Stoichiometric Characteristics of Soil-Microbial-Mineralization Carbon and Nitrogen under Different Land Use Types in the Mao' ershan Region (Postprint)

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Date: 2017-04-18T00:00:00+00:00

Abstract

Changes in land use patterns lead to variations in soil carbon and nitrogen contents and their stoichiometric relationships; however, the response of soil microbial stoichiometry and its driven carbon and nitrogen mineralization processes to such changes remains unclear. This study examined four different land use types in the Mao' er Mountain region—natural deciduous broad-leaved forest, artificial Korean pine forest, grassland, and cropland—and measured soil organic carbon (Csoil), total nitrogen (Nsoil), microbial biomass carbon and nitrogen (Cmic and Nmic), and soil carbon and nitrogen mineralization rates (Cmin and Nmin). The objectives were to compare the effects of different land use patterns on soil and microbial carbon-nitrogen stoichiometric characteristics and mineralization rates, explore the correlations in carbon-nitrogen stoichiometric characteristics among the soil-microbe-mineralization continuum, and reveal the response and regulatory mechanisms of microorganisms to changes in soil carbon-nitrogen stoichiometry. The results demonstrated that Csoil, Nsoil, Cmic, Nmic, and Cmin all followed the trend: natural deciduous broad-leaved forest > artificial Korean pine forest > grassland > cropland, whereas Nmin in natural deciduous broad-leaved forest and grassland was significantly higher than that in artificial Korean pine forest and cropland. Land use pattern significantly influenced both soil and microbial carbon-to-nitrogen ratios (C Nsoil and C Nmic), with the highest values observed in cropland. Comprehensive analysis of data across different land use patterns revealed that the carbon-to-nitrogen mineralization rate ratio was negatively correlated with C Nmic, but significantly positively correlated with the stoichiometric imbalance between microorganisms and soil (C Nimb). The carbon mineralization rate per unit microbial biomass (qCO2) decreased with increasing C Nmic, while the nitrogen mineralization rate per unit microbial biomass (qAN) increased with increasing

C Nmic. C Nimb exhibited a positive correlation with qCO2 and a negative correlation with qAN. These findings indicate that microorganisms adapt to the variability in soil carbon and nitrogen and their stoichiometry induced by land use changes by altering their own carbon-nitrogen stoichiometry and adjusting the relative mineralization rates of carbon and nitrogen, thereby satisfying the carbon-nitrogen balance required for their growth and metabolic demands.

Full Text

Preamble

ACTA ECOLOGICA SINICA ChinaXiv Partner Journal
Vol. 37, No. 7, Apr. 2017
DOI: 10.5846/stxb201512242569

Soil-Microbe-Mineralization Carbon and Nitrogen Stoichiometry Under Different Land-Uses in the Maoershan Region

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Abstract

Land-use changes lead to significant alterations in soil carbon and nitrogen contents and their stoichiometric relationships. However, how soil microbial stoichiometry and the associated mineralization processes of soil carbon and nitrogen respond to these changes remains inconclusive. This study investigated natural deciduous broad-leaved forest, artificial Korean pine plantation, grassland, and cropland—four different land-use types in the Maoershan Forest Ecosystem Research Station, Northeast China (45°20 N, 127°30 E). Our objectives were to: (1) examine the effects of land-use on soil organic carbon (Csoil), total nitrogen (Nsoil), soil microbial biomass carbon and nitrogen (Cmic and Nmic), carbon and nitrogen mineralization rates (Cmin and Nmin), and their stoichiometric ratios; and (2) explore the carbon-nitrogen interactions among soil-microbe-mineralization to mechanistically understand microbial responses and adaptation to resource stoichiometry.

The results showed that the contents of Csoil, Nsoil, Cmic, Nmic, and Cmin decreased in the following order: natural forest > plantation > grassland > cropland, whereas natural forest and grassland had significantly higher Nmin than plantation and cropland. Land-use significantly affected soil and microbial biomass carbon-to-nitrogen ratios (C:Nsoil and C:Nmic), with cropland exhibiting the greatest values among the four land-uses. Analysis of pooled data across all land-uses revealed that the ratio of carbon to nitrogen mineralization rates correlated negatively with C:Nmic but positively with the stoichiometric imbalance between microbes and their soil resources (C:Nimb, defined as C:Nsoil divided by C:Nmic). The carbon mineralization rate per unit microbial biomass carbon (CO2) decreased with increasing C:Nmic, whereas the nitrogen mineralization rate per unit microbial biomass nitrogen (AN) increased with increasing C:Nmic. The C:Nimb correlated positively with CO2 but negatively with AN. Our study suggests that soil microbes may adapt to changes in soil stoichiometry induced by land-use for their carbon and nitrogen requirements for growth and metabolism by adjusting their biomass stoichiometry and carbon-to-nitrogen mineralization rates.

Keywords: stoichiometry; carbon mineralization; nitrogen mineralization; soil microbe; land use change

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1. Study Area Overview

The study site was located at the Maoershan Forest Ecosystem Research Station (45°20 N, 127°30 E). The region has a continental monsoon climate with warm, humid summers and cold, dry winters. Mean annual precipitation is approximately 629 mm, concentrated in summer, while mean annual evaporation is about 864 mm. The average annual temperature is 3.1°C, with January and July mean temperatures of -18.5°C and 22.0°C, respectively. The frost-free period is approximately 120–140 days. The zonal soil type is dark brown forest soil, and the vegetation belongs to the Changbai flora. The existing vegetation represents typical secondary and artificial forest ecosystems in the mountainous areas of eastern Northeast China, formed through secondary succession after repeated human disturbances to the original zonal vegetation.

We selected four adjacent land-use types: (1) natural deciduous broad-leaved forest, the typical hardwood forest in the region dominated by *Phellodendron amurense*, *Juglans mandshurica*, *Fraxinus mandshurica*, and *Acer mono*; (2) artificial Korean pine plantation dominated by planted *Pinus koraiensis* with naturally regenerated *Fraxinus mandshurica* and *Betula platyphylla*; (3) grassland formed after clear-cutting of natural forest with regular shrub and grass removal; and (4) cropland converted from clear-cut sites, primarily planted with corn without fertilizer or pesticide application. Both forest stands were 47–58 years old. Further details are provided in Zhang et al. [19].

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2. Sample Collection and Analysis

Because soil microbial biomass and mineralization are concentrated in the topsoil and more sensitive to environmental changes [8–9], we collected surface soil samples (0–5 cm) in 2015. Twelve surface soil samples were randomly taken from each land-use type and transported to the laboratory immediately. Soil samples were passed through a 2 mm sieve. A portion was air-dried and ground for determination of soil organic carbon (Csoil) and total nitrogen (Nsoil). Fresh soil was used for microbial biomass carbon and nitrogen (Cmic and Nmic) and carbon and nitrogen mineralization analysis. Soil moisture content was measured on fresh subsamples.

For Csoil and Nsoil analysis, approximately 0.100 g of oven-dried, ground soil was analyzed using a multi N/C 3000 HT 1500 Solids Module analyzer (Analytik Jena AG, Germany) via combustion method. For Nsoil, another 0.200 g of oven-dried, ground soil was digested with 3 mL H2O2 at 420°C for 90 minutes until completely white, cooled, and brought to volume. Total nitrogen was then measured using a continuous flow analyzer (BRAN+LUEBBE-AA3, Germany).

Soil microbial biomass was determined using chloroform fumigation-K2SO4 extraction (0.5 mol/L, 1:2.5 soil-to-solution ratio) [21]. Carbon and nitrogen concentrations in extracts were measured using the multi N/C 3000 analyzer. For mineralization, soil water content was adjusted to field capacity and incubated at 25°C for 21 days. CO2 released by soil microbes was measured on days 1, 3, 5, 7, 10, 14, and 21. Carbon mineralization rate (Cmin, g CO2-C g ^{1} d ^{1}) was calculated as CO2-C released per gram of soil per day. Nitrogen mineralization rate (Nmin, g AN-N g ^{1} d ^{1}) was calculated as the change in available nitrogen (ammonium + nitrate) between the start and end of incubation divided by incubation days.

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3. Data Analysis

We standardized carbon and nitrogen mineralization rates as specific rates per unit microbial biomass. Specific carbon mineralization rate (CO2, mg CO2-C g ^{1} Cmic d ^{1}) was calculated as Cmin divided by microbial biomass carbon. Specific nitrogen mineralization rate (AN, mg AN-N g ^{1} Nmic d ^{1}) was calculated as Nmin divided by microbial biomass nitrogen. Stoichiometric imbalance between microbes and soil resources (C:Nimb) was defined as the ratio of soil carbon-to-nitrogen ratio (C:Nsoil) to microbial biomass carbon-to-nitrogen ratio (C:Nmic).

Data were processed and analyzed using SPSS 19.0 software. One-way ANOVA with Duncan' s test was used to assess differences among land-use types. Pearson correlation coefficients evaluated relationships between parameters. Regression analysis established models relating soil, microbial, and mineralization stoichiometry.

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4. Results

4.1 Soil Carbon and Nitrogen Stoichiometry Under Different Land Uses

Land-use change significantly altered topsoil Csoil (F , = 38.77, $P < 0.001$) and Nsoil (F , = 33.52, $P < 0.001$). Both Csoil and Nsoil decreased in the order: natural deciduous broad-leaved forest > artificial Korean pine plantation > grassland > cropland. Csoil and Nsoil were highly correlated (r = 0.97, P < 0.01), though Nsoil showed less variability than Csoil. C:Nsoil also differed significantly among land-uses (F , = 4.27, P = 0.010), with cropland having the highest ratio (22.9), followed by artificial pine plantation (18.2), grassland (10.2), and natural forest (9.1). Further regression analysis revealed a significant positive relationship between C:Nmic and C:Nsoil ($R^2 = 0.21$, P = 0.001).

4.2 Soil Microbial Biomass Carbon and Nitrogen Stoichiometry

Cmic, Nmic, Csoil, and Nsoil were all significantly positively correlated, with correlation coefficients ranging from 0.57 to 0.70 (P < 0.01). Land-use significantly affected Cmic and Nmic, following the same trend as Csoil and Nsoil: natural forest > plantation > grassland > cropland. C:Nmic also varied significantly among land-uses (F , = 4.27, P = 0.010), with cropland showing the highest value (15.8), approximately double that of other land-uses. Natural forest and grassland had significantly lower C:Nmic (7.3 and 8.1, respectively) compared to artificial pine plantation (10.2) and cropland.

4.3 Soil Mineralization Carbon and Nitrogen Stoichiometry

Land-use significantly changed topsoil carbon mineralization rate (Cmin, F , = 49.60, P < 0.001), nitrogen mineralization rate (Nmin, F , = 30.47, P < 0.001), and their ratio (C:Nmin, F , = 18.53, P < 0.001). Cmin and Nmin were significantly positively correlated with soil carbon and nitrogen content. Cmin decreased in the order: natural forest (121.3 g CO2-C g ^{1} d ^{1}) > grassland (73.4 g CO2-C g ^{1} d ^{1}) > artificial pine plantation (54.3 g CO2-C g ^{1} d ^{1}) > cropland (25.1 g CO2-C g ^{1} d ^{1}). Natural forest and grassland had significantly higher Nmin (3.6 and 3.4 g AN-N g ^{1} d ^{1}, respectively) than artificial pine plantation and cropland (1.7 and 1.2 g AN-N g ^{1} d ^{1}). C:Nmin was highest in natural forest (38.1) and artificial pine plantation (23.4), significantly higher than in grassland (15.8).

Correlation analysis revealed significant positive relationships between C:Nmin and both C:Nsoil and C:Nmic. Specific carbon mineralization rate (CO2) decreased with increasing C:Nmic, while specific nitrogen mineralization rate (AN) increased with increasing C:Nmic. C:Nimb correlated positively with CO2 but negatively with AN.

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5. Discussion and Conclusions

5.1 Effects of Land-Use on Soil and Microbial Stoichiometry and Mineralization Rates

When natural deciduous broad-leaved forest was converted to artificial Korean pine plantation, grassland, and cropland, soil carbon and nitrogen contents decreased significantly, consistent with previous studies [24]. Although the natural forest and artificial pine plantation had similar stand ages, long-term monitoring showed the latter had higher annual litterfall (4.0 t hm ^{2} vs. 3.7 t hm ^{2}), yet lower surface soil carbon and nitrogen contents. This is likely because Korean pine needles decompose more slowly than broadleaf litter, and the pine plantation had higher surface litter carbon storage (4.1 t C hm ^{2}) than the natural forest (2.6 t C hm ^{2}), resulting in lower carbon and nitrogen inputs to soil.

Interestingly, cropland had the highest C:Nsoil, contrary to some studies [26,27]. This lack of consistent patterns across forest successional series suggests that soil C:Nsoil responses to land-use and environmental factors are complex. Because soil microbial biomass correlates significantly with soil carbon and nitrogen, land-use effects on soil resources inevitably alter microbial biomass, leading to the observed trend: natural forest > plantation > grassland > cropland for Cmic and Nmic.

C:Nmic also varied significantly among land-uses, with cropland values approximately double those of other land-uses. This aligns with findings that C:Nmic increases with C:Nsoil [8,29], as fast-growing, high-turnover microbes (r-strategists) dominate in low C:Nsoil environments and have lower C:Nmic, while slow-growing microbes (K-strategists) dominate in high C:Nsoil environments with higher C:Nmic [30–31]. Fertilization experiments have shown nitrogen addition significantly reduces C:Nmic [30–31], supporting this pattern.

Land-use significantly affected mineralization rates and their ratios. Natural forest and grassland had higher Nmin than plantation and cropland, possibly because herbaceous litter decomposes more readily than tree litter, and local herbaceous plants have significantly higher leaf nitrogen content than trees [33]. C:Nmin followed the same trend as C:Nsoil and C:Nmic: natural forest > plantation > grassland > cropland.

5.2 Coupling Relationships Among Soil-Microbe-Mineralization Stoichiometry

The consumer-driven nutrient cycling theory [34] posits that the balance between resource and consumer stoichiometry and consumer elemental use efficiency directly affects ecosystem carbon and nutrient fluxes. Microbes retain limiting elements and excrete excess elements, reflecting their energy requirements and carbon use efficiency. Higher specific carbon mineralization rates indicate lower carbon use efficiency, as more assimilated carbon is respired rather than converted to biomass [35].

Microbial carbon and nitrogen mineralization are influenced by substrate C:N ratios. While some studies report positive correlations between specific carbon mineralization rate and C:Nsoil [15–16], we did not find this relationship. Instead, our results show that specific carbon mineralization rate decreases with increasing C:Nmic, while specific nitrogen mineralization rate increases with increasing C:Nmic. This pattern was also observed in subtropical soils [8]. The positive relationship between C:Nmic and C:Nsoil mitigates the effects of increasing soil C:N ratio on carbon and nitrogen mineralization, representing an important microbial adaptation mechanism to soil stoichiometric variability [2,36].

Furthermore, C:Nimb significantly influenced mineralization rates, correlating positively with specific carbon mineralization but negatively with specific nitrogen mineralization. This demonstrates that the stoichiometric disparity between soil and microbes significantly affects mineralization processes. Microbes adjust their biomass stoichiometry and relative mineralization rates to adapt to land-use-induced variability in soil carbon and nitrogen stoichiometry, thereby meeting their growth and metabolic carbon-nitrogen balance requirements. Integrating these coupled relationships among soil, microbial, and mineralization stoichiometry is essential for comprehensively understanding ecosystem carbon and nitrogen cycling.

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