Trends in soil microbial communities during secondary succession

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ABSTRACT

Succession, a central theme throughout the history of ecology, has been well studied predominantly in plant communities, but the general trends in soil microbial communities during succession remain unclear. Here, we compiled a comprehensive data set of 85 age sequences worldwide with the aims to (1) examine the trends in soil microbial composition, bioenergetics, and activity during secondary succession, and (2) explore their coordinating changes with soil properties. The results showed that the fungi to bacteria ratio (*fungi:bacteria*) increased, while the microbial respiration per unit biomass carbon (R/C_{mic}) decreased as the succession proceeds. Secondary succession had the rising trends in microbial biomass carbon to soil carbon ratio (C_{mic}/C_{soil}) and microbial biomass nitrogen to soil nitrogen ratio (N_{mic}/N_{soil}). These successional trends in microbes were coincident with the macro-ecological succession theory in plants and animals. Specifically, early successional stages tended to be dominated by *r*-strategists (bacteria) that had higher R/C_{mic} and lower C_{mic}/C_{soil} and N_{mic}/N_{soil}, whereas late successional stages tended to be dominated by *K*-strategists (fungi) that behaved oppositely. The soil C to N ratio ($C:N_{soil}$) increased significantly with the successional stage, with a fast increasing $C:N_{soil}$ ratio being accompanied by a fast increase of *fungi:bacteria*, a slow decrease of R/C_{mic}, and a slow increase of C_{mic}/C_{soil}. This result suggests that the stoichiometry theory may provide a feasible approach to explain the divergent successional trends in microbial communities. In conclusion, our global synthesis highlights the application of the existing macro-ecological theory to soil microbial ecology studies.

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1. Introduction

Secondary succession is the process of reestablishment of a reasonable facsimile of the original community after a disturbance (Horn, 1974). Currently, three-quarters of Earth's ice-free terrestrial biomes are being disturbed by humans (Ellis, 2011). Meanwhile, many degraded areas are under natural or/and anthropogenic recovery state (Watson et al., 2014). Secondary succession is consequently becoming the primary focus of terrestrial natural resource management under projected global land-use changes (Sitzia et al., 2010). Generally, investigations of successional dynamics have been a central theme of plant community ecology (Odum, 1969; Bardgett et al., 2005; Kardol et al., 2006; Prach and Walker, 2011). Soil microbes, possessing enormous physiological and functional versatility, are ubiquitous and vital to the biogeochemical cycling processes in terrestrial ecosystems, including plant nutrient acquisition, litter decomposition, soil organic matter

transformations, carbon (C) cycling, and nutrient availability (Kardol et al., 2006; Prosser et al., 2007; Serna-Chavez et al., 2013; Freedman and Zak, 2015; Soudzilovskaia et al., 2015; Schimel, 2016; Allison and Goulden, 2017; Xu et al., 2017). However, the general trends in soil microbial communities during secondary succession remain unclear.

Numerous individual studies have examined the successional patterns of microbial community composition, bioenergetics, and activity during secondary succession. However, their changing directions and rates remain controversial. For example, based on a forest chronosequence of open-pit mine reclamation soils, Insam and Domsch (1988) found that both the microbial C to soil organic C ratio (C_{mic}/C_{soil}) and the microbial respiration to biomass C ratio (R/C_{mic}) decreased as succession proceeds. However, Jangid et al. (2010) demonstrated increasing trends in both R/C_{mic} and C_{mic}/C_{soil} during tallgrass prairie restoration. Moreover, Jia et al. (2010) found a significant positive trend in the fungi to bacteria ratio (*fungi:bacteria*) during the natural succession from the abandoned agricultural land on the semi-arid Loess Plateau in China, while Kuramae et al. (2010) reported a negative trend in the

fungi:bacteria across chronosequences of chalk grasslands after the arable field being abandoned for 66 years in Limburg, the Netherlands. Therefore, it is imperative to synthesize the diverse results among various studies in order to explore general trends in soil microbial communities during post-disturbance succession.

Understanding microbiology ecology is arguably one of the most compelling challenges facing contemporary ecology, while application of the concept of the existing macro-ecological theory may provide a feasible approach to microbial ecology studies (Bardgett et al., 2005; Fierer et al., 2007, 2010; Prosser et al., 2007; Stegen et al., 2016; Shoemaker et al., 2017). According to the Odum's (1969) theory on ecosystem succession, *r*-strategy species with a faster growth rate, higher turnover rate, and smaller body size predominate in early successional stages, while *K*-strategy species with a slower growth rate, lower turnover rate, and larger body size predominate in mature stages. In microbial ecology, Fierer et al. (2007) suggested that microbial groups could be differentiated into copiotrophic and oligotrophic categories in correspondence to the *r*-strategist and *K*-strategist, respectively. Broadly speaking, fungi are regarded as *K*-strategists, whereas bacteria are considered as *r*-strategists (Bardgett et al., 2005; Kaiser et al., 2014; Zechmeister-Boltenstern et al., 2015; Chen et al., 2016). Meanwhile, within bacteria, Gram-positive bacteria, bacterial phyla of *Acidobacteria* and *Actinobacteria* are within the oligotrophic category, while Gram-negative bacteria, *Proteobacteria* and *Bacteroidetes* are within the copiotrophic category (Fierer et al., 2007; Zechmeister-Boltenstern et al., 2015). Therefore, we expect that the ratio of *K*-strategists to *r*-strategists would increase with successional stage. Another concept of the Odum's (1969) theory states that the ratio of total community respiration to biomass decreases, but the biomass supported per unit energy or resource increases as succession proceeds. Similar to this concept in microbial ecology, two corresponding parameters are proposed (Anderson, 2003; Anderson and Domsch, 1990, 2010), i.e., microbial community respiration to biomass ratio (R/C_{mic}) and microbial biomass C to soil organic C ratio (C_{mic}/C_{soil}). To link the micro-with macro-scales, we hypothesized that the R/C_{mic} would decrease but the C_{mic}/C_{soil} increase with ecosystem successional stage globally.

Besides, we are also interested in whether changes in microbial composition and bioenergetics are associated with changes in enzyme activity and soil properties during secondary succession. Specifically, microbial extracellular enzyme production depends much on microbial community composition (Cusack et al., 2011; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). So we expect that changes in microbial community composition would have significant effects on extracellular enzyme activity during secondary succession. In addition, soil properties (e.g., soil resource quality and quantity, soil pH, and soil moisture) remarkably influence microbial biomass, stoichiometry, community compositions, extracellular enzyme production, and activity (Xu et al., 2014, 2017; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015; Zhou and Wang, 2015; Zhou et al., 2017), but little is known about their coordinating patterns as secondary succession proceeds.

In this study, our goals are to (1) examine the trends in microbial composition, bioenergetics, and activity during secondary succession; and (2) explore their coordinating changes with soil properties. With these expected results, we test if the theory of macro-ecological succession can be applied to soil microbial studies. Specifically, we hypothesized that the ratio of *K*-strategists to *r*-strategists would increase, the ratio of microbial respiration to biomass would decrease, and the ratio of microbial biomass to soil resource would increase as secondary succession proceeds.

2. Materials and methods

2.1. Literature synthesis

Literature searches were conducted using ISI Web of Science database (http://apps.webofknowledge.com), Google Scholar (https://scholar.google.com), and China National Knowledge Infrastructure (CNKI, http://www.cnki.net) with the key words of "succession or recovery or restoration or development or age sequences or chronosequences" and "microbial biomass or microbial community or extracellular enzyme or enzyme or microbial respiration or qCO_2 or fungi or bacteria." Up to December 2016, 72 published experimental studies reported the composition, bioenergetics, and activity of soil microbial communities during secondary succession, including 85 age sequences in total (Fig. 1; Text S1) that met the following two criteria: (1) each age sequence should include at least four age stages for detecting the statistical trends of these variables along the succession gradient; and (2) each age sequence should contain quantitative information about successional stage (year). All average values were obtained from the tables or extracted from the figures with the Origin7.0 digital plugin (Digitize) (OriginLab Ltd., USA).

The compiled database included: (1) soil properties: soil water content, soil bulk density, soil pH, soil organic C (C_{soil}), soil total nitrogen (N_{soil}), soil organic C to total N ratio ($C:N_{soil}$), and soil available N; (2) microbial biomass and composition: microbial biomass C (C_{mic}), microbial biomass N (N_{mic}), microbial biomass C to N ratio ($C:N_{mic}$), fungi to bacteria ratio (*fungi:bacteria*), Gram-positive bacteria to Gram-negative bacteria ratio, the abundance of arbuscular mycorrhizal fungi, bacterial richness (Operational Taxonomic Units, OTUs), and *Acidobacteria* + *Actinobacteria* to *Proteobacteria* + *Bacteroidetes* ratio; (3) microbial bioenergetics and activities: microbial C to soil C ratio (C_{mic}/C_{soil}), microbial N to soil N ratio (N_{mic}/N_{soil}), N mineralization, microbial respiration (the amount of CO_2 produced during laboratory incubations of soil in the absence of carbon or nutrient additions), microbial respiration to biomass ratio (R/C_{mic}). If microbial biomass was determined by phospholipid fatty acid method, we used the conversion factor of 2.4 to calculate microbial C (1 nmol of phospholipid fatty acid corresponded to a flush of 2.4 μg C released by fumigation; Rinklebe and Langer, 2010). And (4) we also collected the most widely assayed extracellular enzymes: oxidase, hydrolase, and invertase. The term 'oxidase' referred to the sum of phenol oxidase and peroxidase; and the term 'hydrolase' referred to the sum of β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase, and phosphatase in this study. Extracellular enzymatic efficiency was calculated as extracellular enzyme activity normalized to per unit microbial biomass C (Allison et al., 2007), i.e., oxidase activity to microbial C ratio, hydrolase activity to microbial C ratio, and invertase activity to microbial C ratio, respectively.

To maximize the comparability, we (1) categorized the data into two types of ecosystems, i.e., forests and grasslands; (2) categorized the 85 age sequences into short-term (<25 year), medium-term (25–50 year), and long-term succession (>50 year) based the maximum age of the corresponding sequence; and (3) categorized the sequences into different types of disturbances by the starting point of the secondary succession, i.e., mining, fire, cultivation, and harvest sites.

2.2. Data analysis

Changes in soil C during secondary succession are expected to exhibit an initial decrease and a subsequent increase, which can be characterized with a gamma function (Covington, 1981; Zak et al., 1990). However, we did not find the initial decrease stages for the
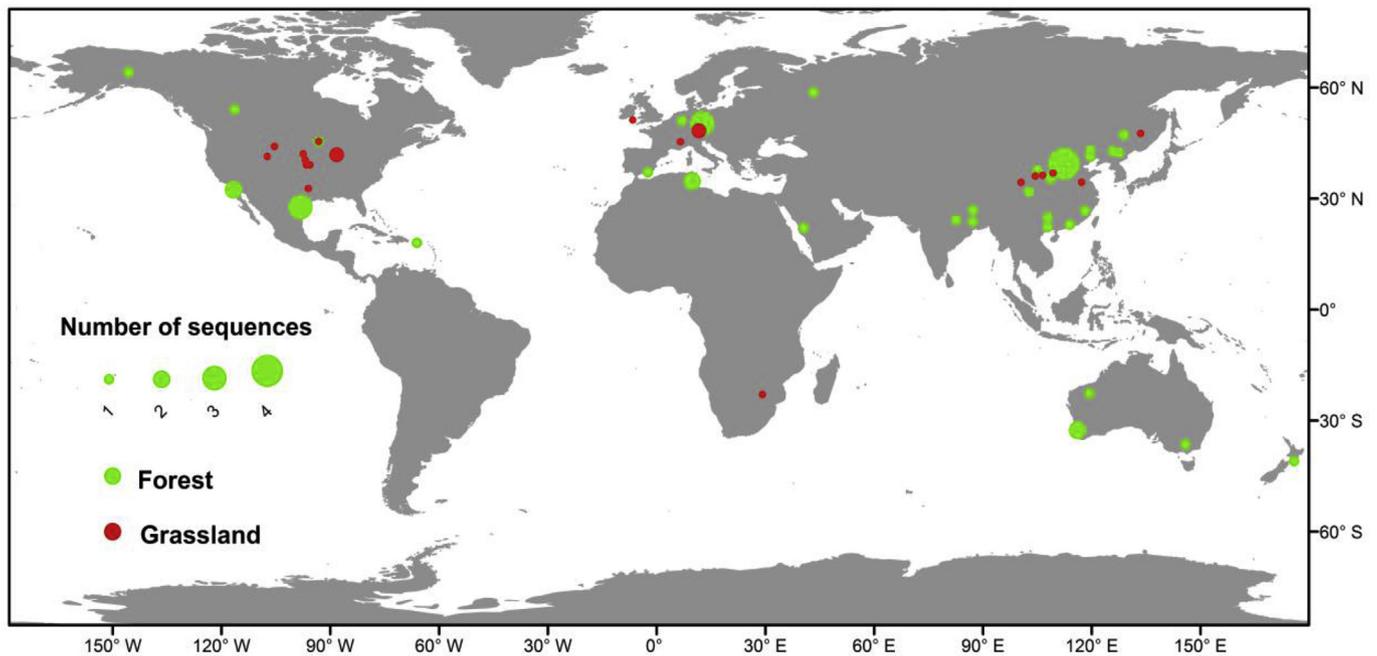
Fig. 1. Distribution of the data points used in this study.

soil- and microbial-related variables with successional stage in most cases, similar to the previous synthesis (Yang and Luo, 2011). In addition, microbe-related variables did not show the initial decrease stages despite the fact that soil C and N displayed a Covington curve (Zak et al., 1990). Therefore, to integrate the general trends in soil microbe-related variables during secondary succession, we first examined the linear relationship between the natural logarithm transformed (ln) microbe-related variables and ln(successional stage) ('successional stage + 1' in order to remove ln 'zero') within each sequence, *i.e.*, a linear ln–ln function was used to examine the relationship between soil- and microbe-related variables and successional stage (equation (1)):

$$\ln(y) = a + b(\ln(x + 1)) \tag{1}$$

where x is successional age (year), y is soil- and microbe-related variable, a is the intercept and b is the slope.

Next, following the methodology of meta-analysis, we calculated the weighted mean slopes (slope$_{++}$) across all individual sequences as following:

$$\text{Slope}_{++} = \frac{\sum_{i=1}^{n} \text{slope}_i \times w_i}{\sum_{i=1}^{n} w_i} \tag{2}$$

where n is the number of the age sequences; and w_i (the weighting factor) was estimated as:

$$w_i = \frac{1}{v} \tag{3}$$

where v is the variance of the slope.

When the number of data points used for assessing slope$_{++}$ of a variable investigated was lower than 20, we used bootstrapping method to obtain the lowest and highest 2.5% values as our bootstrap confidence based on 5000 iterations. If that number was larger than 20, 95% confidence interval (CI) was calculated as:

$$95\% \, \text{CI} = \text{slope}_{++} \pm 1.96s(\text{slope}_{++}) \tag{4}$$

If the 95% CI of the slope$_{++}$ covered zero, the variable was considered to be relatively stable during the succession; else if the slope$_{++}$ was higher than zero, the variable was considered to increase significantly, otherwise to decrease significantly as the succession proceeds. Meta-analysis was conducted with MetaWin 2.1 software (Sinauer Associates Inc., Sunderland, MA, USA; Rosenberg et al., 2000).

3. Results

3.1. Successional trends in soil properties

The successional dynamics in soil properties showed remarkable variability among individual studies (Figs. 2 and S1). The weighted mean slope (slope$_{++}$) of soil water content *vs.* successional stage was significantly higher than zero. Also, the slope$_{++}$ of soil organic C, soil total N, soil C to N ratio, and soil available N all significantly increased as the succession proceeds (Fig. 2). However, the slope$_{++}$ of soil bulk density and soil pH significantly decreased with the secondary succession (Fig. 2).

3.2. Successional trends in microbial community

Secondary succession significantly increased microbial biomass C and N, and the abundance of arbuscular mycorrhizal fungi (Fig. 3A). The slope$_{++}$ of *fungi:bacteria vs.* successional stage was significantly higher than zero. However, the slope$_{++}$ of microbial C:N, Gram-positive bacteria to Gram-negative bacteria ratio, *Acidobacteria + Actinobacteria* to *Proteobacteria + Bacteroidetes* ratio, and bacterial OTU richness were insignificant from zero (Fig. 3A).

Further analysis by ecosystem types showed that the slope$_{++}$ of *fungi:bacteria* was significantly higher than zero in forest rather than grassland (Fig. 4A). The short-term (<25 year) and medium-term sequences (25–50 year) had significant positive trends in the *fungi:bacteria*, but the *fungi:bacteria* for long-term sequences was relatively stable during the secondary succession (Fig. 4A). All
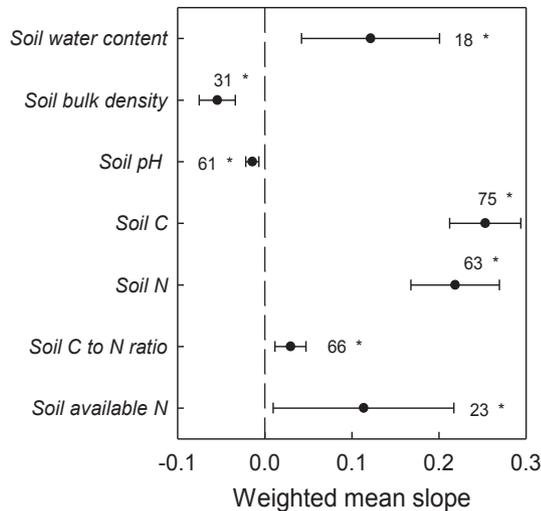
Fig. 2. Trends in soil properties during secondary succession. The dots and error bars indicate the weighted mean slopes and 95% confidence intervals (CIs) of the individual slopes of the target variable versus successional age. The dashed vertical line denotes that the slope is equal to zero (i.e., the target variables do not change with successional age). The sample size for each variable is shown next to the CI bar. The asterisk indicates a significant response.

disturbance types had positive trends in the *fungi:bacteria* ratio as the succession proceeds (Fig. 4A).

Pooling the data across all age sequences, we found a significant positive relationship between ln(*fungi:bacteria*) and ln($C:N_{soil}$) ($R^2 = 0.10$, $P < 0.01$, Fig. S4). Also, ln(Gram-positive bacteria to Gram-negative bacteria ratio) was positively correlated with ln($C:N_{soil}$) ($R^2 = 0.16$, $P < 0.01$, Fig. S4). Moreover, the slopes of microbial *C:N vs.* successional stage ($R^2 = 0.41$, $P < 0.01$, Fig. 5A) and *fungi:bacteria vs.* successional stage ($R^2 = 0.19$, $P = 0.05$, Fig. 5B) tended to increase as the slope of soil *C:N vs.* successional stage increased.

3.3. Successional trends in microbial bioenergetics

The slope$_{++}$ of C_{mic}/C_{soil} or N_{mic}/N_{soil} *vs.* successional stage was significantly higher than zero (Fig. 3B). The slope of C_{mic}/C_{soil} *vs.* successional stage significantly decreased as the slope of soil *C:N vs.* successional stage increased ($R^2 = 0.46$, $P < 0.01$, Fig. 5D). Pooling all data together, ln(C_{mic}/C_{soil}) decreased as the ln($C:N_{soil}$) increased ($R^2 = 0.14$, $P < 0.01$, Fig. S4). Across all age sequences, the slope$_{++}$ of microbial respiration and N mineralization *vs.* successional stage were significantly higher than zero, whereas the slope$_{++}$ of R/C_{mic} *vs.* successional stage was significantly lower than zero (Fig. 3B).

The C_{mic}/C_{soil} consistently increased with the succession across different ecosystems and lengths of age sequences (Fig. 4B). The R/C_{mic} significantly decreased in forest succession rather than in grassland (Fig. 4C). The medium- and long-term sequences had significant negative trends in R/C_{mic}, but the R/C_{mic} for the short-term sequences was relatively stable (Fig. 4C). Mining disturbances had higher slope$_{++}$ of C_{mic}/C_{soil} and R/C_{mic} than fire, cultivation, or harvest disturbances (Fig. 4B and C).

In addition, the slopes of R/C_{mic} *vs.* successional stage significantly increased as the slopes of soil *C:N vs.* successional stage increased ($R^2 = 0.23$, $P = 0.01$, Fig. 5C). Taking all data together, ln(R/C_{mic}) increased with increasing the ln($C:N_{soil}$) ($P < 0.01$, Fig. S4).

3.4. Successional trends in microbial enzymes

The slope$_{++}$ of microbial oxidase efficiency (oxidase activity to

microbial C ratio) or invertase efficiency *vs.* successional stage was significantly lower than zero, but the slope$_{++}$ of microbial hydrolase efficiency *vs.* successional stage was higher than zero despite the 95% CI covered zero (Fig. 3C). All of the six age sequences showed decreasing patterns of oxidase to hydrolase ratio (Fig. 3C). Similarly, the slope$_{++}$ of oxidase to invertase ratio *vs.* successional stage was significantly lower than zero. However, no general successional dynamic of hydrolase to invertase ratio was found (Fig. 3C).

4. Discussion

4.1. The successional trends in soil microbes support the application of the macro-ecological succession theory to microbial ecology

In accord with our initial hypothesis, our global synthesis supported that the Odum's (1969) macro-ecological succession theory of species selection and bioenergetics could be used to explain the trends in microbial communities during secondary succession. We summarized it as a conceptual diagram in Fig. 6: (1) *r*-selected bacteria were more prevalent in the early successional stages while *K*-selected fungi were more prevalent in the late succession stages; and (2) the microbial respiration to biomass ratio (R/C_{mic}) decreased while the microbial biomass to resource ratio (C_{mic}/C_{soil} and N_{mic}/N_{soil}) increased as the secondary succession proceeds (Fig. 3A and B).

Fungal cells differ in size by up to three orders of magnitude from bacterial cells, reflecting a lower surface to volume ratio in fungi than in bacteria. The cell walls of fungi also contain more carbon polymers than those of bacteria; thus fungi have lower growth and turnover rates than bacteria (Kaiser et al., 2014; Zechmeister-Boltenstern et al., 2015). High growth and turnover rates are supported by high metabolism and respiration rates per unit biomass (Kaiser et al., 2014; Xu et al., 2017). Therefore, the early succession stage tends to be dominated by bacteria that have higher R/C_{mic}, while fungi are more prevalent in the late succession stage that have lower R/C_{mic}. Assuming that microbial acquisition and uptake rates of organic C are constant (following previous studies; Anderson, 2003; Anderson and Domsch, 2010; Manzoni et al., 2012; Xu et al., 2014, 2017), the lower R/C_{mic} in the late successional stages implies more C available for microbial biomass production, which should in turn exhibits a higher C_{mic}/C_{soil} (Anderson, 2003; Anderson and Domsch, 2010; Manzoni et al., 2012; Xu et al., 2014). These coordinated changes among the *fungi:bacteria*, R/C_{mic}, and C_{mic}/C_{soil} during the secondary succession found in the present study (Fig. 6) are consistent with previous studies (Bailey et al., 2002; Bardgett et al., 2005; Six et al., 2006; Manzoni et al., 2012; Waring et al., 2013; Xu et al., 2014). Nevertheless, caution should be taken when interpreting these results, because the physiology and functionality of microbial communities may be plastic, rather than static, due to soil environmental changes resulting from the succession (Fig. 2) (Burns et al., 2013; Xu et al., 2017). Environmental changes probably influence microbial acquisition and uptake rates of organic C, which is still far from being understood (Bailey et al., 2002; Bardgett et al., 2005; Six et al., 2006; Manzoni et al., 2012; Waring et al., 2013; Xu et al., 2014, 2017).

Besides the *fungi:bacteria* ratio, the copiotrophic *vs.* oligotrophic spectrum also exists within bacteria (Fierer et al., 2007; Zechmeister-Boltenstern et al., 2015). We found a positive relationship between the ratio of Gram-positive bacteria to Gram-negative bacteria and the $C:N_{soil}$ (Fig. S4), implying that Gram-positive bacteria are more prevalent in soils of lower-quality while Gram-negative bacteria are dominated in soils of higher-quality (Zechmeister-Boltenstern et al., 2015). However, we did
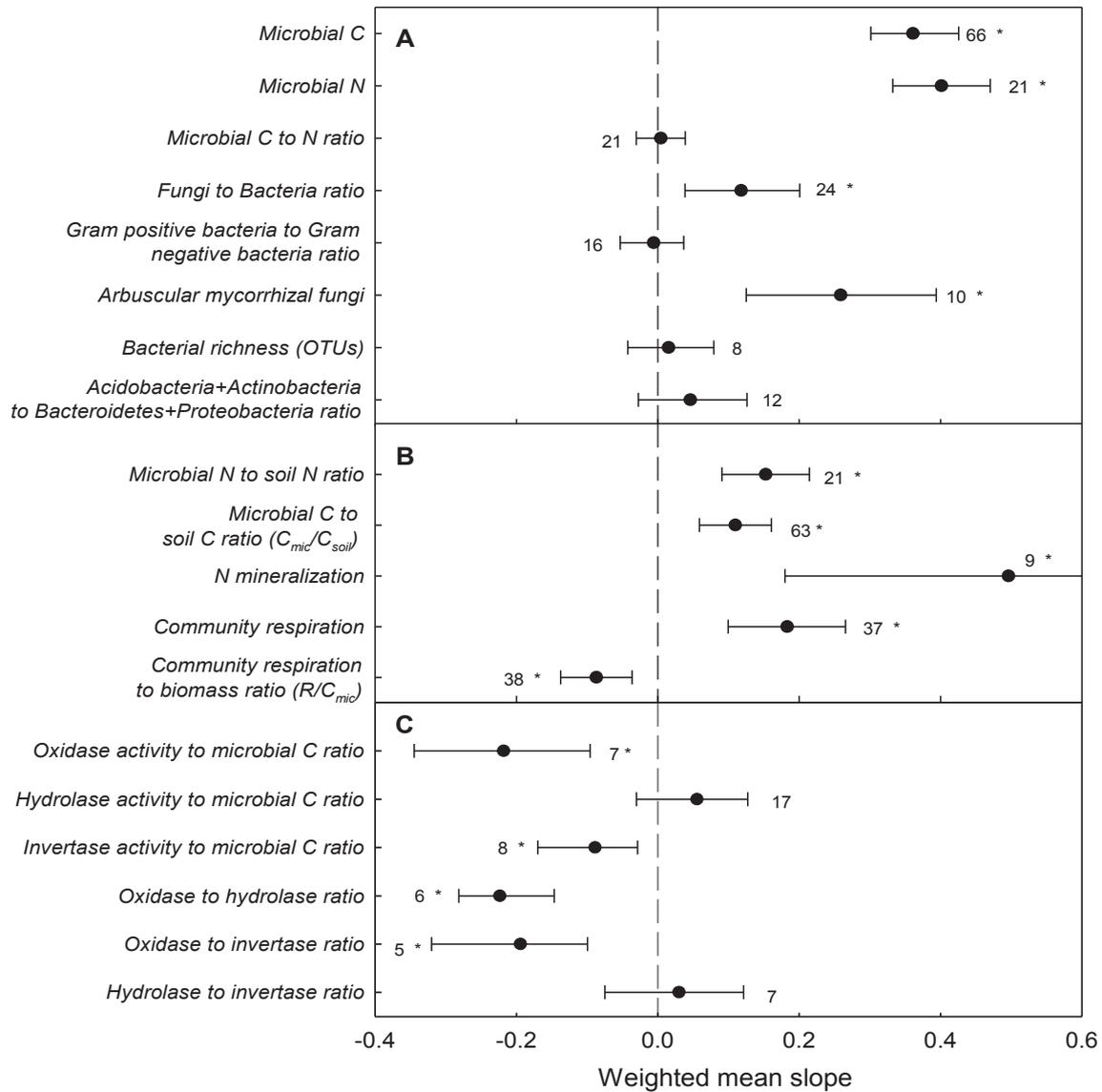
Fig. 3. Trends in microbial biomass, composition, bioenergetics, and activity during secondary succession. The dots and error bars indicate the weighted mean slopes and 95% confidence intervals (CIs) of the individual slopes of the target variable versus successional age. The dashed vertical line denotes that the slope is equal to zero (i.e., the target variables do not change with successional age). The sample size for each variable is shown next to the CI bar. The asterisk indicates a significant response.

not find a positive trend in the Gram-positive bacteria to Gram-negative bacteria ratio and the *Acidobacteria + Actinobacteria* to *Proteobacteria + Bacteroidetes* ratio as succession proceeds (Fig. 3A). One explanation for this discrepancy is that categorizing bacteria into Gram-positive/negative bacteria or even the bacterial phyla examined in this study is still a relatively coarse taxonomic resolution, which may hence induce uncertainty. For example, within the phyla of *Proteobacteria*, Fierer et al. (2007) found that only *β-Proteobacteria* could be categorized into copiotrophic groups, but the *α-Proteobacteria* could be assigned into neither copiotrophic nor oligotrophic groups. To date studies with fine-taxonomic resolutions of microbes are challenging and remain scarce.

4.2. Factors influencing the successional trends in microbial communities

The soil C:N stoichiometry was one important driver of the trends in microbial composition and bioenergetics (Figs. 5 and 6). The C:N$_{soil}$ ratio increased significantly with the successional stage,

which might be attributed to the succession being coupled to changes in the litter chemistry, i.e., plant tissue C:N ratios were found to increase with ecosystem development in a global database (Yang and Luo, 2011). We found the slope of *fungi:bacteria vs.* succession stage increased as the slope of C:N$_{soil}$ *vs.* succession stage increased (Fig. 5B), indicating that a faster increasing rate of C:N$_{soil}$ ratio will lead to a faster increasing rate of *fungi:bacteria* ratio as succession proceeds. Since the *fungi:bacteria* ratio was generally positively correlated with the C:N$_{soil}$ ratio (Fig. S4; Fierer et al., 2009; Waring et al., 2013; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015), it may be concluded that bacteria consequently dominate in the early successional stages that are often associated with high resource quality (i.e., low C:N$_{soil}$), while fungi dominate in the late successional stages that are associated with low resource quality (i.e., high C:N$_{soil}$).

Moreover, the C:N$_{soil}$ ratio was correlated positively with the R/C$_{mic}$ ratio but negatively with the C$_{mic}$/C$_{soil}$ ratio (Fig. S4), suggesting soil resource quality have a positive effect on microbial assimilation of soil organic C. This is consistent with recent results from
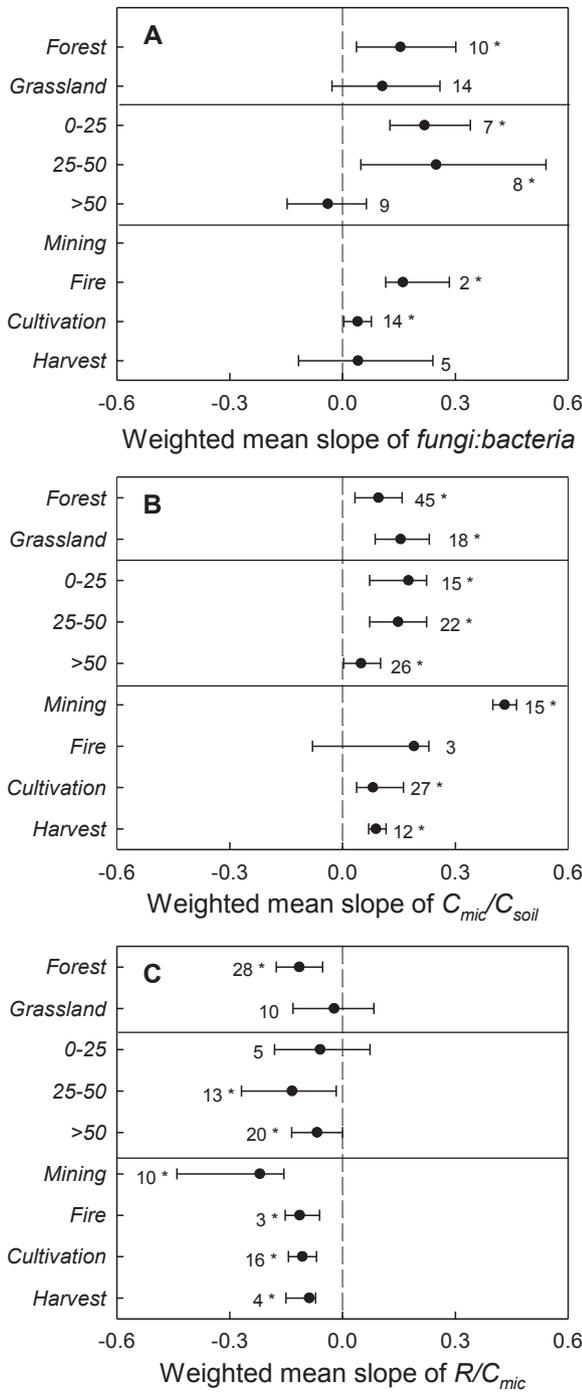
Fig. 4. Trends in fungi to bacteria ratio (*fungi:bacteria*), microbial carbon to soil carbon ratio (C_{mic}/C_{soil}), and microbial respiration to biomass ratio (R/C_{mic}) for different eco-systems, lengths of age sequences, and disturbance types. The dots and error bars indicate the weighted mean slopes and 95% confidence intervals (CIs) of the individual slopes of the target variable versus successional age. The dashed vertical line denotes that the slope is equal to zero (i.e., the target variables do not change with successional age). The sample size for each variable is shown next to the CI bar. The asterisk indicates a significant response.

Fig. 5. Relationships between the slopes of the soil carbon to nitrogen ratio ($C:N_{soil}$) versus successional age and (A) the slopes of microbial biomass carbon to nitrogen ratio ($C:N_{mic}$) versus successional age, (B) the slopes of fungi to bacteria ratio *vs.* successional age, (C) the slopes of microbial community respiration to biomass ratio (R/C_{mic}) versus successional age, and (D) the slopes of microbial biomass carbon to soil organic carbon ratio (C_{mic}/C_{soil}) versus successional age. Open circles are grasslands, while solid circles are forests.

experiments (Spohn and Chodak, 2015; Zhou et al., 2017), meta-analyses (Spohn, 2015; Zhou and Wang, 2015; Xu et al., 2017), and modeling studies (Xu et al., 2014). Theoretically, microbes fed with lower *C:N* ratio resources would have higher growth efficiency and lower release of C through respiration than microbes fed with higher *C:N* ratio resources, and more C available to be converted to biomass (Manzoni et al., 2012; Xu et al., 2014, 2017). Therefore, according to the concept of stoichiometry theory, an increase in the $C:N_{soil}$ ratio with secondary succession (Fig. 2) implies that the R/C_{mic} ratio should increase but the C_{mic}/C_{soil} ratio should decrease. However, these trends are contradictory to the predictions of the ecosystem succession theory in which succession decreases the R/C_{mic} but increases the R/C_{mic}. Interestingly, the "trade-off"
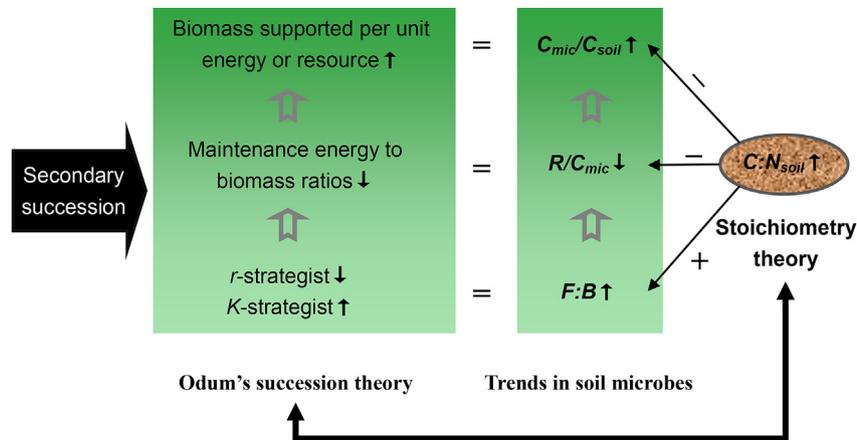
Fig. 6. Conceptual diagram illustrates how the macro-ecology theories explain the trends in soil microbial community with secondary succession. *F:B*, fungi to bacteria ratio; R/C_{mic}, microbial respiration per unit microbial biomass carbon; C_{mic}/C_{soil}, microbial biomass carbon to soil organic carbon rate. The "=" stands for the analogies between the macro-ecology and soil microbial ecology; The "+" stands for a positive effect of the soil carbon to nitrogen ratio ($C:N_{soil}$), while the "−" stands for a negative effect. Up and down arrows indicate increasing and decreasing trends, respectively.

relationships were found in our study: slow increasing rates of $C:N_{soil}$ were accompanied by fast decreasing rates of R/C_{mic} and fast increasing rates of C_{mic}/C_{soil} (Fig. 5C and D). Thus, the concept of stoichiometry theory offers a potential way to explain the divergent successional dynamics (both changes in direction and rate) in the R/C_{mic} and C_{mic}/C_{soil} across different studies (Fig. 6).

Microbial extracellular enzymes are the proximate agents of organic matter decomposition (breakdown complex substrates into compounds small enough for uptake), and are crucial for microbial resource assimilation, biochemistry of decomposition, and nutrient cycling. Microbial oxidases are produced predominantly by fungi to degrade poor quality, chemically complex compounds (Cusack et al., 2011). Given that plant litter quality significantly decreased with secondary succession (Fig. 2; Yang and Luo, 2011), increasing *fungi:bacteria* ratio with ecosystem development would result in higher activity of oxidase in the late succession stage than in the early succession stage. However, we found that the secondary succession decreased both oxidase efficiency and oxidase to hydrolase ratio (Fig. 3C). Soil may be characterized of having a redundancy of functions, a reduction in any group of species that has little effect on overall processes in soil because other microorganisms can take (Nannipieri et al., 2003). Therefore, we should be careful about jumping to conclusion that changes in extracellular enzymes did not result from changes in microbial community composition along the secondary succession. This phenomenon may also be caused by the methodological constraints. As a kinetic cascade, extracellular enzyme activity is as much constrained by soil moisture, temperature, pH, substrate availability, and enzyme concentration (Allison et al., 2010; Sinsabaugh and Follstad Shah, 2012). As these variables all change with secondary succession (Fig. 2), enzyme assays are a measure of potential activity under optimal enzyme reaction conditions that could be different with realistic or in situ activity (Burns et al., 2013). Another potential explanation is that the products of enzymatic deconstruction diffuse in the soil solution and become also accessible to other microbes, resulting not only in competitive but also synergistic interactions among microbes (Allison et al., 2010; Kaiser et al., 2014). Furthermore, many enzymes become stabilized through association with clay minerals, humic acids, and particulate organic matter, and retain significant levels of activity for prolonged periods of time (Allison et al., 2010; Nannipieri et al., 2012; Burns et al., 2013). Changes in microbial community composition do not necessarily match the patterns of enzyme activity.

Secondary succession from mining sites had the steepest slope of microbial variables *vs.* succession stage than the successions after other disturbance types (Fig. 4), most likely because mining destroying on soils is the most serious. The trends in the *fungi:bacteria* ratio for the long-term sequences showed a bit differences with the short- and medium-term sequences (Fig. 4A). Unfortunately, little was known about the potential mechanism. Moreover, chronosequence approaches have provided valuable insight into patterns of ecosystem succession and soil development, but they can be subjected to error in the space-for-time substitution if sites differ in respect to the factors other than their age (Yanai et al., 2003).

In summary, the present study attempted to use the simplistic approach to synthesize the general succession patterns of microbial communities during secondary succession. Our global synthesis found that the changes in the *fungi:bacteria*, C_{mic}/C_{soil}, and R/C_{mic} are reciprocal causation and could be explained by the Odum's (1969) succession theory. In addition, the concept of stoichiometry theory offers an approach to explain the divergent successional trends of microbial community composition and bioenergetics across different age sequences (Fig. 6). Overall, our study highlights the application of the existing macro-ecological theory to soil microbial ecology studies.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.08.014.

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