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Artificial reforestation produces less diverse soil nitrogen-cycling genes than natural restoration

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Abstract. Reforestation is effective in restoring ecosystem functions and enhancing ecosystem services of degraded land. The three most commonly employed reforestation methods of natural reforestation, artificial reforestation with native Masson pine, and introduced slash pine plantations were equally successful in biomass yield in southern China. However, it is not known whether soil ecosystem functions, such as nitrogen (N) cycling, are also successfully restored. Here, we employed a functional microarray to illustrate soil N-cycling. The composition of N-cycling genes in soils varied significantly with reforestation method and varied with constructive species identity and plant diversity. Artificial reforestation had less superior organization of N-cycling genes than natural reforestation, as indicated by the less diverse and less stable pathways to perform the biogeochemical N-cycling processes. Besides, artificial reforestation had lower functional potential (abundance of ammonification, denitrification, assimilatory, and dissimilatory nitrate reduction to ammonium genes) in soils than natural method. Evaluations of the abundance and interactions of N-cycling genes in soils showed that plantations, especially artificial reforestation with slash pine plantations, possessed a smaller range of ecosystem functions that provide a less diverse array of N-related substrates and nutrients to microbial communities compared with natural restoration. This might lead to a lower independence of N-cycling, which indicated a higher risk of N release in plantations. The unfavorable N-cycling conditions in plantations were corroborated by the lower contents of available N, ammonium N, and nitrate N. These findings demonstrate that reforestation methods could have broad regional and possibly global implications for N-cycling.

Key words: artificial restoration; functional potential; genes; natural restoration; network; nitrogen-cycling.

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INTRODUCTION

Reforestation is imperative for restoring ecosystem services of environmentally devastated natural forest lands. Reforestation effectively reverses soil degradation (Chazdon 2008), mitigates the potentially damaging consequences of deforestation leading to climate change (Field and Mach 2017), and provides other ecosystem services (Jayachandran et al. 2017). Restored plantations now cover more than 200 million ha worldwide, 25% of which was accomplished by the introduction

and cultivation of fast-growing tree species that produce harvested timber (FRA 2010). Since the 1970s, China has developed plantation policies and has made considerable progress in reforesting land suffering from excessive deforestation. Consequently, China now leads the world in plantation forest area, with 77.6 million ha in total, 28% of which consists of introduced species (FRA 2010). In central and southern China, naturally reforested secondary forests (NRSF), both artificially reforested native Masson pine (*Pinus massoniana* Lamb., an evergreen pine, ARMP) and introduced slash pine (*Pinus elliottii* Engelm., from southeastern United States, an evergreen pine, ARSP) plantations, are equally successful in biomass yield (Appendix S1: Table S1). Although the natural approach has gained support for its cost-effectiveness and potential high-yield return in ecosystem services (Chazdon 2008), natural forest has dwindled to merely 28% of the forest area nationwide, with mono-species plantations of Masson pine and slash pine accounting for 59% of the plantation area in central and south China (Ren et al. 2007). What will happen to humanity depending ecosystem services, such as fertility maintenance and nutrient cycling, in such large plantation areas?

Nitrogen (N) is the most common limiting nutrient in terrestrial ecosystems (Vitousek et al. 2010). We take N as an example of the nutrient cycling processes in soils. Anthropogenic disturbances have unprecedentedly altered and will continue to alter N transformation in soils (Cui et al. 2013, Moreno-Mateos et al. 2017). Soil N is higher in natural forests than plantations, regardless of stand age, leaf form, leaf seasonality, land-use history, site preparation, or biogeographical zone (Liao et al. 2010). Moreover, ammonium and nitrate contents differed with reforestation methods. However, question remains: Have the N-cycling processes in the underlying soils of restored forests been altered?

Reforestation changed nitrogen-cycling process. Afforestation with *Eucalypts* reduced ammonification and N fixation genes, which correlated with more NH_4^+ as ammonium in plantation soils (Berthrong et al. 2009). Natural reforestation had higher nitrification rate than reforestation with plantations (Paul et al. 2010). Reforestation with different plantation types led to different potential net N mineralization rates. As reforestation method has been shown to influence the abundance and function of N-cycling genes, we are still faced with several questions regarding the impact of reforestation method on N-cycling. What roles would the modes of reforestation play on the diversity, and structure of soil microbial communities that largely determine N-cycling? What roles would the mode of reforestation have on metabolic processes in the affected soil ecosystems, especially on the soil N-cycling potential and the N pool?

The functional genes which address functional potential governing N-cycling processes in the soils of NRSF, indigenous ARMP, and introduced ARSP in southern China would provide valuable insights. We hypothesized that the soil microbial communities of the naturally regenerated secondary forests provided the most favorable environment for compositions of N-cycling genes as the highest quantity and quality of litters.

Materials and Methods

Study area

Artificially reforested slash pine, ARMP, and NRSF from which soil nitrogen-cycling genes were extracted were located in the hilly red soil region of southern China within 24°53′24″–27°19′4.8″ N and 110°9′0″–114°32′56.4″ E. The altitude range was 90–295 m. The climate is subtropical monsoon climate with an annual average temperature of 18.7°C and precipitation is approximately 1600 mm. The soil was Ultisols. The stand age of forests was between 15 and 25 yr. The NRSF have been little disturbed since restoration. After planting, the ARSP and ARMP were cultivated only for the first three to four years, including loosening surface soils and cutting understory vegetation. More stand characteristics including altitude, slope, and vegetation cover were described previously (Wang et al. 2011).

Soil sampling

Fifteen 500 × 500 m soil-sampling plots were randomly selected for each type of forest. Fifteen soil cores 3.5 cm in diameter and of depth 10 cm were collected from each plot, 50 cm from the trunk of the dominant trees, and samples were then combined to create a composite sample. Soil

samples were packed in ice box and transported to the laboratory within 72 h. On arrival, soils were passed through a 2-mm sieve and a half of each sample was stored at −80°C for later molecular analysis and the other half air-dried and stored for biogeochemical analyses. Soil samples were collected in October 2008, August 2009, September 2009 and in Hengyang County in Hunan Province, Anfu County in Jiangxi Province, and Guilin City in Guangxi Province, respectively.

Laboratory analysis

The DNA was extracted from soil specimens using the methods of Peršoh et al. (2008) with the final step modified to be ethanol (reagent grade, Sinopharm) precipitation (Sambrook and Russell 2001). Double distilled water was used instead of diethyl pyrocarbonate-treated water. In total, 14, 15, and 13 DNA samples were extracted for ARSP, ARMP, and NRSF, respectively.

The metagenomic DNA was labeled directly with Cy5 dye, and dissolved in hybridization solution (Wu et al. 2006). After labeling, samples were kept in darkness and hybridized for 10 h at 42°C with a HS4800 Pro hybridization station (Tecan US, Durham, North Carolina, USA). The hybridized GeoChip 3.0 was scanned with a ScanArray 5000 (Perkin-Elmer, Wellesley, Massachusetts, USA) at 95% laser energy and 85% photomultiplier tube gain, and digitally analyzed using ImaGene software (6.0 premium version; Biodiscovery, El Segundo, California, USA).

Statistical analysis

Patterns of N-cycling genes.—Because GeoChip 3.0 is a functional gene microarray, N-cycling genes could be easily identified as indicated by the probe labels. The data were processed as follows: (1) Probes flagged as 1 or 3 by ImaGene and probes with signal-to-noise ratio <2.0 were removed (He et al. 2010); (2) data were normalized firstly within a slide, then among samples, and finally divided by the mean of the signal intensities of all probes (Wu et al. 2006, He et al. 2010); (3) probes appeared less than three times for each reforestation approach were deleted (He et al. 2010); (4) samples with probe numbers outside of mean ± (2 × standard error) were considered outliers (ARMP1, NRSF2, and NRSF7) and removed (Wu et al. 2006). In total, 14, 14, and 11 microarray replicates were obtained for ARSP, ARMP, and NRSF, respectively.

The differences of microbial diversity due to reforestation method (natural, native species, and introduced species restoration) were inferred based on outcomes of one-way analysis of variance (Wu et al. 2013, 2015) followed by Duncan tests (variance homogeneity) or Dunnett tests (variance not heterogeneous) for normal data, or non-parametric test for non-normal data followed by two-tailed Kruskal–Wallis *H* testing. When comparisons were made between two groups, Student's *t*-test was used for normal data, and non-parametric test followed by two-sided Mann–Whitney U testing for non-normal data.

The compositions of functional genes governing N-cycling in forest soils were sorted using detrended correspondence analysis (DCA). The Bray–Curtis distance-based Adonis procedure was used to test whether the N-cycling functional gene compositions were significantly affected by reforestation method. Sample scores on the first axis of the DCA plot were used for correlation with environmental factors using Spearman correlation. The correlations between the abundance of functional genes and environmental factors were examined according to results of Pearson analysis for normal data and Spearman analysis for non-normal data. The normality of the data was checked by Shapiro–Wilk test.

The variance analysis, correlation analysis, and normality test were performed with SPSS 16.0 (SPSS, Chicago, Illinois, USA). The DCA was carried out in CANOCO for Windows 4.5.

Functional molecular ecological network analysis.—We used functional molecular ecological network (fMEN) analyses to illustrate interactions of N-cycling functional genes in forest soils on the pipeline (http://ieg2.ou.edu/MENA, Deng et al. 2012). For soil samples of one forest type, only those genes that appeared in more than half of the specimens were included in analyses. Only genes appeared in at least 11, 11, and eight soil specimens for ARSP, ARMP, and NRSF were used to constructed fMEN, respectively (Appendix S2). Microarray data have been deposited in the National Center for Biotechnology Information (NCBI)'s GSE100379.

Results

Compositions of N-cycling genes

The composition of N-cycling genes shows differences in diversity and relative abundance of respective soil microbial communities involved in N-cycling and would be helpful to deduce the impacts of the reforestation methods employed. The composition of functional genes regulating N-cycling in the soils of the three types of restored forest ecosystems differed significantly ($F < 3.36$, $P < 0.05$, Appendix S1: Table S2). It showed that N-cycling gene composition varied with constructive species identity and plant diversity (Appendix S1: Tables S1, S3), which resulted from reforestation approaches (Figs. 1A, 2). The main factors separating the N-cycling gene compositions of ARMP vs. NRSF were plant diversity (Figs. 1B, 2, Appendix S1: Tables S4, S5) and nutrient availability (Fig. 2, Appendix S1: Table S5). For ARMP vs. ARSP, the main factor was constructive species identity (indigenous vs. exotic; Fig. 1C, Appendix S1: Table S1). For ARSP vs. NRSF, the main factors were constructive species identity (Fig. 1D, Appendix S1: Table S1), plant diversity (Fig. 2, Appendix S1: Tables S3–S5), and nutrient availability (Fig. 2, Table 1). The reforesting approach influenced the composition of N-cycling genes through tree species selection, plant diversity, and nutrient availability.

Interactions of N-cycling genes

To assess potential changes in ecosystem function and understand potential interactions of N-cycling genes, a fMEN was constructed for each of the reforestation strategies (Table 2, Appendix S1: Table S6, and Fig. S1). The soils of ARSP showed eight clusters of functional N-cycling genes (modules, usually regarded as co-occurrence patterns or niches (Williams et al. 2014) able to perform the entire or part of the biogeochemical N-cycling processes, lower than those of ARMP with 15 clusters, which were in turn lower than those of NRSF with 17 clusters (Table 2). The lower modularity for fMENs of the plantations indicated that the biogeochemical processes of N-cycling in these soils were less robust at the ecosystem level and permutations could not be effectively dealt with at local scales compared with natural reforestation (Table 2).

We defined a pathway as the presence of genes in a module with the potential to complete the entire N-cycling process. Microbes from the NRSF soils had 12 pathways to perform the entire N-cycling within the four largest modules, while ARMP and ARSP had nine and 10 pathways, respectively (Table 2, Appendix S1: Fig. S2). Compared with NRSF, ARMP lacked assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium pathways, and denitrification; and ARSP lacked the assimilatory nitrate reduction to ammonium and anammox pathways (Table 2). Due to a lack of functional niches to independently complete N-cycling, these plantations might have low N-cycling potential. This was further indicated by the fact that these two methods of restoration had lower N concentrations compared to the NRSF (Table 1). The N-cycling pathways in plantation soils were less diverse, less stable, and perhaps involved fewer types of soil microbes compared with the NRSF. As an essential but not well-studied part of diversity, the interaction provides insight into the lower ecological stability in plantation soils.

Functional genes of N-cycling vs. soil N

A greater abundance of N-cycling genes reflects relatively greater N-cycling potential in soils (He et al. 2010). There was no significant difference in the abundance of N fixation (process that fixes N_2 to ammonium) genes of *nifH* ($F = 1.77$, $P = 0.185$) and anammox genes (process that converts nitrite and ammonium to N_2) of *hzo* ($\chi^2 = 1.02$, $P = 0.601$) among the reforestation approaches (Fig. 3A, B), indicating that reforestation did not significantly influence the potential of N fixation and anammox. However, reforestation significantly influenced the abundance of genes involved in ammonification, nitrification, assimilatory nitrate reduction to ammonium, dissimilatory nitrate reduction to ammonium, and denitrification ($F > 3.26$, $P < 0.050$, Fig. 3A, B).

Ammonification (microbial process that transforms from organic N to ammonium).—The ARSP soils exhibited a lower abundance of *gdh* (encoding glutamate dehydrogenase) than those of ARMP and NRSF (Fig. 3A, B). This showed that ammonification potential was lower in ARSP than ARMP and NRSF soils.

Nitrification (biological oxidation process that converts ammonia firstly to nitrite, and then to nitrate).—The *amoA* genes (encoding ammonia monooxygenase) were less abundant in soils of

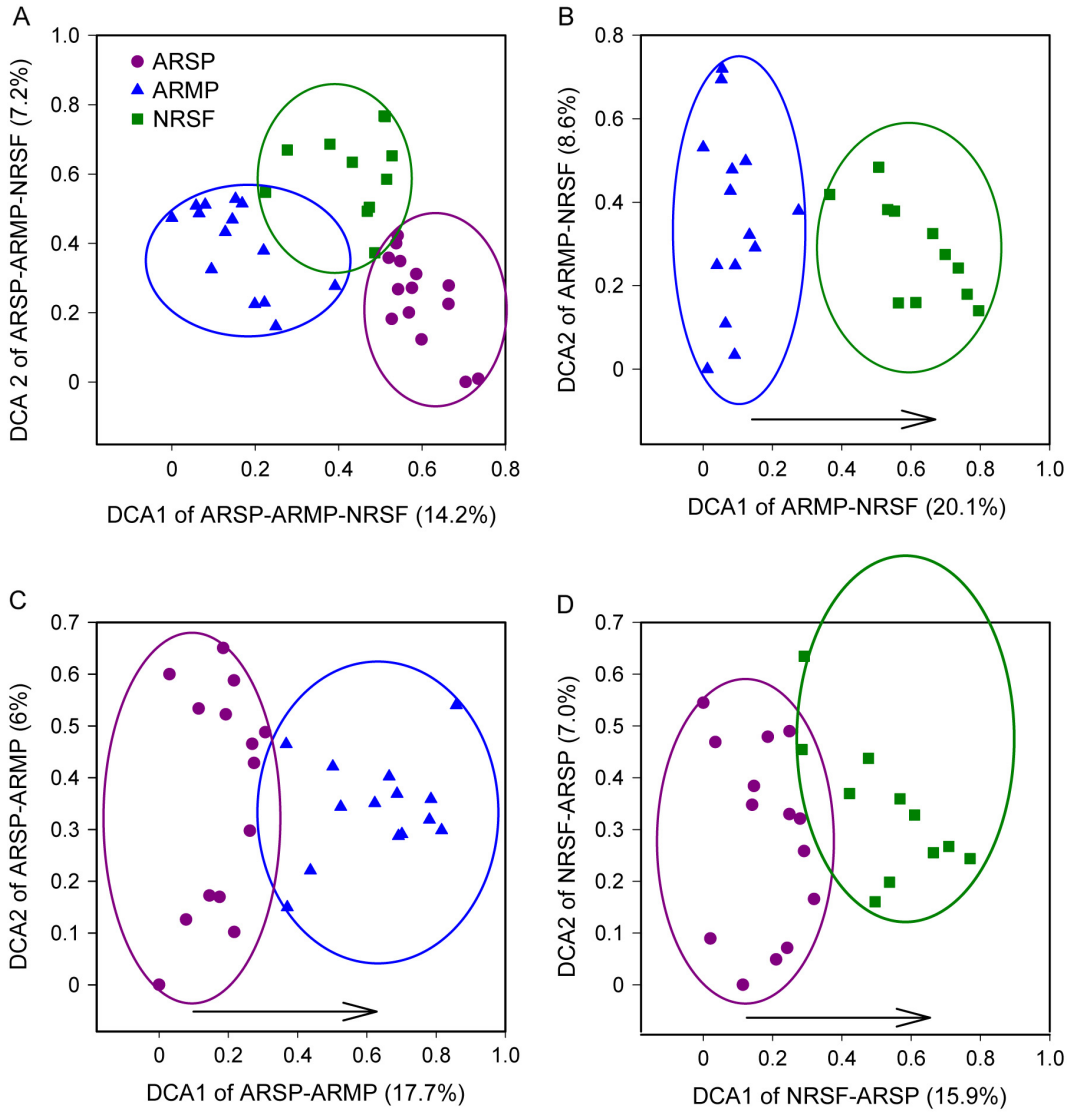

Fig. 1. Distribution of N-cycling functional gene compositions in soils of restored forest stands based on detrended correspondence analyses (DCA). (A) Three reforestation approaches; (B) artificially reforested Masson pine plantations (ARMP) vs. naturally reforested secondary forests (NRSF); (C) ARMP vs. artificially reforested slash pine plantations (ARSP); (D) ARSP vs. NRSF. Fourteen stands of ARSP (pink circles); 14 stands of ARMP (blue triangles); and 11 stands of NRSF (green squares). In part A, there were significant differences in sample scores on the first DCA axis for different reforestation approaches ($\chi^2 = 27.7$, $P = 8.49 \times 10^{-7}$). The arrows in parts B ($t = 12.3$, $P = 4.90 \times 10^{-9}$), C ($t = 8.99$, $P = 1.39 \times 10^{-8}$), and D ($t = 6.72$, $P = 7.37 \times 10^{-7}$) denote significant differences in sample scores of different reforestation approaches on the first DCA axis.

ARSP than those of NRSF, which were in turn less abundant than those of ARMP (Fig. 3A, B). Thus, the ARSP soils exhibited lower potential for nitrification than those of NRSF, which were lower than those of ARMP.

Assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium (ammonium was used for microbial synthesis and released to environment, respectively).—The abundances of assimilatory nitrate reduction to

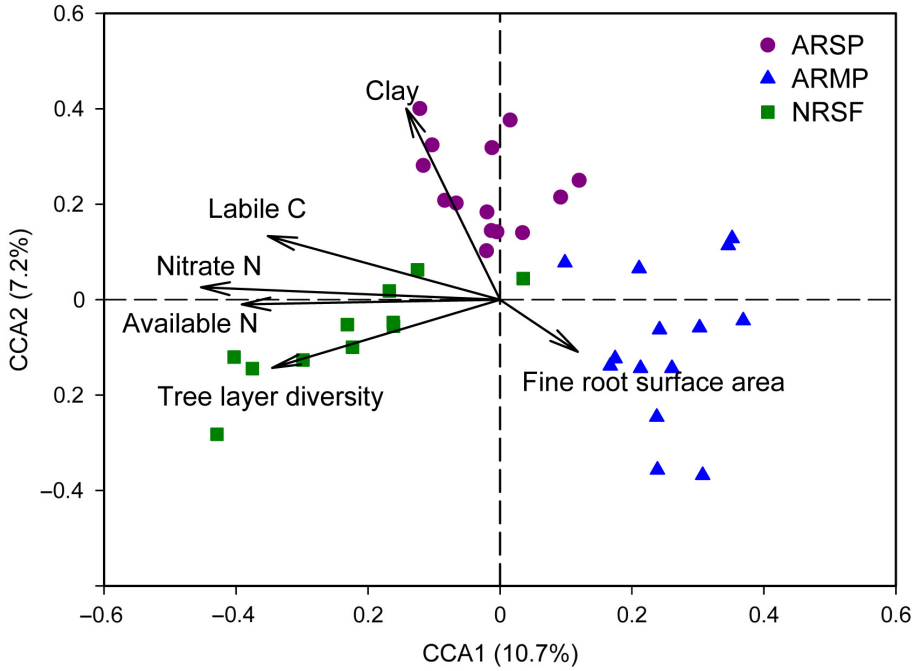

Fig. 2. Biplot of canonical correspondence analysis (CCA) of abundance of N-cycling genes and plant and soil factors. Fourteen stands of artificially reforested slash pine plantations (pink circles), 14 stands of artificially reforested Masson pine plantations (blue triangles), and 11 stands of naturally reforested secondary forests (green squares).

Table 1. Comparisons of N forms of soils in forests restored by three reforestation approaches.

Reforestation approach	Organic N (mg/g)	Nitrate N (μg/g)	Ammonium N (μg/g)	Nitrite N (μg/g)
ARSP	1.40 ± 0.44 b	2.18 (0.84, 5.79) ab	11.4 ± 2.5 b	0.061 ± 0.012 a
ARMP	1.27 ± 0.35 b	1.23 (0.76, 1.68) b	12.0 ± 2.9 b	0.0500 (0.045 ,0.0527) b
NRSF	2.26 ± 0.63 a	4.48 ± 2.07 a	18.2 ± 5.6 a	0.0501 (0.049, 0.0591) ab

Notes: Mean ± standard deviation is shown for normal and approximately normal data. Median (25% quartile, 75% quartile) is shown for non-normal data. ARSP, artificially reforested slash pine plantations; ARMP, artificially reforested Masson pine plantations; and NRSF, naturally reforested secondary forests. Different letters attached to the numerals within a column denote that the indicated N content significantly differs between forest types at $P < 0.05$. Organic N ($F = 15.2$, $P = 1.60 \times 10^{-5}$); nitrate N ($\chi^2 = 9.74$, $P = 0.008$); ammonium N ($F = 12.2$ $P = 9.06 \times 10^{-5}$); nitrite N ($\chi^2 = 7.58$, $P = 0.023$).

ammonium (*nasA* and *nirA*/*nirB*) and dissimilatory nitrate reduction to ammonium (*napA* and *nrfA*) genes in ARSP soils were lower than those of ARMP, which were lower than those of NRSF (Fig. 3A, B). Thus, soils of ARSP and NRSF exhibited the lowest and the highest potential for assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium, respectively.

Denitrification (reductive process that converts nitrate firstly to nitrite, then nitric oxide, nitrous oxide, and finally to nitrogen).—The soils of ARSP showed lower abundance of *narG* (encoding nitrate reductase) than those of ARMP, which were in turn lower than those of NRSF. The soils of ARSP showed lower abundance of *nirS* (encoding nitrite reductase) than those of NRSF, which were lower than those of ARMP (Fig. 3A, B). These indicated that ARSP had the lowest potential of denitrification. There was no significant difference in the potential of nitric oxide and nitrous oxide reduction among the three reforestation approaches, as indicated by the non-significant difference in abundances of *norB* (encoding nitric oxide reductase, $F = 1.95$, $P = 0.157$) and *nosZ* (encoding nitrous oxide reductase, $F = 2.09$, $P = 0.139$), respectively (Fig. 3A, B).

Table 2. Topological properties of molecular ecological networks of N-cycling genes in soils of forests restored using three approaches.

Reforestation approach	Node†	Similarity threshold‡	Module number	Modularity§	Pathway number	Lacked pathways¶
ARSP	149	0.900	8	0.156 ± 0.005 c	10	Dissimilatory nitrate reduction to ammonium, anammox
ARMP	192	0.880	15	0.233 ± 0.005 b	9	Assimilatory and dissimilatory nitrate reduction to ammonium, denitrification
NRSF	203	0.870	17	0.341 ± 0.007 a	12	None

Notes: ARSP, artificially reforested slash pine plantations (n = 14); ARMP, artificially reforested Masson pine plantations (n = 14); and NRSF, naturally reforested secondary forests (n = 11).

† Number of functional genes in the molecular ecological network.

‡ Similarity threshold, the value for transforming similarity matrix to neighboring matrix.

§ If the letters following the numerals differ, the values of the indicated network parameter significantly ($P < 0.05$) differ among the soils of the three forest types.

¶ Information from the four prominent modules compared with NRSF. Modularity, $F = 3331.6$, $P < 0.001$.

There were significant correlations between the abundance or diversity of N-cycling genes and concentrations of different N forms that acted as either products or starting metabolites for enzymes encoded by corresponding genes. For example, the abundance of *amoA* genes was significantly correlated with nitrate N concentration as revealed by the Mantel test ($r = 0.107$, $P = 0.023$); the diversity of assimilatory nitrate reduction to ammonium genes (*nasA*) was significantly correlated with nitrate N concentration (Spearman correlation, $r = 0.500$, $P = 0.001$), which is the starting metabolite and driver of nitrification.

Discussion

The changes in the abundance of N-cycling genes resulting from reforestation have an important impact on N-cycling and maintenance. The lowest abundance of ammonification genes, organic N, and ammonium N levels (Fig. 3) indicated that the potential for ammonification, which is often regarded as a regulator of N availability in plants (Yang et al. 2017), was the least robust in the ARSP. Consistently, with the conversion of natural systems to artificial systems, both the abundances of ammonification genes (Berthrong et al. 2009) and ammonification rate in soils are significantly reduced. As nitrifying microorganisms reduce nitrate (Kessel et al. 2015), the lower abundance of *amoA* genes in ARSP might lead to the lower nitrate concentrations (Fig. 3, Table 1). Because N in assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium was transformed throughout the N cycle (Fig. 3A) to a less mobile form of ammonium (Yang et al. 2017), the lower abundance of assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium genes might lead to lower nitrate and ammonium N contents in ARSP and ARMP than in NRSF soils (Fig. 3, Table 1). The lowest abundance of assimilatory nitrate reduction to ammonium and dissimilatory nitrate reduction to ammonium genes also increased the risk of nitrite leaching in ARSP plantations, further aggravated by its highest nitrite concentrations (Table 1) and lack of genes downstream of *narG* in module three (Appendix S1: Fig. S2). In addition to the lower abundance of assimilatory nitrate reduction to ammonium genes, the lack of assimilatory nitrate reduction to ammonium, dissimilatory nitrate reduction to ammonium and the denitrification pathways, and denitrification genes in module three increased the risk of nitrate leaching in ARMP (Table 2, Appendix S1: Fig. S2). The higher runoff in plantations compared with NRSF (Zheng et al. 2008) further increased the risk of N leaching. The reductions in abundance of N-cycling genes reduced the potential of ammonification, nitrification, ANRA, DNRA, and denitrification in plantations. These changes might reduce contents of ammonium and nitrate in plantation soils, especially in ARSP.

The N-cycling potential was affected by the reforestation approach. These changes in potential were driven by complex interactions among the N-cycling genes and soil N quality. Artificial plantations, especially ARSP, had a lower abundance of functional genes for ammonification, ANRA, DNRA, and denitrification, and less pathways of

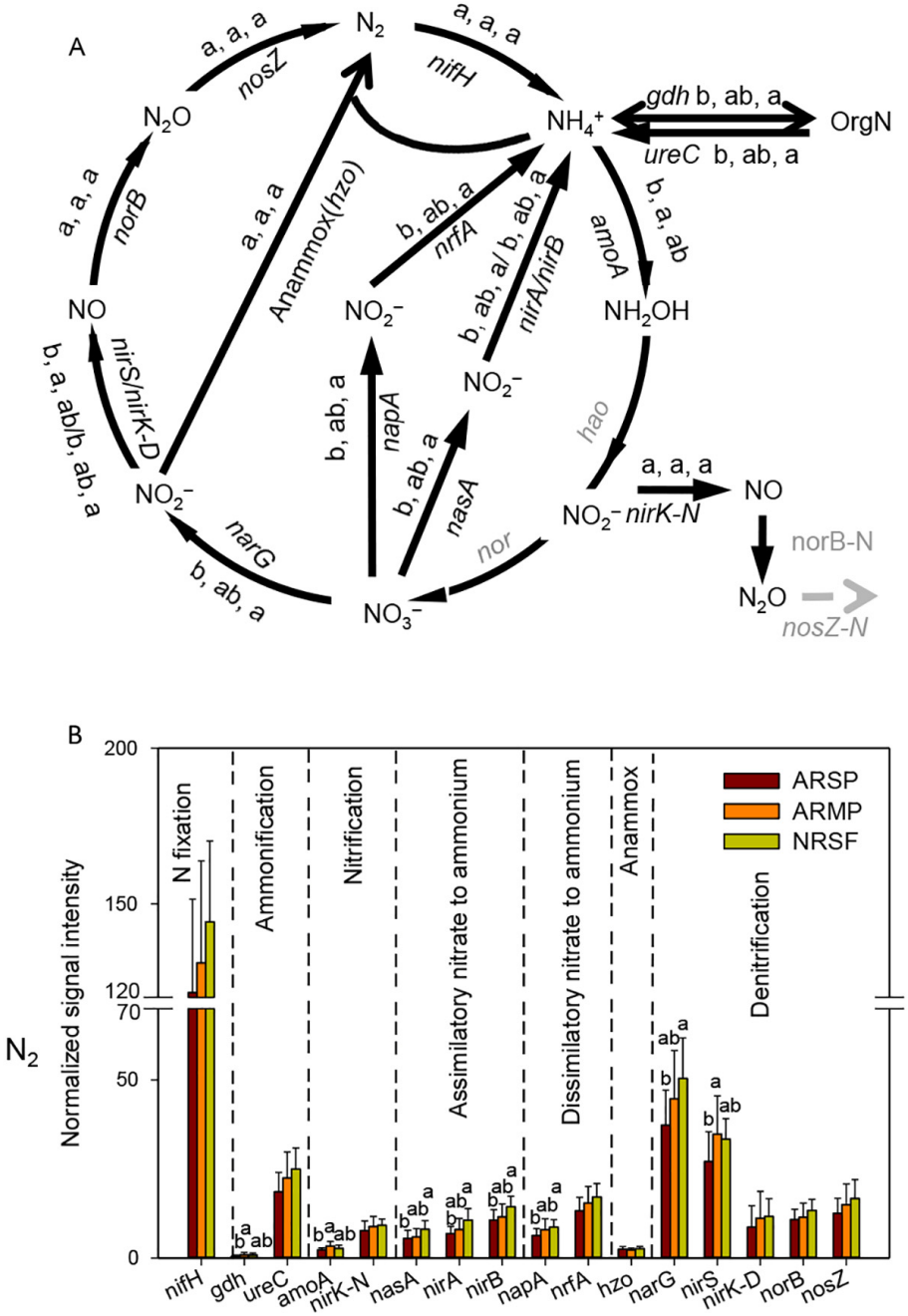

Fig. 3. Relative (A) and normalized signal intensity (B) of N-cycling functional genes in soils of artificially reforested slash pine plantations (ARSP), artificially reforested Masson pine plantations (ARMP), and naturally reforested secondary forests (NRSF). The comparisons were made according to outcomes of Duncan tests at $P < 0.05$; the letters a and b lined up from left to right adjacent to respective genes denote differences of abundance levels of ARSP ($n = 14$), ARMP ($n = 14$), and NRSF ($n = 11$); forest types represented by different letters indicate a significant difference in gene abundances between corresponding soils. Black genes without letters indicate no significant difference among soils of different forest types. Gray genes are not included in microarrays. The data for the columns and error bars are means and standard deviations. *nirK-N* and *nirK-D* are *nirk*

(Fig. 3. *Continued*)
genes involved in nitrification and denitrification, respectively. ARSP, red columns; ARMP, orange columns; and NRSF, yellow columns. *nifH* ($F = 1.77$, $P = 0.185$), *gdh* ($F = 4.70$, $P = 0.015$), *ureC* ($F = 1.14$, $P = 0.331$), *amoA* ($F = 4.27$, $P = 0.022$), *nirK-N* ($F = 1.16$, $P = 0.325$), *nasA* ($F = 4.07$, $P = 0.025$), *nirA* ($F = 5.50$, $P = 0.008$), *nirB* ($F = 4.47$, $P = 0.018$), *napA* ($F = 3.88$, $P = 0.030$), *nrfA* ($F = 2.76$, $P = 0.077$), *hzo* ($\chi^2 = 1.02$, $P = 0.601$, ARSP, median (quartiles) (2.28, (1.83, 3.15)), *narG* ($F = 3.91$, $P = 0.029$), *nirS* ($F = 3.03$, $P = 0.061$), *nirk-D* ($F = 2.92$, $P = 0.067$), *norB* ($F = 1.95$, $P = 0.157$), *nosZ* ($F = 2.09$, $P = 0.139$).

N metabolism within modules (niches) than natural restoration. Evaluations of the abundance and interactions of N-cycling genes in soils showed that plantations, especially ARSP, possessed a smaller range of ecosystem functions that provide a less diverse array of N-related substrates and nutrients to microbial communities compared with natural restoration. This might lead to a lower independence of N-cycling, which indicated a higher risk of N release (Wakelin et al. 2013) either leached as nitrate or emitted as the greenhouse gas nitrous oxide, thus reducing ecosystem stability and diversity in plantation soils (Eisenhauer et al. 2012). In comparison, natural reforestation was more dependent on internally closed N-cycling, with a lower risk of N leakage, and was less vulnerable to environmental changes or ecological disturbances.

The unfavorable N-cycling conditions in plantations were corroborated by the lower concentrations of available N, ammonium N, and nitrate N, which are the main forms of N assimilated by plant (Ashton et al. 2010), providing critical insight into N maintenance and N-cycling of life supporting systems. Due to changes in composition and interactions of N-cycling genes, N-cycling in plantation soils would differ from those in NRSF soil ecosystems. The robust soil microbial ecosystem supported by diverse sources of substrates and nutrients in NRSF resulted in a more stable soil N pool and a far superior N-cycling potential than plantations of either native or introduced tree species. The introduction of plantations significantly changes the aboveground plant community composition, reduces plant diversity, which in turn reduces N-cycling gene diversity and interactions, and consequently reduces N availability, particularly for introduced tree plantations. The reduction of ammonification, nitrification, assimilatory nitrate reduction to ammonium, and dissimilatory nitrate reduction to ammonium potential and thus available ammonium and nitrate N concentrations in plantations probably lead to progressive N limitation (Li et al. 2014), lower carbon use efficiency (Li et al. 2014), thereby reducing soil fertility and productivity, carbon sequestration, and even overall ecosystem function in the long term (Li et al. 2014, Wu et al. 2016). These findings demonstrate that reforestation methods could have broad regional and possibly global implications for N-cycling.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2562/full