RESEARCH PAPER

Carbon : nitrogen stoichiometry in forest ecosystems during stand development

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ABSTRACT

Aim Carbon (C) and nitrogen (N) stoichiometry is a critical indicator of biogeochemical coupling in terrestrial ecosystems. However, our current understanding of C : N stoichiometry is mainly derived from observations across space, and little is known about its dynamics through time.

Location Global secondary forests.

Methods We examined temporal variations in C : N ratios and scaling relationships between N and C for various ecosystem components (i.e. plant tissue, litter, forest floor and mineral soil) using data extracted from 39 chronosequences in forest ecosystems around the world.

Results The C : N ratio in plant tissue, litter, forest floor and mineral soil exhibited large variation across various sequences, with an average of 145.8 ± 9.4 (mean $\pm$ SE), 49.9 ± 3.0, 38.2 ± 3.1 and 18.5 ± 0.9, respectively. In most sequences, the plant tissue C : N ratio increased significantly with stand age, while the C : N ratio in litter, forest floor and mineral soil remained relatively constant over the age sequence. N and C scaled isometrically (i.e. the slope of the relationship between log-transformed N and C is not significantly different from 1.0) in litter, forest floor and mineral soil both within and across sequences, but not in plant tissue either within or across sequences. The C : N ratio was larger in coniferous forests than in broadleaf forests and in temperate forests than in tropical forests. In contrast, the N–C scaling slope did not reveal significant differences either between coniferous and broadleaf forests or between temperate and tropical forests.

Main conclusions These results suggest that C and N become decoupled in plants but remain coupled in other ecosystem components during stand development.

Keywords
Carbon : nitrogen ratio, isometric scaling, litter, plant, secondary forests, soil, time.

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INTRODUCTION

Since Redfield (1958) documented a well-constrained ratio of carbon (C) : nitrogen (N) : phosphorus (P) (i.e. $C_{106}:N_{16}:P_1$) in marine plankton, the C : N : P ratio has been widely used to indicate the elemental composition of living organisms (Sterner & Elser, 2002; Elser *et al.*, 2009). Though the Redfield C : N : P ratio was originally proposed as an indicator of the elemental status of marine plankton (Sterner *et al.*, 2008), this approach has also proved to be valuable in understanding ocean–atmosphere carbon dioxide (CO_2) exchange and the extent of nutrient limitation of marine net primary production (NPP) and biogeochemical cycles in ocean ecosystems (Field *et al.*, 1998). More importantly, ecological stoichiometry, which aims to understand the balance of the multiple chemical elements required by organisms, has become a new discipline in ecology and provided new perspectives for understanding ecosystem processes from the individual organism to the ecosystem level (Elser, 2000; Sterner & Elser, 2002).

The predictive power of the C : N : P ratio, together with its importance in understanding elemental components and biogeochemical cycles in ocean ecosystems, has motivated ecologists to search for similar stoichiometric patterns in terrestrial ecosystems (e.g. Güsewell, 2004; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Cleveland & Liptzin, 2007; Townsend *et al.*, 2007). Previous studies have revealed that despite remarkable variation among different functional groups, the C : N ratio is well constrained (i.e. it shows relatively small variation) in foliage (McGroddy *et al.*, 2004; Han *et al.*, 2005), litter (McGroddy *et al.*, 2004) and mineral soil (Cleveland & Liptzin, 2007; Yang *et al.*, 2010a). Previous analyses have also shown that N scaled isometrically with respect to C (i.e. the slope of the relationship between log-transformed N and C is not significantly different from 1.0) in both foliage (McGroddy *et al.*, 2004; Niklas & Cobb, 2005) and mineral soil (Cleveland & Liptzin, 2007; Yang *et al.*, 2010a) but not in litter (McGroddy *et al.*, 2004). These studies have greatly advanced our understanding of C : N stoichiometry in terrestrial ecosystems across space. However, our knowledge of the temporal variability of C : N stoichiometry is much less developed than its spatial patterns (Ågren, 2008). It has been suggested that ecological patterns occurring through time may differ from those observed across space (Lauenroth & Sala, 1992; Jobbágy *et al.*, 2002; Fisher *et al.*, 2010). Thus, it remains unknown whether the C : N ratio is also well constrained through time and whether the isometric pattern between N and C observed across space also exists through time.

Secondary forests, with naturally occurring C and N dynamics, possess an ideal age gradient for exploring the temporal variability of C : N stoichiometry in terrestrial ecosystems (Sterner & Elser, 2002). Numerous studies have examined C and N dynamics during stand development (e.g. Hooker & Compton, 2003), and thus offer the possibility to quantify the temporal pattern of C : N stoichiometry over the age sequence in different ecosystems. However, the direction of C : N ratio dynamics during stand development remains controversial. For instance, Hooker & Compton (2003) demonstrated that the C : N ratio in plant tissue increased dramatically with stand age since agricultural abandonment. In contrast, Clinton *et al.* (2002) observed that plant tissue C : N ratio did not experience a significant change over age sequence. Therefore, it is imperative to synthesize diverse results among various studies to reveal generalized patterns and advance our understanding of C : N stoichiometry during stand development.

In this study, we examined temporal patterns of the C : N ratio for major ecosystem components (i.e. plant tissue, litter, forest floor and mineral soil) in secondary forests by synthesizing data extracted from 39 peer-reviewed papers. We also investigated the stoichiometric relationship between N and C along the successional gradient. This synthesis aimed to test the following two hypotheses: (1) the C : N ratio remains relatively constant over the age sequence, and (2) N scales isometrically with respect to C during stand development.

MATERIALS AND METHODS

Literature synthesis

We collected data from 39 published studies that quantified changes in C, N and the C : N ratio in forest ecosystems over the age sequence (Appendices S1 & S2 in Supporting Information). These studies provided 10 sequences for plant tissue, 10 for litter, 8 for forest floor and 21 for mineral soil. These papers were assembled from a range of literature sources according to the following two criteria: (1) each sequence should include at least five age stages to detect the statistical trends of the C : N ratio along the successional gradient, and (2) each sequence should contain quantitative information about stand age. The raw data were extracted from published tables or published graphs using the software ORIGINPRO 7.5 (OriginLab, Northampton, MA, USA). Our database recorded geographic location (longitude and latitude), climatic information (mean annual temperature and mean annual precipitation), forest type (coniferous and broadleaf forests or temperate and tropical forests), stand age and 12 target variables for each sequence. These 12 variables included C pools, N pools and C : N ratios in plant tissue, litter, forest floor and mineral soil. It should be noted that the term 'plant tissue' used in this study referred to the whole above-ground plant since there were few data available for *specific* plant tissue (e.g. leaf, wood, root). In addition, soil C and N content were derived from the same soil depth within each sequence, although soil depth was inconsistent among various sequences (Appendix S1).

Both C and N pools were estimated using similar methods among different studies. Specifically, the allometric function between vegetation biomass and diameter at breast height, together with N concentration measured with the Kjeldahl methodology, were frequently used to evaluate plant C and N pools (e.g. Compton *et al.*, 2007). Litter biomass was mostly determined with harvesting methods using litter bags, and then converted to litter N content using litter biomass and N concentration measured with the Kjeldahl approach (i.e. Ostertag *et al.*, 2008). In addition, both forest floor and mineral soil were sampled by digging soil pits, and then the C and N concentrations were measured using chemical methods (e.g. the Walkley–Black method and Kjeldahl method) and further converted to C and N content by multiplying by soil bulk density (e.g. Hooker & Compton, 2003).

Data analysis

Data were processed through the following two steps. First, to test whether the C : N ratio remains relatively constant over age sequence, we examined the relationship between the C : N ratio and stand age for major ecosystem components (i.e. plant tissue, litter, forest floor and mineral soil) within each sequence. If the slope of the relationship between C : N ratio and stand age was not statistically different from zero (i.e. the 95% of confidence interval of the slope covered zero), the C : N ratio was considered to be relatively stable during stand development. To further
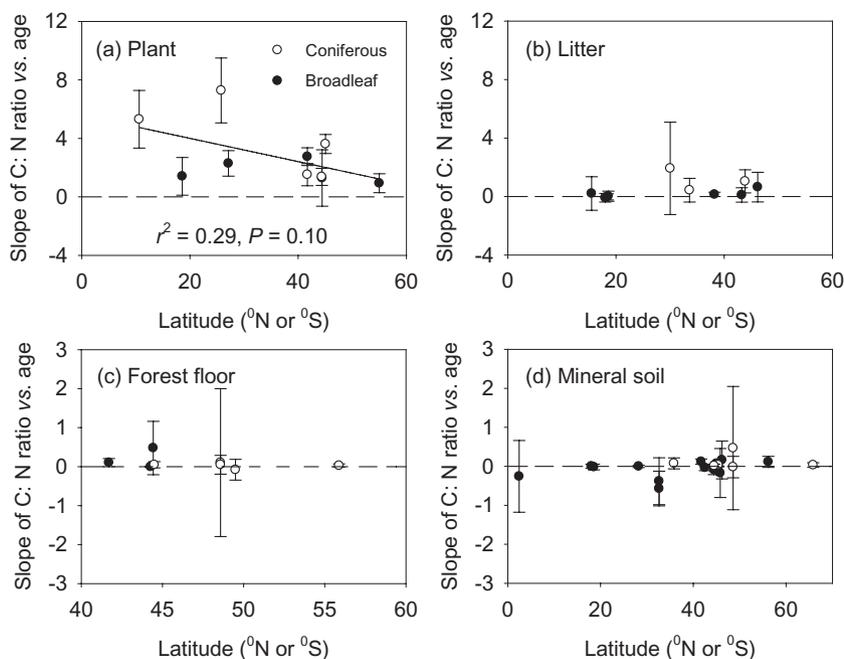
Figure 1 Variations in the slopes of the relationships between C : N ratio and stand age along the latitudinal gradient for (a) plant tissue, (b) litter, (c) forest floor and (d) mineral soil. Each data point represents an individual sequence. The error bar indicates the 95% confidence interval of the slope of the C : N ratio versus stand age relationship. The dashed line denotes that the slope is equal to zero (i.e. the C : N ratio does not change over age sequence).

examine potential differences in the C : N ratio among different forest types, we then compared C : N ratios between coniferous and broadleaf forests and between temperate and tropical forests.

Second, to test whether an isometric relationship between N and C (i.e. the slope of the relationship between log N and log C is not significantly different from 1.0) occurs through time, we explored the N–C stoichiometric relationship in major ecosystem components for each sequence. If the slope of the N–C stoichiometric relationship was not significantly different from 1.0 (i.e. the 95% of confidence interval of the slope covered 1.0), the isometric hypothesis held true during stand development. Compared with the C : N ratio, the N–C scaling relationship could indicate the proportional changes between N and C content through time. Biologically, the isometric scaling relationship between N and C reflects that plant physiological N investment is independent of plant biomass (McGroddy *et al.*, 2004; Niklas & Cobb, 2005; Kerkhoff *et al.*, 2006). Similar to other scaling studies (e.g. McGroddy *et al.*, 2004; Niklas & Cobb, 2005; Kerkhoff *et al.*, 2006; Yang *et al.*, 2009, 2010a,b), we used a log–log function to quantify the stoichiometric relationship between N and C in various ecosystem components (equation 1)

$$\log y = a + b(\log x) \qquad (1)$$

where x is C content (Mg C ha^{-1}), y is N content (Mg N ha^{-1}), a is the intercept and b is the scaling slope.

The original data were log_{10}-transformed before analysis due to the lognormal distributions of both C and N content. Reduced major axis (RMA) regression (i.e. type II regression) was performed to examine the N–C stoichiometric relationship (e.g. McGroddy *et al.*, 2004; Niklas & Cobb, 2005; Cleveland & Liptzin, 2007; Sterner *et al.*, 2008; Yang *et al.*, 2010a). The scaling slope of the stoichiometric function was determined using the

software package 'Standardised Major Axis Tests and Routines' (Falster *et al.*, 2003). In addition, to explore potential differences in N–C scaling relationships among different forest types, we then compared the scaling slopes between coniferous and broadleaf forests and between temperate and tropical forests.

RESULTS

The temporal dynamics of the C : N ratio showed remarkable differences among various ecosystem components (Fig. 1). In most sequences, the slope of the relationship between the C : N ratio of plant tissue and stand age was significantly larger than zero ($P < 0.05$) (Fig. 1a), demonstrating an increasing trend of plant tissue C : N ratio over age sequence. By contrast, the slopes of the relationships between C : N ratio and stand age in litter, forest floor and mineral soil did not reveal significant differences from zero in most cases ($P > 0.05$) (Fig. 1b–d), indicating that C : N ratios in these ecosystem components did not experience significant change during stand development. In addition, the slope of plant C : N ratio versus stand age exhibited a marginally significant decreasing trend with latitude (Fig. 1a), but the slopes of C : N ratio versus stand age in other ecosystem components did not show any significant change along the latitudinal gradient ($P > 0.05$) (Fig. 1b–d). C : N ratios in all ecosystem components varied widely across various sequences, with an average of 145.8 ± 9.4 (mean ± SE) for plant tissue, 49.9 ± 3.0 for litter, 38.2 ± 3.1 for forest floor and 18.5 ± 0.9 for mineral soil (Appendix S3). Besides that in forest floor, C : N ratios in other ecosystem components significantly differed among various forest types ($P < 0.05$) (Fig. 2). Specifically, C : N ratios were larger in coniferous forests than in broadleaf forests and in temperate forests than in tropical forests.

The stoichiometric relationship between N and C within a sequence revealed substantial differences among various ecosys-
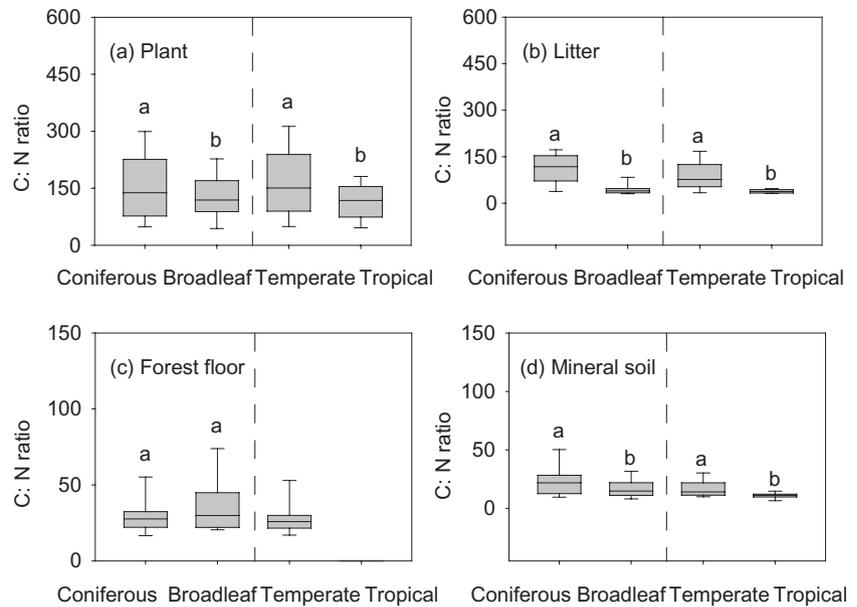
Figure 2 Comparison of the C : N ratio in various ecosystem components between coniferous and broadleaf forests and between temperate and tropical forests for (a) plant tissue, (b) litter, (c) forest floor and (d) mineral soil. Statistical differences are denoted by different letters (Tukey test, $P < 0.05$).
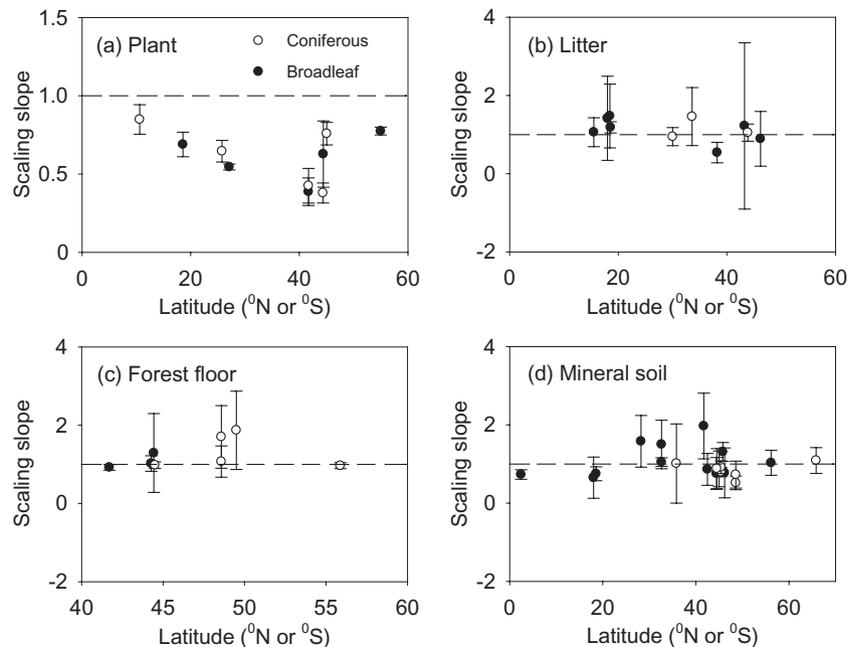
Figure 3 Variations in the scaling slopes of the N–C stoichiometric relationships along the latitudinal gradient for (a) plant tissue, (b) litter, (c) forest floor and (d) mineral soil. Each data point represents an individual sequence. The scaling slope indicates the slope of the type II (i.e. reduced major axis, RMA) relationship between log-transformed N and C. The error bar shows the 95% confidence interval of the scaling slope of the relationship between log N and log C. The dashed line denotes the scaling slope is equal to 1.0, indicating the isometric scaling between N and C along the age gradient.

tem components (Fig. 3). In most sequences, the scaling slope (i.e. the slope of the N–C stoichiometric relationship between log N and log C) in plant tissue was significantly lower than 1.0 ($P < 0.05$) (Fig. 3a). However, the allometry of N with respect to C was predictable and consistent in litter, forest floor and mineral soil over age sequence (Fig. 3b–d). The slopes of N–C stoichiometric relationships in these ecosystem components were not statistically different from 1.0 in most cases ($P > 0.05$) (Fig. 3b–d). The stoichiometric relationship between N and C across various sequences was different among various ecosystem components (Fig. 4). The scaling slope in plant tissue was significantly lower than 1.0 (Table 1), indicating that the isometric pattern did not occur in plant tissue across various sequences

(Fig. 4a). However, N and C did exhibit isometric relationships in other ecosystem components across various sequences (Fig. 4b–d, Table 1). Interestingly, the scaling slope did not reveal significant differences either between coniferous and broadleaf forests or between temperate and tropical forests ($P > 0.05$) (Table 1).

DISCUSSION

Variation in the C : N ratio over age sequence

The C : N ratio in plant tissue increased during stand development, contrary to Hypothesis 1. The increase in the C : N ratio
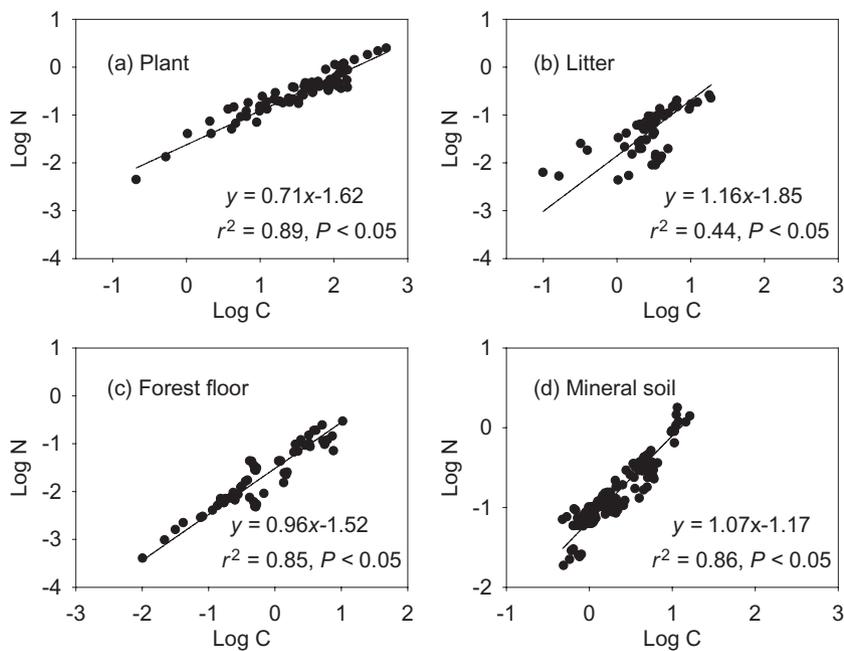
Figure 4 N–C stoichiometric relationships in different ecosystem components across various sequences for (a) plant tissue, (b) litter, (c) forest floor and (d) mineral soil.

in plant tissue may be due to shifts in biomass dominance from photosynthetic to structural tissues (Sterner & Elser, 2002; Ågren, 2008). The proportion of woody biomass increases substantially during stand development. The increasing proportion of woody biomass could lead to an increase in the C : N ratio in plant tissue (Sterner & Elser, 2002; Ågren, 2008) because the C : N ratio in wood is much larger than that in leaves or branches (Ågren, 2008; Janssens & Luyssaert, 2009). Interestingly, the increasing rate of plant tissue C : N ratio over age sequence (i.e. the slope of the relationship between plant tissue C : N ratio and stand age within each sequence) decreased with stand age ($r^2 = 0.52$, $P < 0.05$) (Appendix S4a). The negative association may also be due to the decrease in the rate of accumulation of wood over the age sequence, which is driven by the decline in net primary production (NPP) during stand development (Ryan *et al.*, 1997). In addition, the increasing rate of plant tissue C : N ratio over age sequence exhibited a marginally significant decreasing trend with latitude ($r^2 = 0.29$, $P = 0.1$) (Fig. 1a), possibly due to the rapid accumulation of wood in tropical regions compared with temperate and boreal regions (Luyssaert *et al.*, 2007). On the other hand, the increase in the plant tissue C : N ratio over age sequence may be induced by shifts in species composition along the successional gradient (Knops & Tilman, 2000). However, in this synthesis, only one of ten sequences reporting plant tissue C : N ratio experienced significant shifts in species composition. Thus, changes in species composition should not make a large contribution to the increase in plant tissue C : N ratio over age sequence observed in this study.

By contrast, C : N ratios in the litter, forest floor and mineral soil remained remarkably stable over age sequence, consistent with Hypothesis 1. The stable C : N ratio in litter over age sequence could be driven by the relatively constant C : N ratio in leaves (e.g. Alberti *et al.*, 2008), as most individual studies col-

lected in this synthesis examined C and N dynamics in leaf litter over age sequence (e.g. Hughes & Fahey, 1994). In addition, part of the variation in litter C : N ratio might be hidden in the variability in C : N ratio in the forest floor and mineral soil. Also, litter production would be dominated by materials with a low C : N ratio over the long-term period of forest rotation (Ågren *et al.*, 2008). Both factors could potentially explain the relatively constrained C : N ratio in litter. The relatively constant C : N ratio in the forest floor and mineral soil may be ascribed to the close C–N coupling in the litter over age sequence. It is well known that both soil C and soil N are largely derived from humus formed after the degradation of dead organisms (Cleveland & Liptzin, 2007). Organisms tend to have a remarkably stable C : N ratio and the close C–N coupling exists even after plant detritus enters into soil as litter (McGroddy *et al.*, 2004). Consequently, the close C–N coupling in litter could lead to the tight linkage between C and N in both forest floor and mineral soil (McGroddy *et al.*, 2004; Cleveland & Liptzin, 2007; Yang *et al.*, 2010a).

N–C scaling patterns during stand development

N scaled isometrically with respect to C in litter, forest floor and mineral soil during stand development, supporting Hypothesis 2. By contrast, the scaling slope of the relationship between N and C in plant tissue was lower than 1.0 over age sequence, indicating that plants display an entirely different pattern of N and C scaling from the isometric pattern proposed in Hypothesis 2. Theoretically, the size of the scaling slope is consistent with the change in C : N ratio over age sequence due to the similar scaling intercept within certain ecosystems during stand development (Kerkhoff *et al.*, 2006). The isometric scaling relationship between N and C should be logically consistent with the relatively stable C : N ratios in these components over age

Table 1 Summary of reduced major axis (RMA) analysis of the log-transformed N–C stoichiometric relationship for different forest types.

Ecosystem component	Overall			Coniferous forests			Broadleaf forests			Temperate forests			Tropical forests		
	Slope	r^2	n	Slope	r^2	n	Slope	r^2	n	Slope	r^2	n	Slope	r^2	n
Plant	**0.71** (0.66,0.77)	0.89	77	**0.64** (0.58,0.71)	0.88	40	**0.72** (0.63,0.81)	0.86	37	**0.65** (0.58,0.73)	0.86	41	**0.74** (0.67,0.81)	0.94	30
Litter	1.12 (0.96,1.29)	0.67	62	0.95 (0.72,1.25)	0.88	18	1.01 (0.84,1.20)	0.71	44	0.99 (0.81,1.20)	0.70	33	0.95 (0.82,1.10)	0.86	29
Forest floor	0.96 (0.88,1.06)	0.85	66	0.97 (0.91,1.03)	0.95	44	1.00 (0.87,1.15)	0.91	22	0.97 (0.90,1.04)	0.95	43	n.a.	n.a.	n.a.
Mineral soil	1.03 (0.96,1.11)	0.74	176	0.99 (0.86,1.13)	0.70	69	1.05 (0.97,1.14)	0.88	107	1.03 (0.96,1.12)	0.82	139	1.03 (0.80,1.33)	0.69	22

n.a., not available.

The numbers in parentheses are 95% confidence intervals of the slopes of N–C stoichiometric relationships. Slopes statistically lower than 1.0 are indicated in bold type, while those not statistically distinguishable from 1.0 are presented in normal type.

sequence, while a lower scaling slope (i.e. scaling slope < 1.0) should result from the increasing C : N ratio over age sequence (Kerkhoff *et al.*, 2006). In this study, C : N ratios in litter, forest floor and mineral soil did not exhibit significant changes along the age gradient (Fig. 1b–d). Then, N accumulates in proportion to accrual of C during stand development and isometric patterns thus occur in these ecosystem components (McGroddy *et al.*, 2004; Niklas & Cobb, 2005; Cleveland & Liptzin, 2007). By contrast, the plant tissue C : N ratio increased dramatically during stand development (Fig. 1a), since N becomes increasingly diluted by metabolically inactive C-rich, N-poor structural components of woody tissues (Kerkhoff *et al.*, 2006). The increase in plant tissue C : N ratio can induce higher N use efficiency, and hence enable forest ecosystems to immobilize more C per unit of N uptake over age sequence (Hessen *et al.*, 2004). The decrease in proportional N investment with increasing forest biomass or production could result in a scaling slope < 1.0 (McGroddy *et al.*, 2004). Interestingly, the scaling slope decreased with stand age (r^2 = 0.48, P < 0.05) (Appendix S4b). The decrease in the scaling slope with stand age indicated that the N–C scaling relationship tended to move progressively away from the isometric pattern with the increasing experimental duration involved in individual studies (i.e. the length of individual sequences). Such a pattern could also be ascribed to the continuous increase of plant tissue C : N ratio induced by the increasing proportion of woody tissues through time.

C : N ratios were larger in coniferous forests than in broadleaf forests and higher in temperate forests than in tropical forests (Fig. 2). These differences may be due to different N use efficiencies among various forest ecosystems, as coniferous forests and temperate forests tend to be strongly N deficient compared with broadleaf forests and tropical forests (Vitousek & Howarth, 1991; McGroddy *et al.*, 2004). However, the N–C scaling slope did not reveal significant differences either between coniferous and broadleaf forests or between temperate and tropical forests (Table 1). Then, why are C : N ratios different when the scaling slopes are similar among various forest ecosystems? Theoretically, under isometric scaling conditions, the different C : N ratios among various ecosystems should be consistent with the different intercepts of the N–C scaling relationships among them (Kerkhoff *et al.*, 2006). It should be noted that these arguments do not conflict with our previous statements about the logical connections between the scaling slope of the N–C relationship and dynamics of the C : N ratio over age sequence. As mentioned above, ecosystems with similar N–C scaling slopes could have different C : N ratios due to different intercepts of the N–C scaling relationships among them. However, changes in the C : N ratio during stand development should be consistent with the N–C scaling slopes due to similar intercepts of the N–C scaling relationship within certain ecosystem over age sequence.

CONCLUSIONS

To our knowledge, this synthesis provides the first comprehensive analysis of terrestrial C : N stoichiometry through time. Our results showed that C : N ratios in litter, forest floor and mineral

soil did not vary with stand age, but the C : N ratio in plant tissue significantly increased with stand age within most sequences. The isometric pattern held true in litter, forest floor and mineral soil over age sequence, but plant N did not scale isometrically with plant C within most sequences. These results highlight that C and N become decoupled in plants but remain coupled in other ecosystem components during stand development. More broadly, these results also imply that plant C : N stoichiometry may exhibit considerable flexibility under global change scenarios, such as elevated CO_2 and enhanced N deposition (Sterner & Elser, 2002; Güsewell, 2004; Elser *et al.*, 2009). Future studies are required to investigate how C : N stoichiometry in terrestrial ecosystems responds to global environmental changes.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Characteristics of 39 age sequences collected in this study.
Appendix S2 A list of papers from which data were extracted for this synthesis.
Appendix S3 Frequency distributions of C : N ratios in different ecosystem components across various sequences.
Appendix S4 Relationships between the slope of C : N ratio versus age and stand age, and between the scaling slope and stand age.

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BIOSKETCHES

The research of **Yuanhe Yang**, PhD, is focused on large-scale ecology, and the responses of terrestrial ecosystems to global change.

Professor **Yiqi Luo** is interested in exploring patterns and processes in terrestrial ecosystems and biogeochemical cycles.

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