

## Linkages of Litter and Soil Carbon, Nitrogen and Phosphorus Stoichiometry in a Temperate Broad -Leaved Forest Stand

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**Background:** Measures of nutrient availability such as concentrations of carbon (C), nitrogen (N) and phosphorus (P) are important indicators of terrestrial ecosystems productivity. Current research illustrates the C, N and P stoichiometry of litter and soil in a coastal mixed forest stand, northern Iran.

**Materials and Methods:** To this, the *Carpinus betulus* (CB), *Acer velutinum* (AV), *Pterocarya fraxinifolia* (PF), *Quercus castaneifolia* (QC) species were considered; litter and soil (0-15cm depth) samples were taken under tree canopy cover.

**Results:** Litter and soil C: N ratio differed among the tree species, showing the highest (61.08 and 31.44) and lowest (21.90 and 3.59) under the QC and CB tree species, respectively. The litter and soil C: P ratio varied among the study sites and ranked in order of QC (52.4 and 27227.04) > PF (30 and 1465.61) > AV (15.74 and 630.54) ≈ CB (13.42 and 566.28). The higher amounts of litter N: P ratio were significantly found under QC (0.86) > PF (0.73) > CB (0.61) ≈ AV (0.55), whereas soil N: P ratio were significantly higher under CB (177.69) > PF (123.53) ≈ AV (121.60) > QC (109.25), respectively.

**Conclusion:** We found the species that differed in traits could influence C, N and P dynamics and its stoichiometry. The *Q. castaneifolia* species with different root traits that resulted in different vertical and horizontal distributions of C, N and P, reflecting differences in nutrient uptake by plants and microbial dynamics, drove the biggest changes in litter and soil C, N and P.

**Keywords:** Carbon, Ecological stoichiometry, Nitrogen, Phosphorus, Tree species

### 1. Background

In recent years a number of leading soil scientists have voiced warnings about developments in soil science and the possible consequences for the role of soil knowledge in major global issues like food production, the loss of biodiversity, and the availability of

ample and clean water resources (1). In the globalized world of the 21<sup>st</sup> century, soil sustainability depends not only on management choices by farmers, foresters and land planners but also on political decisions on rules and regulations, marketing and subsidies, while public perceptions are perhaps the most

important issue. The United Nations has proposed 17 sustainable development goals, which present a clear challenge to not only national governments but also a wide range of stakeholders (2). Soil is the complex expression of physical, chemical and biological processes occurring across spatial and temporal scales. Soil properties at a specific location integrate and reflect both past and present conditions. Above and belowground components of ecosystems are tightly linked and their interactions greatly affect ecosystem processes and properties (3, 4, 5). The feedbacks between above-and belowground components influence ecosystem functions and are affected by environmental factors (6). Soil nutrient concentrations, particularly nitrogen (N) and phosphorus (P), are important indicators of terrestrial ecosystems productivity. Equally important are their stoichiometric balances in relation to carbon (C) concentration, since strict proportions of C, N and P are key regulatory mechanisms for optimal plant growth and ecosystem functioning (7). The C: N: P ratio in soil directly reflected soil fertility and indirectly indicated plant nutritional status (8). These stoichiometric ratios are important from a functional perspective as indicators of substrate quality for decomposers (9), thus these stoichiometric ratios can influence important ecosystem processes such as detrital turnover and trophic dynamics in food webs (10).

Although it is widely recognized that vegetation canopy is important in hindering soil erosion, by comparison, the role of vegetation litter layers in modulating surface runoff and soil erosion remains poorly understood. Litter layers are known to protect soil from raindrop splashes by intercepting rainfall, preventing surface sealing and crusting of soil, extend the time of soil infiltration, and enhance sediment deposition by increasing soil surface roughness (2, 11, 12). In the forest ecosystems, leaf litter is of great ecological importance as a source of

nutrients in soils (13). The annual fall of fresh litter contributes up to 60-70% of N and up to 75-95% of P supply to living organisms. The amount of organic matter and nutrients that return to the soil through litter decomposition is key to the biogeochemistry of forest ecosystem (14). Different plant types can change soil C, N, and P through detritus and litter decomposition (which in turn depend on the chemical and physical composition of living plants), root secretion and N fixation, soil mineralization, and contributions to soil ecosystems from resident animals, insects and microorganisms (15). Furthermore, different plant species differ in their capacity to utilize and capture essential resources supplied by C, N and P (16). Several studies have shown that tree species can have an impact on the litter quality as well as soil physico-chemical and biological features (17, 18, 19, 20). In fact, litter quality characters, as the main driving force for soil processes, especially relative proportions of C, N and P contents vary between different tree species (19). Neiryneck *et al.* (21) found that C: N ratio in topsoil was variable under different tree species (*i.e. Tilia platyphyllos*, *Fraxinus excelsior* and *Acer psedoplatanus*) that was related to litter quality of these species.

The forests Hyrcanian vegetation zone of Iran are among the oldest forests in Asia and the northern hemisphere (22). The natural forest vegetation is temperate deciduous forests containing broadleaved species such as beech (*Fagus orientalis* Lipsky), oak (*Quercus castaneifolia* C. A. M. *macranthera* F. & M.), hornbeam (*Carpinus betulus* L.), maple (*Acer velutinum* Boiss., *Acer cappadocium* Gled.), ash (*Fraxinus excelsior* L.), alder (*Alnus subcordata* C. A. M., *Alnus glutinosa* Gaertn.), elm (*Ulmus glabra* Huds.), wild cherry (*Prunus avium* L), wild service tree (*Sorbus torminalis* Crantz), and lime tree (*Tilia platyphylus* Scop.). These forests appear to be very similar to broadleaf forests typical of central Europe,

northern Turkey and the Caucasus (23). In Hyrcanian forests, decomposition of organic matter plays a vital role in nutrient cycling, driving the mineralization of organically bound nutrients, and making them available for plant uptake (24). In infertile soils the role of decomposition processes becomes even more significant in nutrient cycling, since almost all plant available nutrients in these ecosystems originate from plant debris (25).

## 2. Objective

This study aims to investigate if different tree species affect litter quality and soil properties and how are the linkages of litter and soil C: N: P stoichiometry. We hypothesized that the influence of individual trees on litter and soil properties is detectable even in mixed stands and the soil landscape may be considered a mosaic of profiles reflecting the litter chemical characters through individuals of the various tree species present. The findings can be useful to knowing the ecosystem processes including nutrient cycling, decomposition and soil C storage involving with climate change scenario.

## 2. Materials and Methods

### 2.1. Study area

The study area is located at the Experimental Forest Station of Tarbiat Modares University, north of Iran (51° 46 " E, 36° 47" N) (Figure 1A, B). The experimental plots were located at an altitude of 15 m above sea level. The area is on flat and uniform terrain (slope 0–3%). Mean annual rainfall is 803.4 mm and mean annual temperature is 17° C with a dry season between May and August. The parent material is dolomite limestone which belongs to upper Jurassic and lower Cretaceous period. The soil order name is Alfisols. The natural forest vegetation is temperate deciduous forests containing broad-leaved species dominated by oak (*Quercus castaneifolia* C. A. M.

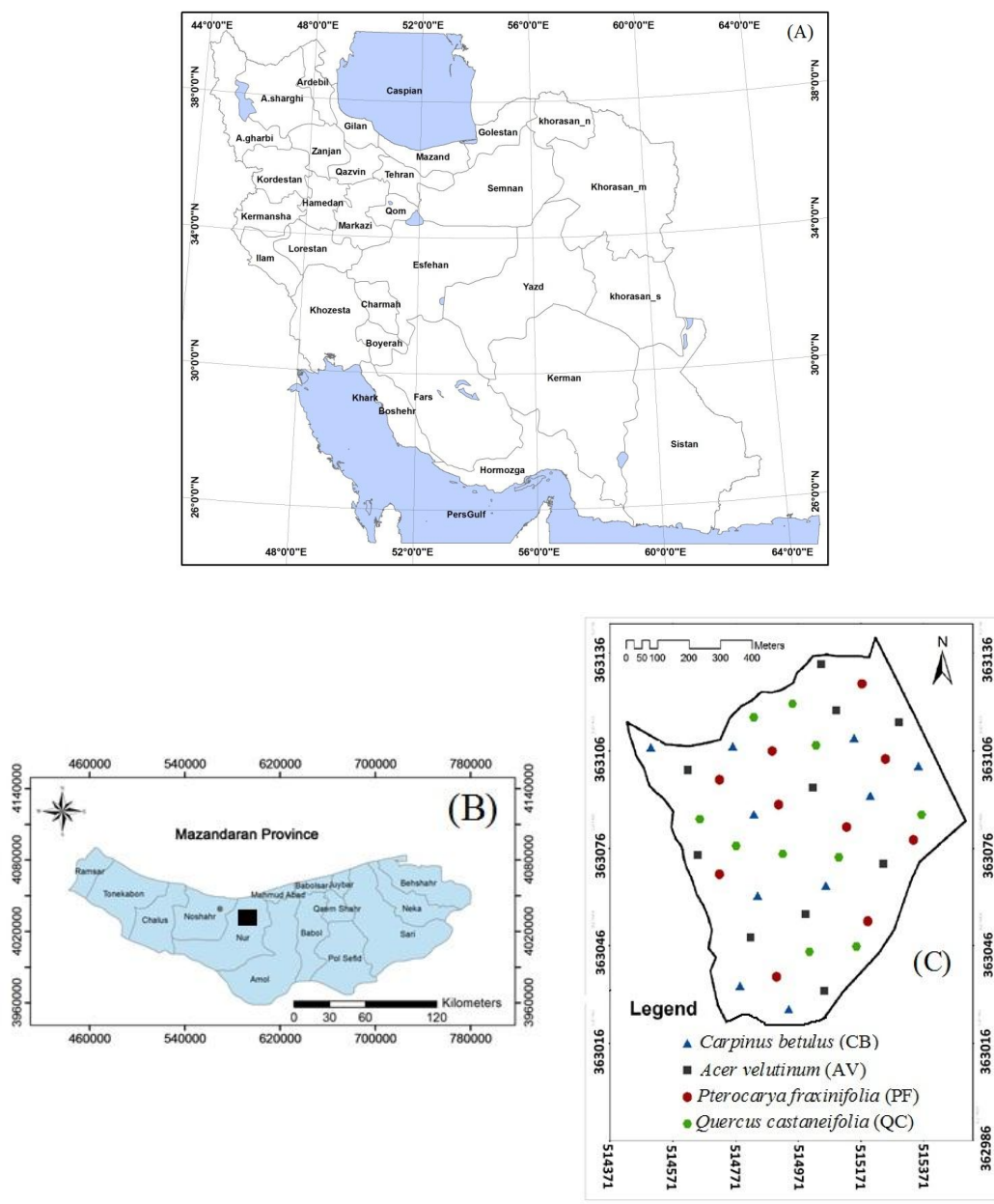
*macranthera* F. & M.), hornbeam (*Carpinus betulus* L.), elm (*Ulmus minor* Mill., *Ulmus glabra* Huds.), Caucasian Wingnut (*Pterocarya fraxinifolia*), Persian poplar (*Populus caspica* Bornm.) with some associated species such as maple (*Acer velutinum* Boiss., *Acer cappadocium* Gled.), ash (*Fraxinus excelsior* L.), alder (*Alnus subcordata* C. A. M., *Alnus glutinosa* Gaertn.), wild cherry (*Prunus avium* L), wild service tree (*Sorbus torminalis* Crantz) and lime tree (*Tilia platyphyllos* Scop.) (26).

### 2.2. Sampling and laboratory analysis

Following a field trip, clumps of tree species were identified in the study area (Figure 1C). Samplings were performed under individual tree of *Carpinus betulus* (CB), *Acer velutinum* (AV), *Pterocarya fraxinifolia* (PF), *Quercus castaneifolia* (QC) species in the same diameter class ( $\approx 50$ cm). These species were surrounded by similar tree species. Ten replication of each species was done; litter and soil (30×30×15 cm) samples were taken under tree canopy cover in the summer (August) to characterize litter and soil properties. Litter was stored in bags for transport to the laboratory, washed gently for 30 s to remove mineral soil, and dried at 70 °C for 48 h. Dried litter samples were finely ground and analyzed. Total C, N and P contents in litter samples were determined in quadruplicate using dry combustion with an elemental analyzer (Fisons EA1108, Milan, Italy) calibrated by the BBOT [2, 5-bis-(5-tert-butyl-benzoxazol-2-yl)-thiophen] standard (Ther moQuest Italia s.p.a.). Soil samples were air dried at room temperature (25°C), and then were passed through a 2 mm mesh sieve to remove coarse living roots and gravel and ground with a mill to pass through a 0.25 mm mesh sieve before chemical analysis. Soil C was determined using the Walkley-Black technique (27). The N was measured using a semi Micro-Kjeldhal technique (28). Also, P was determined with a spectrophotometer and

the Olsen method (29). Whole of concentrations were measured as percent and used to calculate stoichiometry ratios.

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**Figure 1** Location of the study site in the Hyrcanian zone, the Central Caspian region of northern Iran (A), Mazandaran Province (B) with sampling points map (C). The schematic design not in scale

### 2.3. Data analyses

Normality of variables was checked by Kolmogorov-Smirnov test, and Levene's test was used to examine the quality of the variances. One-way analysis of variance (ANOVA) was used to compare litter and soil C: N: P stoichiometry data among the different tree species. Duncan test was used to separate the means of dependent variables that were significantly affected by the treatments. Pearson linear correlation analysis was used to examine the relationships between C: N: P ratios of litter and soil. All statistical analyses were performed in SPSS v. 20 software.

## 3. Results

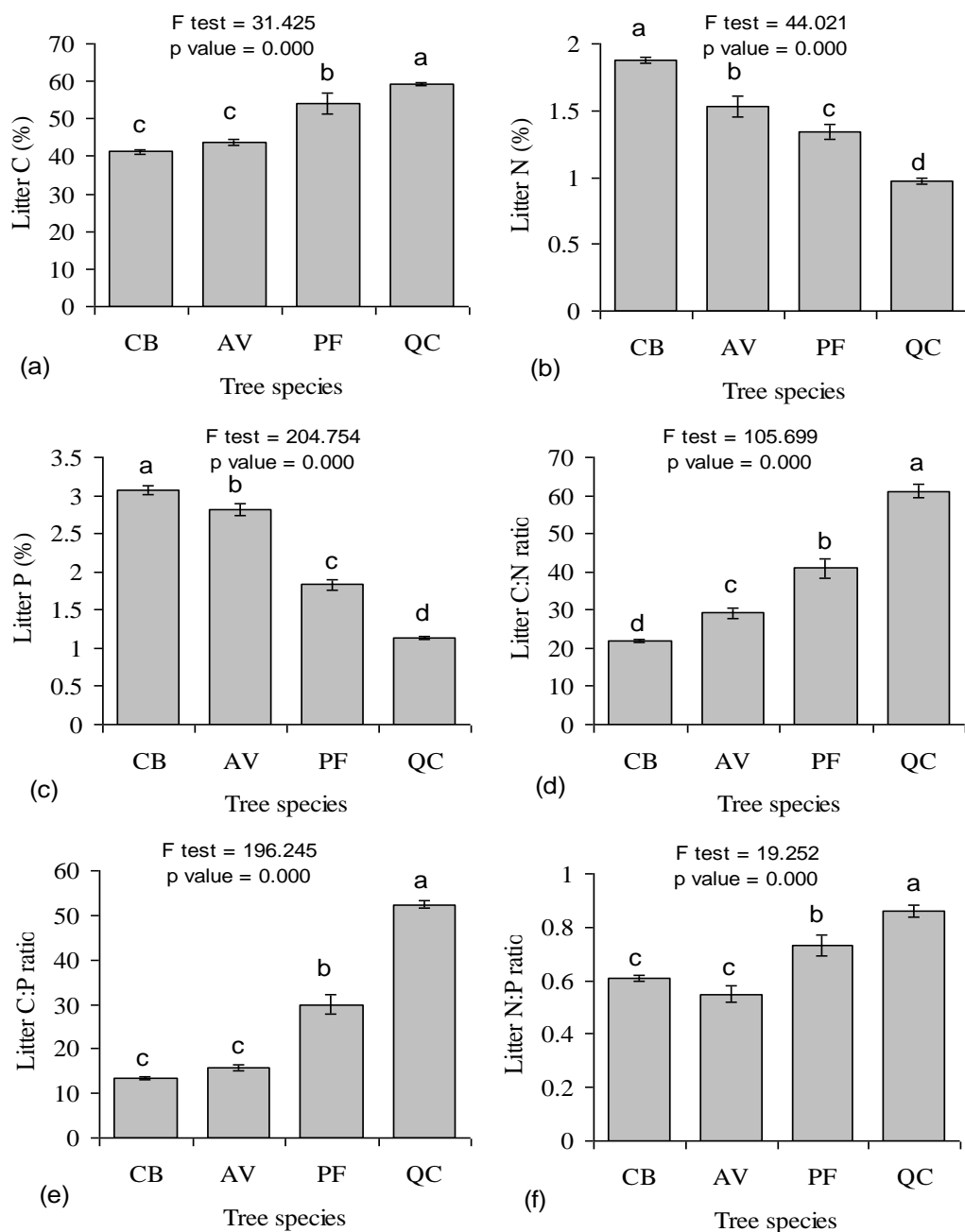
### 3.1. Litter chemistry

All tested litter properties were influenced by tree characteristics (Figure 2). The greatest values of litter C were observed in soils under QC followed by PF, AV  $\approx$  CB tree species (Figure 2a). Litter N and P were found in ranked order of CB > AV > PF > QC species (Figure 2b, c). There were significant differences in litter C: N, with the highest values in the QC site and decreasing values for PF, AV and CB tree types, respectively (Figure 2d). Litter C: P in the QC site showed

significantly higher values when compared with the PF > AV  $\approx$  CB tree species (Figure 2e). The N: P ratio of litter was significantly higher under QC species than under PF > CB  $\approx$  AV tree types (Figure 2f).

### 3.2. Soil chemistry

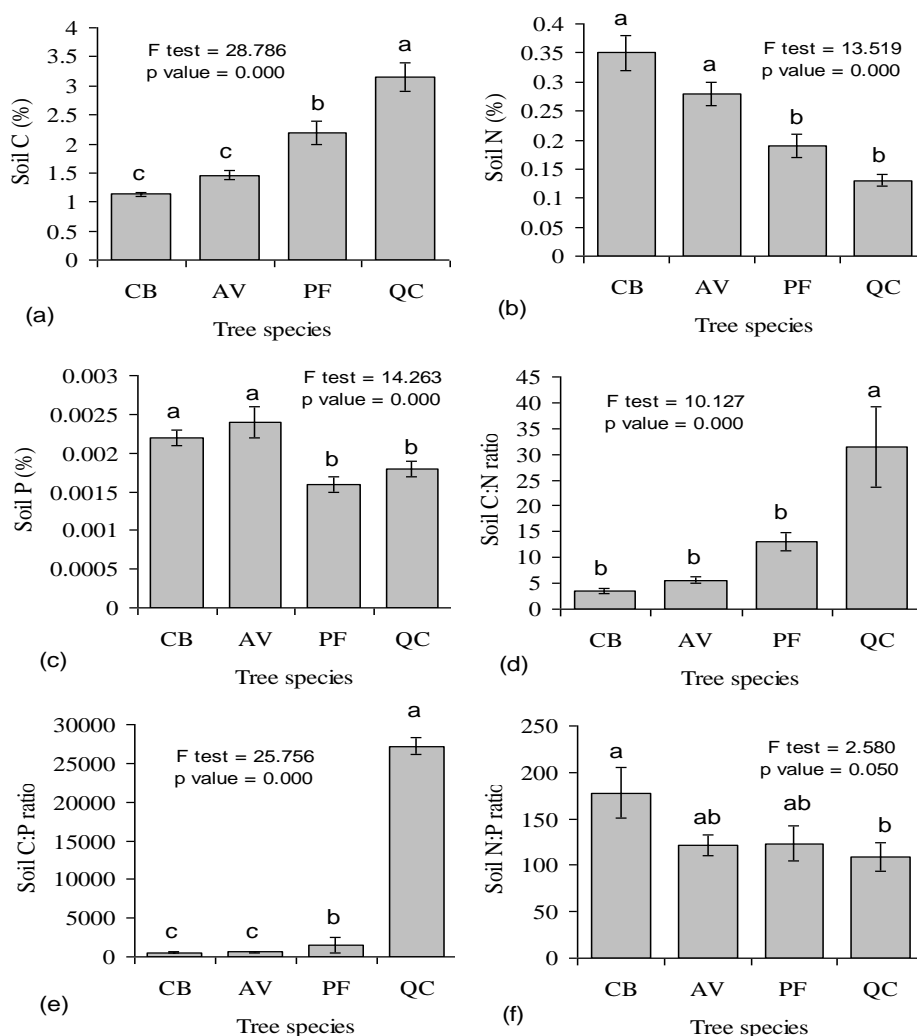
Soil C was significantly higher under QC trees when compared with the other stands (Figure 3a). A significantly higher content of N content was found under CB  $\approx$  AV compared with PF  $\approx$  QC tree types (Figure 3b). Soil P was significantly higher under AV  $\approx$  CB when compared with the QC  $\approx$  PF tree species (Figure 3c). Soil C: N ratio was significantly higher under QC than in the other tree species (Figure 3d). Higher values of soil C: P observed in the QC site compared with the PF > AV  $\approx$  CB trees (Figure 3e). Soil C: N and C: P ratio were positively correlated with C, C: N, C: P and N: P of litter but negatively related to litter N and P (Table 1). The amount of soil N: P varied among the study sites in the ranked order CB > PF  $\approx$  AV > QC tree types (Figure 3f), and it had a positive correlation with litter N and P; it correlated negatively with the litter C, C: N, C: P and N: P ratio (Table 1).



**Figure 2** Mean ( $\pm$ SE) of litter carbon (a), nitrogen (b), phosphorus (c), carbon: nitrogen ratio (d), carbon: phosphorus ratio (e) and nitrogen: phosphorus ratio (f) under different tree species. Lowercase letters over the bars indicate statistical significance at  $P < 0.05$ . The studied trees species were the *Carpinus betulus* (CB), *Acer velutinum* (AV), *Pterocarya fraxinifolia* (PF), *Quercus castaneifolia* (QC)

**Table 1** Pearson correlation coefficients (with *p*-value in parenthesis) of soil characters with litter features under different tree species

	Litter C	Litter N	Litter P	Litter C: N ratio	Litter C: P ratio	Litter N: P ratio
Soil C	0.817 (0.000)	-0.762 (0.000)	-0.812 (0.000)	0.855 (0.000)	0.846 (0.000)	0.599 (0.000)
Soil N	-0.551 (0.000)	0.630 (0.000)	0.722 (0.000)	-0.617 (0.000)	-0.646 (0.000)	-0.553 (0.000)
Soil P	-0.605 (0.000)	0.501 (0.001)	0.687 (0.000)	-0.622 (0.000)	-0.672 (0.000)	-0.620 (0.000)
Soil C:N ratio	0.592 (0.000)	-0.535 (0.000)	-0.619 (0.000)	0.625 (0.000)	0.653 (0.000)	0.506 (0.000)
Soil C:P ratio	0.730 (0.000)	-0.693 (0.000)	-0.782 (0.000)	0.799 (0.000)	0.821 (0.000)	0.625 (0.000)
Soil N:P ratio	-0.245 (0.128)	0.324 (0.042)	0.334 (0.035)	-0.259 (0.106)	-0.275 (0.086)	-0.230 (0.154)



**Figure 3** Mean (±SE) of soil carbon (a), nitrogen (b), phosphor (c), carbon: nitrogen ratio (d), carbon: phosphor ratio (e) and nitrogen: phosphor ratio (f) under different tree species. Lowercase letters over the bars indicate statistical significance at  $P < 0.05$ . The studied trees species were the *Carpinus betulus* (CB), *Acer velutinum* (AV), *Pterocarya fraxinifolia* (PF), *Quercus castaneifolia* (QC)

#### 4. Discussions

Our data showed that the influence of individual tree species could be detected in litter chemistry and topsoil even within a mixed stand. In addition to effects on soil C and nutrient pools and stoichiometry, species also appear to differentially regulate belowground nutrient cycling through N  $\times$  P interactions. For example, soil P content under AV and CB trees was higher than in other trees. Under nutrient-rich conditions, high foliar concentrations of N and P are mostly accompanied by low N: P in litter (30, 31, 32, 33). When the foliar concentrations of N and P are higher, less P is retranslocated while translocation of N seems independent of foliar N. Similarly, our data showed that where litter N and P concentration were high, the N: P ratio was low. The findings of this research demonstrated that C content in both of litter and soil were significantly higher under QC trees when compared to the other trees. This indicates that QC litter decomposed at a slower rate independent of soil N availability. The QC trees produce litter with lower N content and decomposition is controlled by N volatilization from litter (34). Increases in soil C through litter fall and rhizodeposition could potentially lead to increases in soil N (35).

Trees types can alter soil C stock through several key factors, litter fall, root turnover, litter quality and soil chemistry (16). In this study, soil C stock was positively correlated with litter C: N and C: P ratios. Litter and soil N content was significantly greater under CB. Lower C: N ratios under CB are driving increased N mineralization under CB compared to other trees (36). Chiti *et al.* (37) concluded that the introduction of good quality of litter caused a statistically significant positive increase in the concentration of both soil N and C. Litter quality is usually defined in terms of several indices, such as C: N ratio and lignin content. Many studies have examined the

relationship between total N and C: N ratio to litter decomposition (38). Decomposing organisms utilize N to breakdown C substrates, therefore, litters with high total N and narrow C: N ratio decomposes more rapidly, such as litter CB trees.

The relative importance of litter quality is influenced by soil properties and may change as decomposition progresses (39). The soil C: N ratio is an important indicator of the intensity of organic matter changes. The observed increase in the soil C: N ratio may result from a reduced decomposition rate of organic matter. This would give rise to a disproportionate increase in C content and a decrease in total N, which indicates that the soil is more resistant to the decomposition of soil organic matter. The C: N ratio in mineral soil decreased due to more a rapid N accumulation rate compared with the C accumulation rate (40). The CB is a symbiotic N<sub>2</sub>-fixer under N-limiting conditions, and thus produces litter of significantly higher N content compared to AV, PF and QC tree species. We observed an increase in soil and litter C, C: P and C: N under QC compared to CB trees. Whole of studied specie are deciduous species, but also clearly differ in litter quality. CB species has generally lower lignin-N ratios, more tannins, and faster decomposition rates than others. For these reasons, the forest floor layers tend to be lower under hornbeam compared to others especially under QC site (41).

Considering the relatively large effects of litter quality on litter decay and soil characteristics, it would be expected that studied trees also differ in N-dynamics. Net N-mineralization may be higher under CB than others, because initial N-content is a key parameter to net N-release, and N-rich litter often show higher net N-mineralization than N-poor litter (42). Aubert, *et al.* (43) pointed that CB trees generally had low lignin-N ratios, high tannins and fast decomposition rates. Low-



quality litter under QC species will release less N than the high quality litter under the other tree species, due to the fact that available nutrients are immobilized more rapidly by microbes decomposing low quality, nutrient-poor litter (44). In temperate forests the main limiting nutrient for plant growth is generally available N. Temperate hardwood stands are generally considered to be N-limited in the absence of anthropogenic N inputs. Our results thus suggest that increased P limitation at the site is driven mainly by increased tree demand under N sufficiency conditions, with a relatively lesser effect attributable to increased P fixation (45). However, the negative correlation with litter P was stronger than the positive correlation with soil N and combining N and P (N: P) gave the moderate correlation. This study emphasizes the differences in decomposition rates among common litter species in a temperate forest of Mazandaran province, northern Iran, based on litter quality and chemistry.

## 5. Conclusions

The objective of this study was to test the effect of *Carpinus betulus* (CB), *Acer velutinum* (AV), *Pterocarya fraxinifolia* (PF), *Quercus castaneifolia* (QC) species on litter and soil stoichiometry. Both litter and soil C content, C: N and C: P ratios were lowest under CB canopy covers. Whereas, soil C: N and C: P ratios were significantly higher under QC than in the other tree species. This study also suggests, however, that tree species with rapidly decomposing litter (i.e., *C betulus*) may be planted to improve soil fertility. But, *Q. castaneifolia* is driving the biggest changes in litter and soil C, N and P.

## Conflicting of Interests

There are no conflicts of interest with respect to the Tarbiat Modares University.

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## Authors' Contributions

Each of the authors contributed to the development of the paper.

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## ارتباط شیمی کربن، نیتروژن و فسفر لاشبرگ و خاک در یک توده جنگلی پهن برگ معتدل

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**مقدمه:** اندازه‌گیری عناصر غذایی قابل دسترس از قبیل کربن، نیتروژن و فسفر به عنوان شاخص‌های مهم حاصل‌خیزی اکوسیستم‌های خشک‌زی محسوب می‌شوند. پژوهش حاضر شیمی کربن، نیتروژن و فسفر لاشبرگ و خاک را در یک توده جنگلی آمیخته ساحلی در شمال ایران مورد بررسی قرار می‌دهد.

**مواد و روش‌ها:** بدین منظور لاشبرگ و خاک (عمق ۰ تا ۱۵ سانتی‌متری) بخش زیر تاج پوشش گونه‌های درختی ممرز، پلت، لرگ و بلوط جمع‌آوری شدند.

**نتایج:** نسبت کربن به نیتروژن لاشبرگ و خاک در بین گونه‌های درختی متفاوت بوده به طوری که بیش‌ترین (۶۱/۰۸ و ۳۱/۴۴) و کم‌ترین (۲۱/۹۰ و ۳/۵۹) مقادیر آنها به ترتیب در بخش تحتانی گونه‌های درختی بلندمازو و ممرز مشاهده شدند. نسبت کربن به فسفر لاشبرگ و خاک نیز در بین گونه‌های درختی مختلف تغییرات معنی‌داری را به صورت بلندمازو (۵۲/۴۰ و ۲۷۲۲۷/۰۴) < لرگ (۳۰) و ۱۴۶۵/۶۱ < پلت (۱۵/۷۴ و ۶۳۰/۵۴) ~ ممرز (۱۳/۴۲ و ۵۶۶/۲۸) نمایش داده است. مقادیر بیش‌تر نسبت نیتروژن به فسفر به طور معنی‌دار در بخش تحتانی بلندمازو (۰/۸۶) < لرگ (۰/۷۳) < ممرز (۰/۶۱) ~ پلت (۰/۵۵) مشاهده شد، در حالی که نسبت نیتروژن به فسفر به طور معنی‌دار دارای بیش‌ترین مقادیر خود در بخش تحتانی ممرز (۱۷۷/۶۹) < لرگ (۱۲۳/۵۳) ~ پلت (۱۲۱/۶۰) < بلندمازو (۱۰۹/۲۵) بوده‌اند.

**نتیجه‌گیری:** در این مطالعه آشکار شد که گونه‌های درختی مختلف می‌توانند بر پویایی کربن، نیتروژن و فسفر و شیمی آن‌ها اثرگذار باشند. گونه بلند مازو باعث تفاوت‌های اساسی در غلظت‌های کربن، نیتروژن و فسفر گردیده که این موضوع منجر به تفاوت‌هایی در جذب بوسيله گیاهان و پویایی‌های میکروبی گردیده است.

**کلمات کلیدی:** شیمی اکولوژیکی، فسفر کربن، گونه درختی، نیتروژن