acidobacteria belonged to order Acidobacteriales (class
407 Acidobacteria) in module 3, while there were many OTUs belonging to Ellin6513 (class DA052) in
408 modules 2 and 4 (Supplementary Fig. 6 and Supplementary Table 4). Modules 5 and 6 had 8 and 10
409 OTUs belonging to the phylum Proteobacteria, respectively (Fig. 5b). Module 1 tended to have
410 many OTUs of the Proteobacteria and Bacteroidetes (Fig. 5b and Supplementary Fig. 4).
411 The OTUs that were predicted to have the genes responsible for N degradation enzymes
412 (chitinase, NAGase, leucine aminopeptidase, arginase, and urease) and for nitrification enzymes
413 (ammonia oxidation, hydroxylamine oxidation, and nitrite oxidation) investigated in this study were
414 depicted in the co-occurrence network, as shown in Figs. 6 and 7, respectively. Most of the OTUs in
415 the microbial co-occurrence network were predicted to have genes encoding NAGase and leucine
416 aminopeptidase regardless of the modules, while the numbers of OTUs having other functional
417 genes differed among modules (Fig. 6). The OTUs belonging to modules 2, 3, and 4 tended to have

418 the gene encoding chitinase, while relatively lower rates of the OTUs belonging to modules 5 and 6
419 were predicted to have that gene (Fig. 6a). In contrast, many OTUs belonging to modules 5 and 6
420 were predicted to have the gene responsible for urease, while modules 2, 3, and 4 had no or very few
421 OTUs predicted to have the gene (Fig. 6d). There was no OTU that was predicted to have the genes
422 responsible for ammonia monooxygenase, which is involved in the step from ammonia to
423 hydroxylamine (K10944, K10945, and K10946) in the co-occurrence network (Fig. 7a). Some links
424 between the OTUs that were predicted to have the gene responsible for hydroxylamine oxidation
425 (K10535), and the OTUs that were predicted to have at least one gene responsible for nitrite
426 oxidation (K00370, K00371) could be detected in module 6 and partly in module 5 (Fig. 7b, c). The
427 genes for denitrification were also depicted in the co-occurrence network (Supplementary Fig. 7).
428 There was only one link in module 1 between the first two steps, while some links were found in
429 modules 4, 5, and 6 for nitric oxide production and consumption, and in modules 2 and 4 for the
430 nitrous oxide production and consumption (Supplementary Fig. 7).

431 The sampling month did not significantly affect the relative abundances of modules 1 to 6
432 (Supplementary Fig. 8). The relative abundances of modules 1 to 3 and 6 had significant positive or
433 negative correlations with forest types; for example, module 1 had a significantly higher relative
434 abundance in the natural forest, while it was significantly lower in the fir forest (Supplementary Fig.
435 8; $R^2 = 0.32$ and $p < 0.001$, and $R^2 = 0.20$ and $p < 0.001$, respectively). There was no significant
436 relationship between modules 4 and 5, and forest types (Supplementary Fig. 8). The net nitrification
437 potential had significant positive correlations with the relative abundances of modules 1 and 5, and
438 negative correlations with modules 2 and 4 (Fig. 8a; $R^2 = 0.40$ and $p < 0.001$, $R^2 = 0.10$ and $p < 0.05$,
439 $R^2 = 0.07$ and $p < 0.05$, and $R^2 = 0.18$ and $p < 0.001$, respectively). On the contrary, the net
440 mineralisation potential only had a significant positive correlation with module 1 (Fig. 8a; $R^2 = 0.16$
441 and $p < 0.01$). The chemical properties were also significantly and positively or negatively correlated

with individual modules, particularly pH, moisture content and the C/N ratio were significantly correlated with the relative abundances of the modules (Supplementary Fig. 9).

The estimated absolute abundances of modules 1 to 6, calculated by multiplying the total bacterial 16S rRNA and fungal ITS genes, were significantly and negatively correlated with the month of August (Supplementary Fig. 10; from module 1 to 6, $R^2 = 0.17$ and $p < 0.01$, $R^2 = 0.16$ and $p < 0.01$, $R^2 = 13$ and $p < 0.01$, $R^2 = 0.16$ and $p < 0.01$, $R^2 = 0.10$ and $p < 0.05$, and $R^2 = 0.18$ and $p < 0.001$, respectively), which reflected the temporal changes of the total bacterial 16S rRNA gene abundance (Fig. 2a). There were no significant correlations between the estimated absolute abundances of the modules and the other sampling months (Supplementary Fig. 10). The effects of forest types on the estimated absolute abundances of modules 1 to 6 showed similar trends with the relative abundances, e.g. the estimated absolute abundance of module 1 was higher in the natural forest and lower in the fir forest (Supplementary Fig. 10; $R^2 = 0.15$ and $p < 0.01$, and $R^2 = 0.16$ and $p < 0.01$, respectively). The net nitrification potential had a significant positive correlation with the estimated absolute abundance of module 1 and a negative correlation with module 4 (Fig. 8b; $R^2 = 0.20$ and $p < 0.001$, and $R^2 = 0.16$ and $p < 0.01$, respectively). There was no significant relationship between the estimated absolute abundances of modules 1 to 6 and the net mineralisation potential (Fig. 8b).

Discussion

Differences in microbial communities and N transformation among seasons and forest types

We hypothesised that microbial abundance and functional communities changed due to the differences in seasons and/or forest types (hypothesis 1). Regarding this hypothesis, the effect of season on microbial abundance and functional communities was more significant than the effect of forest type (Figs. 2 and 4). However, the effect of season differed between bacteria and fungi, i.e. seasonal differences were apparent for the total abundance of bacteria and for the functional compositions of the fungi (Figs. 2 and 4). In the mid-summer, trees allocate more of their newly synthesised carbon to ectomycorrhizal fungi (EMF) (Högberg *et al.*, 2010) in order to take up more nutrients (Gessler *et al.*, 1998). The seasonal differences of carbon allocation from trees to EMF would explain the higher relative abundance of ectomycorrhizal guilds in mid-summer. In contrast, the abundance of saprotrophic fungi was suppressed in the mid-summer. Saprotrophic fungi decrease in abundance with increasing aridity (Tatsumi *et al.,* 2019). This suggests that the lower soil moisture content in the mid-summer (Supplementary Table 1) could lead to the lower relative abundance of saprotrophic fungi. Another explanation of a lower relative abundance of saprotrophic fungi was the suppression by the EMF. Ectomycorrhizal fungi have been reported to compete for resources with saprotrophic fungi (Lindahl *et al.*, 2002; Bödeker *et al.*, 2016). EMF are also strong competitors for nutrients with free-living microbes, including bacteria (Averill *et al.*, 2014; Tatsumi *et al.*, 2020). Furthermore, EMF have the ability to produce antibacterial material that could suppress bacterial species (Assigbetse *et al.*, 2005; Brooks *et al.*, 2011; Shirakawa *et al.*, 2019). Thus, the total bacterial abundance was probably suppressed in mid-summer due to the increase in EMF (Fig. 2). Another possible explanation for the seasonality of bacterial abundances was the seasonal changes in

environmental conditions. Among the chemical properties, only the pH significantly and positively affected bacterial gene abundance (Supplementary Table 2). This was consistent with previous findings (Rousk *et al.*, 2009, 2010). Thus, the lower pH in the mid-summer also might affect the bacterial abundance. Osburn et al. (2018) found that similar seasonal trends of bacterial and fungal abundances and reported that soil drying in summer decreased the bacterial abundance, while fungal abundance was maintained potentially by the stimulation of mycorrhizal growth by carbon supply from tree roots. In the present study, we could not find such interactions based on the correlational analysis using all data; however, there was still the possibility that the soil moisture content regulated the balance of soil microbial abundance. Although the environmental preferences differ among bacterial species (Supplementary Fig. 9), the soil environmental condition might strongly affect the total bacterial abundance and functional gene abundances.

The abundances of the fungal functional groups, particularly the saprotrophic fungi, varied among forest types (Supplementary Fig. 2). The saprotrophic fungi are the main decomposers of recalcitrant organic matter and obtain their energy and carbon sources from litter decomposition (Osono, 2007; Uroz *et al.*, 2016). In general, the evergreen litter (Fir) decomposed slower than the deciduous litters (Larch and Natural), partly because of the accumulation of recalcitrant organic matter in the fir litter (Cornwell *et al.*, 2008). This difference of decomposability would affect the energy acquisition of the saprotrophic fungi. Therefore, the EMF would be still active in the fir forest due to the carbon supply from symbiotic trees, while the saprotrophic fungi might be suppressed by the substrate recalcitrance.

Significant differences in the bacterial community structures among seasons and forest types were observed (Nakayama *et al.,* 2019). Surprisingly, the relative abundances of the bacterial functional genes concerning the N mineralisation process were stable across seasons and forest types (Fig. 3). One explanation for this is the functional redundancy of the bacterial community

508 (Nannipieri *et al.*, 2003; Wertz *et al.*, 2006). Frossard *et al.* (2012) reported that there were no
509 relationships between bacterial community structures and potential enzymatic activities because of
510 bacterial functional redundancy. Thus, even if the microbial community structures changed among
511 forest types, the potential to produce extracellular enzymes by the microbes would be maintained in
512 these sites. Another explanation is the methodological limitations of PICRUSt, such as shortage of
513 referenced reference database and restricted OTUs generation method (Douglas et al., 2020).
514 Although PICRUSt has been widely used in the study of soil bacterial functional community (e.g.,
515 Isobe et al., 2018), other methods such as shotgun metagenomic sequencing should be considered for
516 more detailed functional community analysis.

517 The N mineralisation potentials had no consistent relationships with total microbial
518 abundances and functional groups among seasons as well as forest types. Thus, our second
519 hypothesis that the differences in microbial abundance and functional communities regulated the
520 changes of N transformation did not hold. In contrast to the microbial communities not varying
521 among forest types, N mineralisation potentials varied among forest types (Fig. 1). There were three
522 possible explanations for this result. First, litter quality may exert a stronger control on N
523 mineralisation than the microbial factors. Based on the mycorrhizal associated nutrient economy
524 theory (Phillips et al., 2013), the EMF-dominated forest stands, such as the plots in this study tend to
525 have “organic nutrient economy” characterized by slow litter decomposition, and the organic N
526 turnover regulates N availability in the soil (Phillips et al., 2013). As we discussed above, deciduous
527 tree has higher decomposition rate than evergreen. In general, conifer leaves (Larch and Fir) also
528 tend to have a slower decomposition rate than broad leaves (Natural) (Singh and Gupta, 1977).
529 Accordingly, the N transformations might be faster in Natural than Larch and Fir, regardless of
530 microbial dynamics. Second, we measured net N mineralisation and the functional attributes we
531 characterized are more specifically focused on N acquiring enzymes. However, microbial

532 communities also consume mineralised N. While microbial N consumption was not measured and
533 not known in this study, Urakawa *et al.* (2016) reported that the net rates of N mineralisation
534 correlated with its gross rates, respectively, by investigating the net and gross rates of the N
535 transformation processes in 38 forest sites across the Japan archipelago including the sites of this
536 study. Further, some previous studies have reported significant relationships between microbial
537 properties and the net N mineralisation rate (Fraterrigo *et al.*, 2006; Kang *et al.*, 2018). However,
538 there is still the possibility that microbial N consumption masks the relationships between microbes
539 and N transformations, and future research using the gross measurement of N mineralisation is
540 needed to test this. The third possible explanation was that a part of microbial dynamics was
541 important for N mineralisation potentials rather than whole microbial and functional gene abundance.
542 Most bacterial species are considered to relate to N mineralisation (Bottomley et al., 2012; Isobe and
543 Ohte, 2014). Furthermore, strong correlations between enzymatic activities and microbial functional
544 predictions have been reported (Trivedi *et al.*, 2016). However, microbial activity varies widely
545 among species (Fierer *et al.*, 2007; Pinnell *et al.*, 2014; Wilhelm *et al.*, 2019). Thus, as we discuss
546 below for our third and fourth hypotheses, there is the possibility that a subsection of the microbial
547 communities is more active and important for N mineralisation.

548 In terms of the nitrification process, positive relationships between ammonia-oxidising
549 communities and nitrification have been previously reported (Hawkes *et al.*, 2005; Jia and Conrad,
550 2009; Isobe *et al.*, 2015). However, we could not find any significant relationships between them
551 (Supplementary Table 2). Although the ammonia-oxidising prokaryotes markedly increased in
552 mid-summer, the nitrification potential was stable across the seasons. The activities of the
553 ammonia-oxidising prokaryotes are sensitive to environmental conditions, including pH and
554 temperature (Jung *et al.*, 2011; Yao *et al.*, 2011; Zhang *et al.*, 2012; Taylor *et al.*, 2017). The
555 activities of nitrifying communities might be low in the mid-summer in our study site because of the

lower pH, although further research focusing on the gross nitrification is needed as we discussed above. Furthermore, the proportions of the net nitrification and mineralisation potentials suggest that most of the mineralised N underwent the steps of nitrification at this study site (Fig. 1). The mineralisation step might limit the nitrification potentials at the site. There was also another explanation. In this study, we measured bacterial and archaeal *amoA* genes as the microbial factors because the *amoA* genes involved in the ammonia-oxidation, the first and rate-limiting step of nitrification (Isobe et al., 2011). Indeed, Isobe et al. (2018) reported that gross nitrification rete and soil NO_3^- concentration related to the AOB population in the natural forest of this study site. However, some bacteria completely oxidize ammonium into nitrate, which is called comammox, and has different enzymes from AOB (Costa et al., 2006). Comammox could be the main driver of nitrification in some ecosystems (Osburn and Barrett, 2020). A part of fungal groups also involved in the nitrification processes (Zhu et al., 2015). Thus, those other nitrifying communities that we did not measure might drive the nitrification process.

Modules in the microbial co-occurrence network

Our results showed that each module was differently related to N transformation processes at the site. Namely, modules 1 and 5 had significant positive relationships with N transformation processes and modules 2 and 4 had significant negative relationships (Fig. 8). These results affirmed our third hypothesis that individual modules in the co-occurrence network each had relationships with N mineralisation and nitrification potentials.

Bacterial species belonging to the Proteobacteria and Bacteroidetes, the main members of modules 1 and 5 (Fig. 5b), tend to have higher abundances in nutrient rich conditions (Fierer *et al.*, 2012; Peiffer *et al.*, 2013), implying their copiotrophic strategy. Copiotrophs are reported to have

high population turnover and short mean generation time (Fierer *et al.*, 2007). The microbial necromass is a significant source of dissolved organic matter and is recycled and mineralised by microbes (Miltner *et al.*, 2012; Huygens *et al.*, 2016). Hence, *r*-selected bacterial species could lead to more active mineralisation. However, module 6, which consisted mainly of Proteobacteria, did not correlate with net mineralisation potentials (Figs. 5 and 8). The phylum Proteobacteria is the largest bacterial phylum and contains diverse OTUs with diverse functions and metabolisms; even within the same class, their metabolisms vary (Kersters *et al.*, 2006). Therefore, our results indicate that modules 1 and 5 might include OTUs responsible for creating the variation of mineralisation potential, while there might be less important Proteobacteria within module 6. Future research highlighting the members within modules 1 and 5 can reveal the detailed relationships between microbial subgroups and the N transformation processes.

In contrast, modules 2 and 4, consisting mainly of the OTUs from the order Ellin6513 (Acidobacterial subdivision 2), significantly and negatively correlated with the net mineralisation potentials (Fig. 6 and Supplementary Table 4). The abundance of module 2 differed among forest types, while there were no clear trends for module 4 (Supplementary Figs. 8 and 10). Some of the members of the Acidobacteria adapt to oligotrophic conditions, having the *K*-selected strategy (Fierer *et al.*, 2007; Kielak *et al.*, 2016). Oligotrophic and *K*-selected bacteria have low growth rates, slow population turnover rates and long generation times (Fierer *et al.*, 2007). Therefore, modules 2 and 4 might have lower mineralisation potentials than the other modules. Further, modules 2 and 4 tended to have higher relative abundances within lower pH conditions (Supplementary Fig. 9). Previous studies have reported that the relative abundance of Acidobacteria, including subdivision 2, was negatively correlated with pH, while other bacterial phyla tended to correlate positively with pH (Lauber *et al.*, 2009; Rousk *et al.*, 2010). In the lower pH environments that the microbes from modules 2 and 4 were abundant in, other bacteria, including the Proteobacteria, would have been

suppressed.

The possibility that the litter quality rather than microbial community determined the N transformation processes discussed above still could not be rejected. However, the microbial subgroups would be important for the net N transformation processes at the site. We expected that the modules that fluctuated with differences in seasons and forest types were particularly important for the N mineralisation and nitrification potentials in our fourth hypothesis. Indeed, module 1, which had the strongest positive correlations with net N mineralisation and nitrification, varied among the forest types (Supplementary Fig. 8). However, module 5, which also had significant and positive relationships with net nitrification, did not vary among forest types (Supplementary Fig. 8).

Further, the relative abundances of analysed microbial modules did not fluctuate seasonally. This was consistent with bacterial community structures and functions (Fig. 3). These results of seasonally stable relative abundances of modules imply that at least core microbial species, which would be important for N transformations, had similar seasonal trends of growth and death. Therefore, our fourth hypothesis was partly affirmed but largely did not hold.

The co-occurrence network was constructed mathematically based only on the relative abundance data (Deng *et al.*, 2012). Therefore, it was difficult to separate whether the co-occurring links between two microbial species were actual interactions, such as substrate giving-receiving, or environmental filtering (e.g. niche preferences). In this study, there were only a few links of predicted enzymatic production within the modules in the co-occurrence network (Fig. 7 and Supplementary Fig. 7). Furthermore, individual modules had individual correlations with environmental factors (Supplementary Fig. 9), as shown in previous studies (Deng *et al.*, 2012; de Menezes *et al.*, 2015; Purahong *et al.*, 2016). Thus, the modules of the microbial co-occurrence networks may represent the niche separations of the microbial communities rather than interactions and physical links. Therefore, our results indicate that the niches of microbial sub-communities are

the factors important for N mineralisation processes.

Conclusions

In this study, the results showed that the abundances of microbial communities differed among seasons. However, these seasonal fluctuations of microbial abundances were not related to the net N mineralisation potentials. Instead, the importance of microbial modular sub-groups that shared similar niches for the net N transformation potentials was revealed by the analysis of the co-occurrence network and functional predictions. In this study, because low sequencing reads were obtained and prevented us from analysing rare microbial communities, we focused on the core soil microbial community in the network analysis. Since the network analysis is based on the correlation of (relative) abundance, the difference in total sequence read numbers is not a major problem. Therefore, in future research, the combination of 16S rRNA, amoA, and other functional genes with the network analysis also could be adapted to analyse rare microbes with the core microbial communities as used in this study for bacterial-fungal relationships. Although further research investigating the detailed ecology and functions of the detected microbial subgroups is needed, our findings and approaches provide a key for revealing N cycling by microbial communities in forest soil.

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925 Figure captions

926

927 **Fig. 1** Temporal changes of (a) the net nitrification and (b) the mineralisation potentials of Larch, Fir
928 and Natural forests. The values are means and bars represent the standard deviation. FT, M, and
929 F×M represent the results of the two-way ANOVA for forest types, sampling months, and the
930 interaction of forest type and sampling month, respectively. Symbols represent the following: n.s.
931 represents $p \geq 0.05$ and ** represents $p < 0.01$.

932

933 **Fig. 2** Gene abundances of (a) total bacteria, (b) total archaea, (c) total fungi, (d) AOB, and (e) and
934 AOA. The values are means and the bars represent standard deviations. FT, M, and F×M represent
935 the results of the two-way ANOVA for forest types, sampling months, and the interaction of forest
936 type and sampling month, respectively. Symbols represent the following: n.s. represents $p \geq 0.05$ and
937 *** represents $p < 0.001$.

938

939 **Fig. 3** Predicted counts of genes involved in nitrogen degradation and mineralisation from the
940 rarefied 1441 16S rRNA gene sequences. The values are means and the bars represent standard
941 deviations. FT, M, and F×M represent the results of two-way ANOVA for forest types, sampling
942 months, and the interaction of forest type and sampling month, respectively. Symbols represent the
943 following: n.s. represents $p \geq 0.05$ and * represents $p < 0.05$.

944

945 **Fig. 4** The predicted relative abundances of guilds within the symbiotrophic fungal group. The
946 values are means and the bars represent standard deviations. FT, M, and F×M represent the results of
947 the two-way ANOVA for forest types, sampling months, and the interaction of forest type and
948 sampling month, respectively. Symbols represent the following: n.s. represents $p \geq 0.05$ and ***

949 represents $p < 0.001$.

950

951 **Fig. 5** (a) The co-occurrence network divided by modules, (b) the taxa of the operational taxonomic
952 units (OTUs) belonging to each module, and (c) the average relative abundance of each module. The
953 average relative abundances of modules were calculated by summing the average relative
954 abundances of the OTUs belonging to each module.

955

956 **Fig. 6** The operational taxonomic units (OTUs) with the genes responsible for (a) chitinase, (b)
957 NAGase, (c) leucine aminopeptidase, (d) arginase, and (e) urease in the co-occurrence network.

958

959 **Fig. 7** The operational taxonomic units (OTUs) with the genes responsible for each nitrification step:
960 (a) ammonia to hydroxylamine, (b) hydroxylamine to nitrite, and (c) nitrite to nitrate in the
961 co-occurrence network.

962

963 **Fig. 8** The relationships between the nitrogen mineralisation potentials and (a) the relative
964 abundances and (b) the estimated absolute abundances of modules 1 to 6. Values in each cell
965 represent correlation coefficients by Pearson's correlation. Symbols represent the following: n.s., *,
966 **, and *** represent $p \geq 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

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Fig. 1 Nakayama et al.

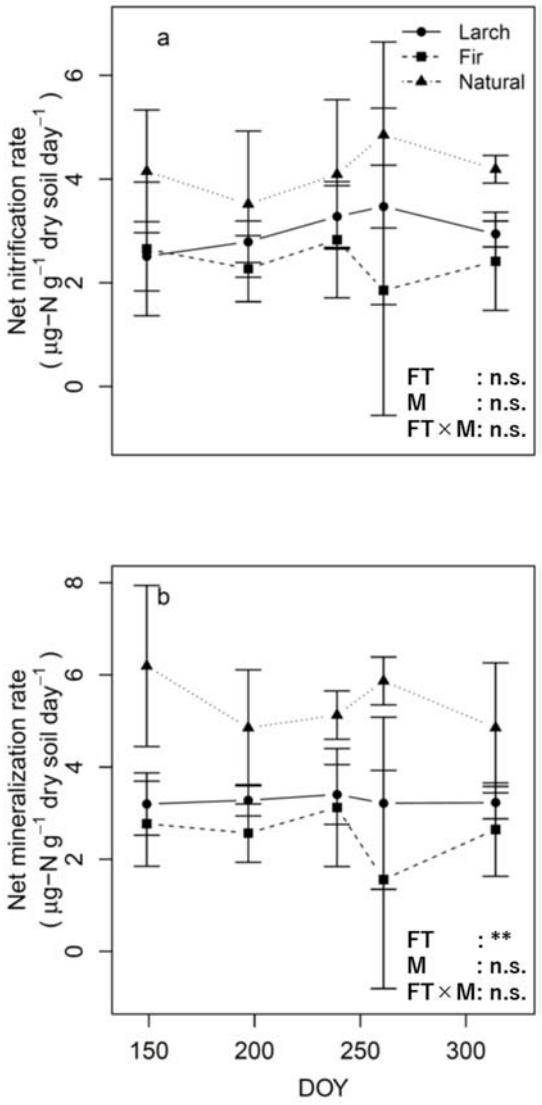
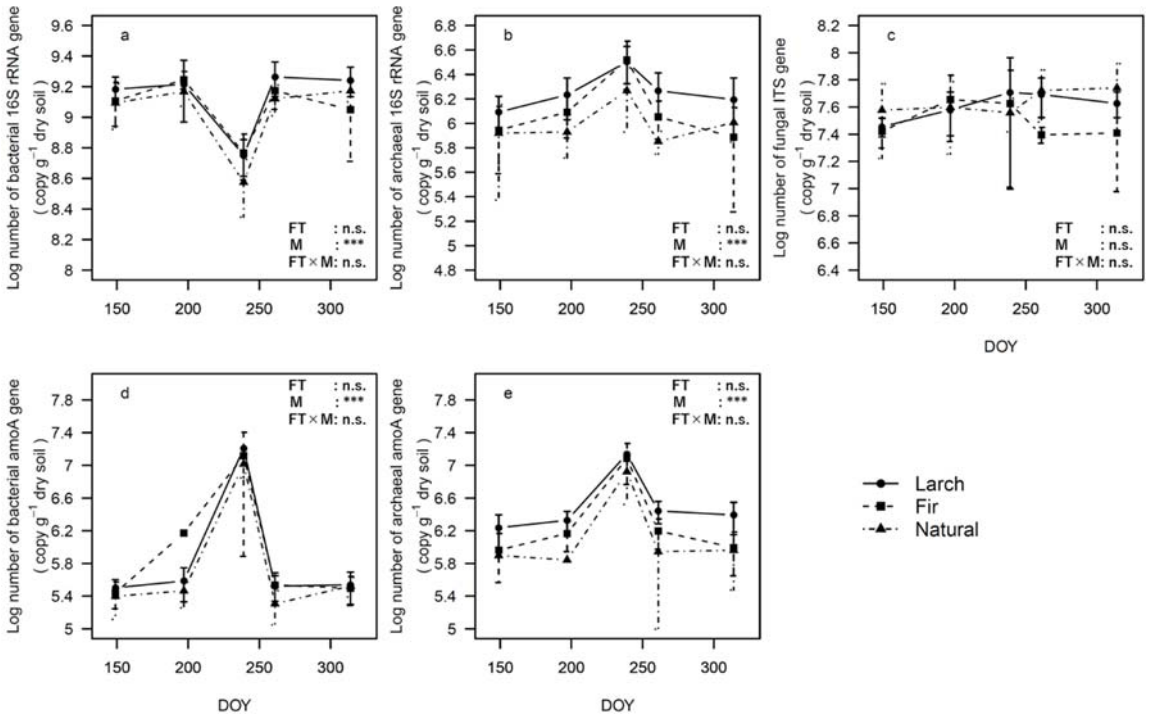


Fig. 2 Nakayama et al.



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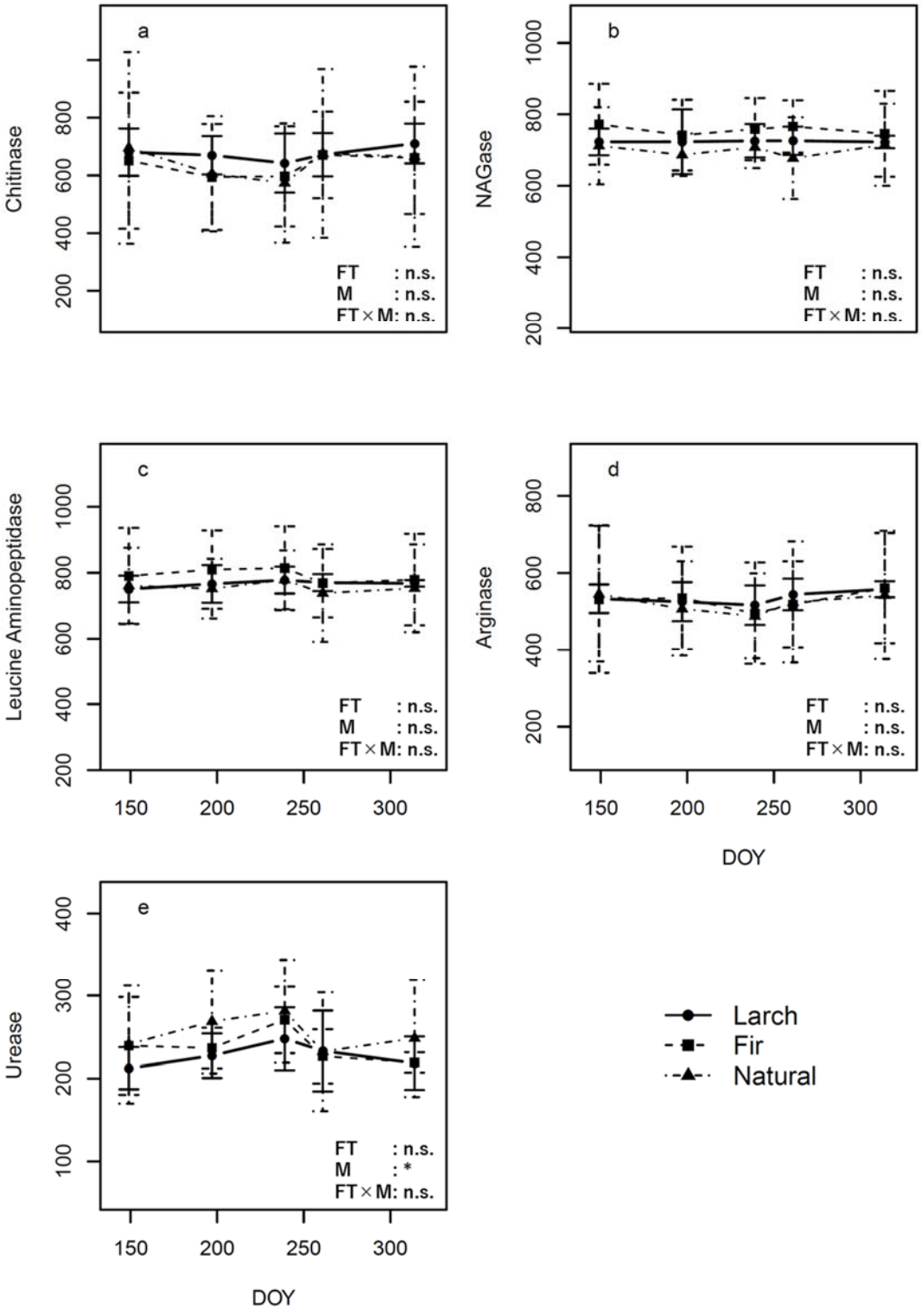

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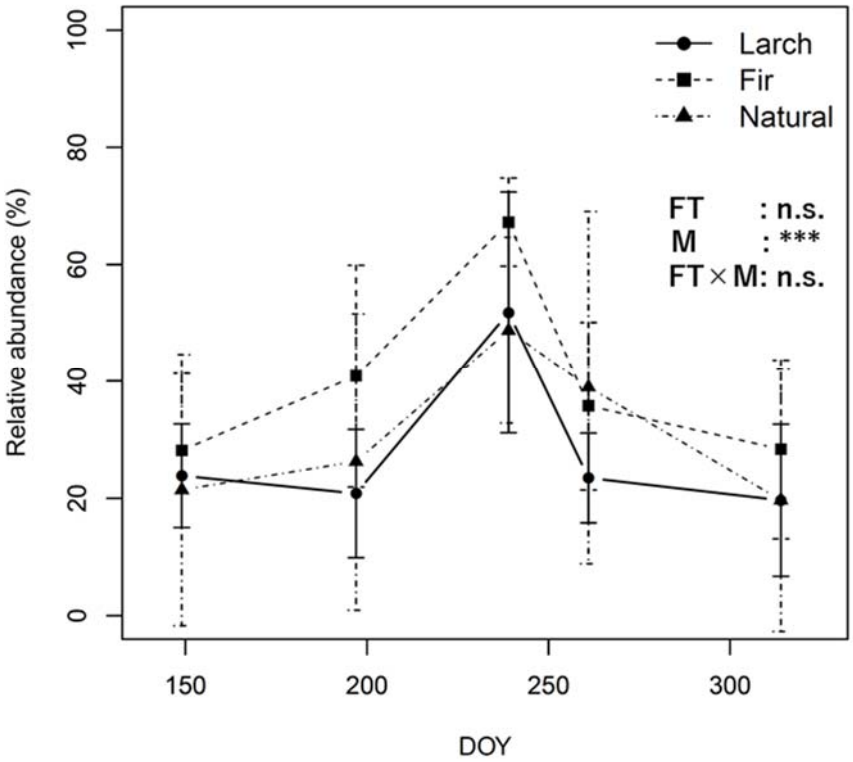

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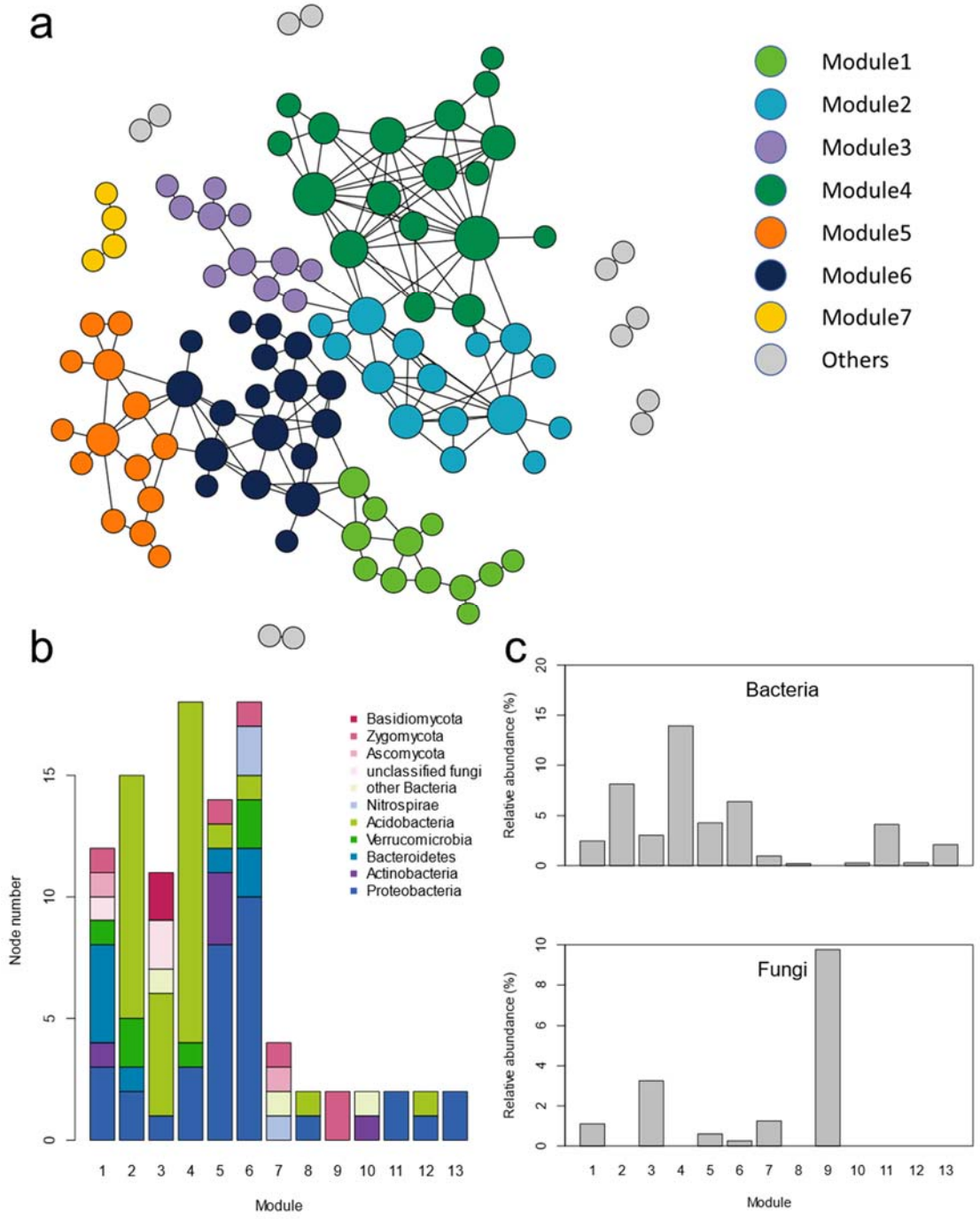

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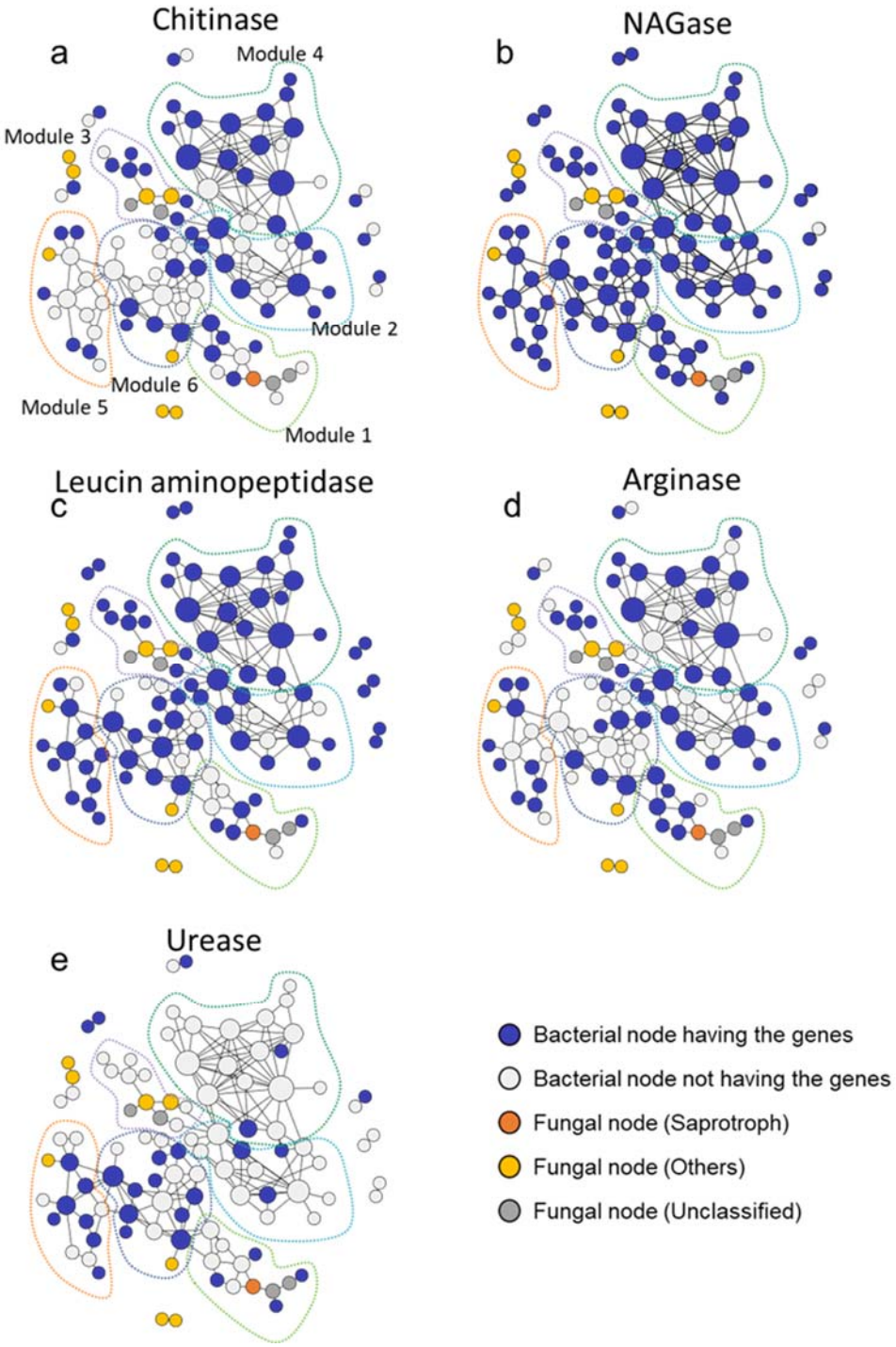

Fig. 7 Nakayama et al.

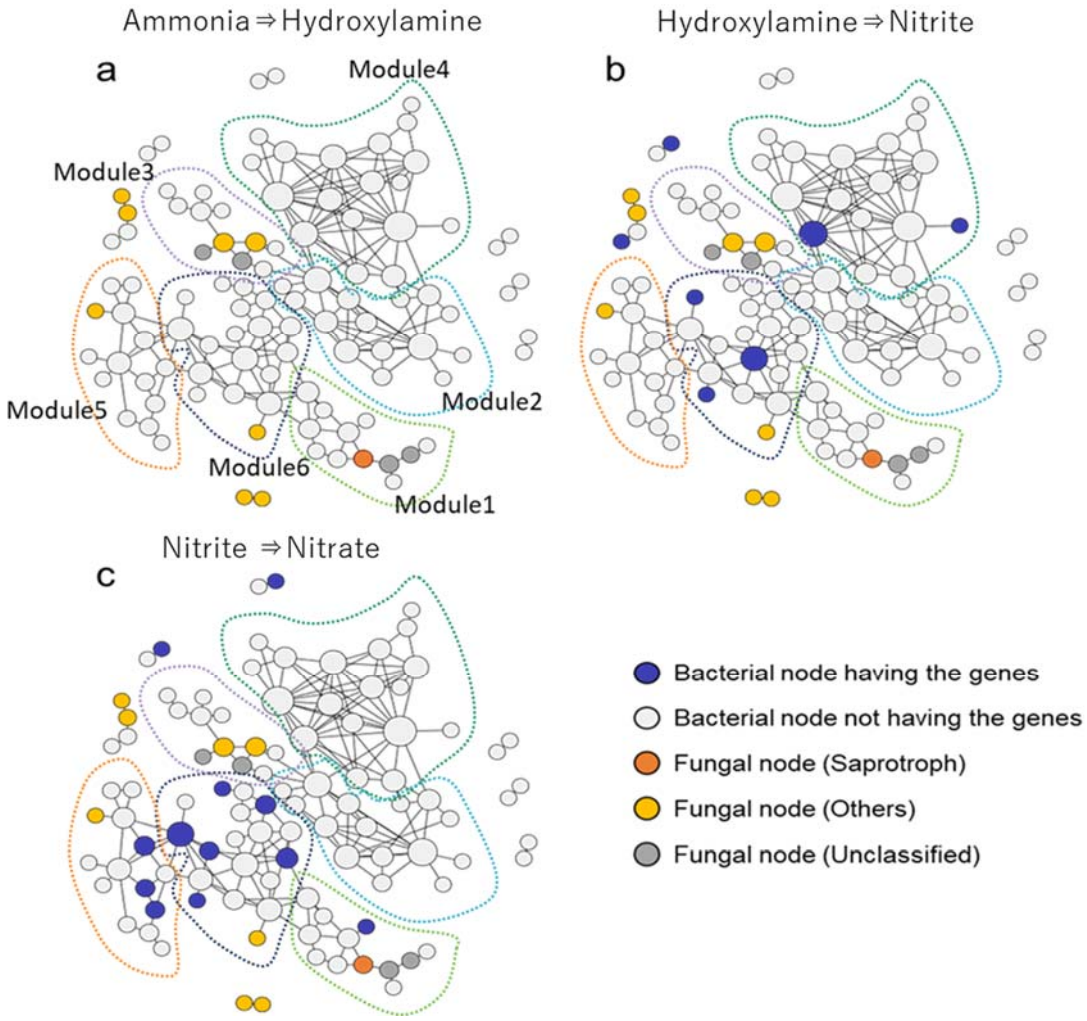

Fig. 8 Nakayama et al.