

Carbon and nitrogen accumulation in forest floor and surface soil under different geographic origins of Maritime pine (*Pinus pinaster* Aiton.) plantations

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Abstract

Aim of study: To determine if plantations consisting of different geographic origins of the Maritime pine (*Pinus pinaster* Aiton.) could have altered C and N stocks in the forest floor and surface soils.

Area of study: Forest floor and mineral soil C and N stocks were measured in four adjacent plantations of different geographic origins of Maritime pine (Gironde, Toulon, Corsica and Spain) and adjacent primary native Sessile oak (*Quercus petraea* L.) at Burunsuz region in Belgrad Forest where is located in the Istanbul province in the Marmara geographical region between 41° 09'-41° 12' N latitude and 28° 54'-29° 00' E longitude in Turkey.

Material and methods: Plots were compared as common garden experiments without replications. 15 surface soil (0-10 cm) and 15 forest floor samples were taken from each Maritime pine origins and adjacent native Sessile oak forest. C and N contents were determined on LECO Truspec 2000 CN analyzer. The statistical significance of the results was evaluated by one-way Analysis of Variance (ANOVA).

Research highlights: Forest floor carbon mass, nitrogen concentration and nitrogen mass of forest floor showed a significant difference among origins. Soil carbon mass and nitrogen mass did not significantly differ among investigated plots.

Key words: carbon sequestration; C/N ratio; decomposition; exotic; tree provenance.

Introduction

Forest ecosystems play an important role in carbon (C) cycle, acting as a carbon sink (Penne *et al.*, 2010). Approximately 40% of the global soil C stocks reside in the forest ecosystems (Matos *et al.*, 2010). The C pool in the forest ecosystem is nowadays an objective to be taken into account in forest management, because C is accumulated in the forests through an increment in biomass, dead organic matter and soil C, and is released through respiration and decomposition (Del Río *et al.*, 2008). The C stored in terrestrial ecosystems is distributed in three compartments: biomass of living plants (stem, branches, foliage and roots), plant detritus (branches and cones, forest litter, tree stumps, toppings and logs) and soil (organic mineral humus,

surface and deep mineral soil). The rate of soil organic C sequestration and the magnitude and quality of soil C stock depend on the complex interaction among climate, soils, tree species, and management, as well as chemical composition of the litter determined by the dominant tree species (Compton *et al.*, 1998; Lal, 2005; Matos *et al.*, 2010). The C accumulation of the pine species has been studied by some researchers (Bravo *et al.*, 2008; Sever and Makineci, 2009; Tolunay, 2009; Fernández-Núñez *et al.*, 2010), however, there has been a gap for understanding of soil C accumulation and decomposition which is currently not sufficient for predicting changes in the amount of carbon sequestered in forest soils in the literature (Gorte, 2009).

Nutrient availability, such as Nitrogen (N), limits primary production in many terrestrial ecosystems (Soudzilovskaia and Onipchenko, 2005; Castro-Díez *et al.*, 2012). One of the important functions of soils

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is their ability to retain N (Vesterdal *et al.*, 2008), as N has a significant role in terms of plant nutrition as well as C. According to Aber *et al.* (1995), recent years has seen a dramatic shift in the focus of nitrogen cycling research in the forest ecosystems. The importance of forest canopy of tree species composition and litter quality as factors controlling N budget in the temperate forest soils studies are showing increasing tendency (Lawrence *et al.*, 2000; Groffman *et al.*, 2001). Consequently, it is important to quantify the N budget in the forest ecosystems.

In general forests have a greater influence on the soil conditions than most of the other plant ecosystem types. For instance forests greatly regulate/influence the developing of soils O horizon, moderating the temperature and humidity at the soil surface input of the litter with high lignin content, and also by high total net primary production and high water and nutrient demand (Binkley and Giardina, 1998). Moreover, different tree species can differ significantly in their influence on the soil properties as well as on the soil fertility (Brüggemann *et al.*, 2005). In this context, forest conversion to tree plantations is regionally important (Sevgi *et al.*, 2011).

Different origins-provenances of the same tree species can also have specific characteristics and effects on the forest ecosystems. Many researchers have indicated these differences in various research approaches, for example; chemical composition of needles of different Spanish Maritime pine provenances-origins (Arrabal *et al.*, 2005), different nutritional status of the Maritime pine populations (three families in NW Spain (Martins *et al.*, 2009), variations in growth, survival and carbon isotope composition among Maritime pine populations of different geographic origins (Correia *et al.*, 2008), variability of abundant proteins in seven geographical origins of Maritime pine (Bahrman *et al.*, 1994), and growth and water relations of three geographically separate origins of Maritime pine under saline conditions (Loustau *et al.*, 1995).

The impacts of different origins of the same tree species on C and N content in the forest floor and soils have been one of the priority research topics in the literature.

The primary objective of this study was to determine if plantations consisting of different geographic origins of the Maritime pine (*Pinus pinaster* Aiton.) with likely varied phenologies, resource requirements, growth rates, and chemical characteristics in litter fall could have altered C and N stocks in the forest floor and sur-

face soils. To address this, we measured forest floor and mineral soil C and N stocks, in four adjacent plantations of different geographic origins of Maritime pine (Gironde, Toulon, Corsica and Spain) and adjacent primary native Sessile oak (*Quercus petraea* L.) forest as common garden experiments without replications. Because this type of Mediterranean forest tree species cover large areas and show up in a wide variety of habitats. Moreover, they have great ecological significances in Mediterranean Basin (Correia *et al.*, 2008). The Maritime pine is also one of the important conifer types from the western Mediterranean Basin with a distribution that exceeds 4 million hectares under the broad ranges of elevation in suitable climate and soil conditions. The Maritime pines have notable genetic and phenotypic variability in their growth and traits of life-stories among populations (Alía *et al.*, 1995; Alía *et al.*, 1997; Fernandes and Rigolot, 2007; Jiménez *et al.*, 2011). In addition, the Maritime pine is one of the most preferable fast growing and exotic tree species in terms of afforestation in Turkey.

Material and methods

Study site

Belgrad Forest is located in the Istanbul province in the Marmara geographical region between 41° 09'–41° 12' N latitude and 28° 54'–29° 00' E longitude in Turkey. The average annual precipitation is 1,074 mm, annual mean temperature is 12.8°C, mean maximum temperature is 17.8°C, and the average minimum temperature is 9°C. The climate of Istanbul Belgrad Forest is a Maritime climate with medium water deficit in summers. All plots have same soil type on classification of Kantarcı (1980). According to the World Reference Base for Soil Resources (WRB), the soil group in the research area is Luvisol. Soils are well-drained and moderately deep. The general texture type of the soil is sandy clay loam. Soils are lime-free with no carbonate reaction, rich in organic matter on top soil and “acidic” around 5 pH. The growing season is for 7.5 months (230 days) on the average.

Four different origins of Maritime pine seeds from Gironde, Toulon, Corsica and Spain were delivered to Turkey in 1950 by Istanbul University, Faculty of Forestry, Department of Forest Yield and Biometry. The seedlings originating from seeds were planted with 2 × 2 m planting space range in their own plots at Bu-

runsuz region in Bahcekoy-Istanbul. The seedlings of Spain, Gironde and Toulon origins were planted in 1953 and the seedlings Corsica origin were planted in 1954. Thus, adjacent plots were established with four origins of Maritime pine. The study sites were established by introducing different Maritime pine origins instead of native Sessile oak forest. The sample plots were located side by side at the research area. The natural development of plantations without applying silvicultural treatments has been monitored over 60 years (Akarp, 2002).

Experimental analysis

We sampled four adjacent plantation plots planted with the different origins of Maritime pine (Gironde, Toulon, Corsica and Spain) and adjacent native Sessile oak (*Quercus petraea* L.) forest. Common garden experiments without replications were applied in the experiment design because these experiment provides an opportunity to minimize confounding effects since the different origins of the same tree species were planted in adjacent plots so that climate, parent material, time, hydrology and previous land use are almost the same (to keep other site-related factors similar between sites) (Sevgi *et al.*, 2011; Akburak *et al.*, 2013). All of the plots had a high homogeneity of abiotic environmental conditions (aspect, slope, elevation, and soil type).

The soil was left relatively undisturbed with no weed control treatments, no fertilization applied, and not subjected to any silvicultural treatment after the plantations were establishment. Before the collection of forest floor and soil samples, we determined the characteristics including density, average diameter (dbh) and height of the trees on the sample plots (Table 1).

Sampling was conducted at the central 20 × 20 m area within each adjacent plot to reduce the negative edge effects. The sample plots had homogeneity of abiotic environmental conditions (the aspect, slope,

elevation and soil type). The forest floor and top soil samples were assessed by collecting 15 samples (randomly selected 5 sampling subplots and 3 sampling occasion in per plots). Samples were taken from each Maritime pine origins and adjacent native Sessile oak forest for the comparison (15 × 5 = 75 soil and 75 forest floor samples, 150 samples in total).

The forest floor in the plots was sampled from the quadrats (0.25 m²). After collection of the forest floor samples, at the same points, the soil samples were taken from 0-10 cm soil depth with the aid of 80 cm³ steel soil cores with an inner diameter of 3.5 cm. The soil samples were dried at 105°C for 24 h and were subsequently weighed for determination of bulk density. The soil samples were sieved through 2 mm sieves to remove stones and roots by hand. Thus, the volumetric content of the fine soil (< 2 mm) was determined as a soil bulk density by omitting the total weight of the stones and roots.

Forest floor samples were dried at 65°C and were subsequently weighed. The samples were ground, and the Dumas dry combustion method was used for the determination of C and N contents using a LECO Truspec 2000 CN analyzer.

Statistical analysis

The statistical significance of the results under Maritime pine origins (Spain, Gironde, Toulon and Corsica) and the primary native Sessile oak forest were evaluated by one-way Analysis of Variance (ANOVA). The homogeneity of the variances were checked by Levene test ($p > 0.05$) and as the result of this analysis, Tukey's HSD (Honestly Significant Difference) test was conducted on equal homogeneity samples and the Tamhane's T2 Test was performed on the samples which were unequal and different. Statistical evaluation of the data was performed using the computer software package IBM SPSS STATISTIC 19 (IBM SPSS STATISTIC, 2010).

Results and discussions

The forest floor mass, soil bulk density, C and N concentrations-C/N ratio C and N masses of the forest floors and surface soils were evaluated according to four different geographic origins of the Maritime pine and native Sessile oak forest.

Table 1. Density, average diameter (dbh) and height of trees on sample plots

Characteristics	Oak	Spain	Corsica	Gironde	Toulon
Number (ha ⁻¹)	475	650	1,100	675	700
Diameter (dbh) (cm)	25.7	34.4	28.5	35.7	34.4
Height (m)	16.3	23.9	21.5	25.4	20.2

Forest floor mass and soil bulk density

In terms of forest floor mass per unit area, the Toulon origin has the lowest (498 g/m^2) and the Corsica origin has the highest value ($2,326 \text{ g/m}^2$). Forest floor masses of the Maritime pine origins indicated a statistically significant difference between each other at $p < 0.05$ level. Forest floor mass of native oak forest did not show any difference in Gironde, Spain and Corsica origins, only Toulon origin showed a statistical difference at $p < 0.05$ level (Table 2).

The Corsica origin has the highest soil bulk density value (808 g/l) and the Toulon origin (694 g/l) has the lowest. On the other hand, soil bulk densities did not present a statistical difference (Table 3).

The possible reason of existence the highest mass of forest floor under Corsica origin was the higher tree density than others (Table 1). The plots of other origins might be subjected a snow break and wind break in the field (Akalp, 2002). The typical thinning applied in pine reforestation, may have modified not only the amount of litter fall produced in the stands, but also the decomposition rates and processes which critically

affects the performance of understory herbs (Roig *et al.*, 2005). Similarly, Rozas *et al.* (2011) underlined that this is particularly true near the boundaries between bio-geographical regions, where the limiting conditions can differentially affect the growth of species with distinct origin.

As known, and as also mentioned in C section below; soil bulk density is highly related to soil organic matter-C content. Corsica origin has the highest bulk density with the lowest C concentration; similarly, Toulon origin has the lowest bulk density with the highest C concentration among plots (Table 3). On the other hand, many undetermined factors can be effective on soil bulk density (*e.g.* biological activity, existence of ground cover and their dead or live roots, soil air and water penetration, leaching or stabilizing of decomposition products in soil).

Carbon

The forest floor of native Sessile oak forest has the lowest C concentration (48.5%) among all the Mariti-

Table 2. Multiple comparisons of forest floor variables under Maritima pine origins and native oak forest by Post-Hoc Tests

Plots	Forest floor mass (g/m^2)		C (%)		N (%)		Carbon mass (g/m^2)		Nitrogen mass (g/m^2)		C/N	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Oak	1,727 ^{a,c,d}	847	48.5 ^a	1.5	1.00 ^a	0.08	841 ^{a,c,d}	418	17.3 ^{a,c}	8.5	48.6 ^{b,c}	4.7
Spain	1,136 ^c	259	51.5 ^{b,d}	2.1	1.23 ^b	0.11	586 ^c	138	14.0 ^a	3.4	42.1 ^a	4.5
Corsica	2,326 ^d	638	53.1 ^{b,c}	1.1	1.04 ^a	0.15	1,237 ^d	347	24.2 ^c	8.4	52.2 ^{c,d}	7.2
Gironde	1,680 ^a	298	50.4 ^d	1.3	0.94 ^a	0.14	845 ^a	143	15.7 ^a	3.2	54.8 ^d	8.0
Toulon	498 ^b	74	54.0 ^c	1.0	1.20 ^b	0.09	269 ^b	43	6.0 ^b	0.9	45.3 ^{a,b}	4.1

Values within columns followed by the same letter (a,b,c,d) are not statistically different at 0.05 significance level. Std.: standard deviation.

Table 3. Multiple comparisons of surface soil (0-10 cm) variables under Maritima pine origins and native oak forest by Post-Hoc Tests

Plots	Soil bulk density ($< 2 \text{ mm}$) (g/l)		C (%)		N (%)		Carbon mass (g/l)		Nitrogen mass (g/l)		C/N	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
Oak	791 ^a	128	3.16 ^{a,c}	0.78	0.20 ^a	0.04	25.0 ^a	7.4	1.60 ^a	0.43	16.3 ^{a,b}	4.7
Spain	760 ^a	58	3.24 ^{a,b,c}	0.99	0.19 ^a	0.04	24.8 ^a	8.5	1.40 ^a	0.27	17.9 ^{a,b}	5.4
Corsica	808 ^a	96	2.95 ^a	0.28	0.19 ^a	0.03	23.8 ^a	3.4	1.52 ^a	0.36	16.1 ^a	2.8
Gironde	706 ^a	132	3.58 ^c	0.19	0.21 ^a	0.05	25.3 ^a	5.1	1.43 ^a	0.30	18.1 ^{a,b}	4.1
Toulon	694 ^a	147	3.93 ^b	0.18	0.20 ^a	0.03	27.2 ^a	5.6	1.35 ^a	0.28	20.4 ^b	2.8

Values within columns followed by the same letter (a,b,c,d) are not statistically different at 0.05 significance level. Std.: standard deviation.

me pine origins, and statistically significantly differs at $p < 0.05$ level. The C concentration in forest floor of Toulon origin (54%) has the highest value and it showed a significant difference from the origins of Gironde and Spain. There was no statistically significant difference on forest floor C concentrations between Corsica origin and other investigated plots except the Gironde origin. Forest floor C mass of the Maritime pine origins as well as forest floor mass among themselves showed a significant statistical difference. The Corsica origin has the highest forest floor carbon mass (1,237 g/m²) while the Toulon origin has the smallest as 269 g/m² depending highly on significant differences forest floor masses. Native oak forest showed a significant difference with Toulon origin of Maritime pine in terms of the forest floor C mass, and it did not show any difference with the other origins (Table 2).

The Corsica origin which has the lowest soil carbon concentration value (2.95%) and showed a statistical significant difference between Gironde and Toulon origins. Toulon which has the highest concentration of soil carbon (3.93%) indicated a significant difference with the oak and other Maritime pine origins, while there was no statistical significance compared to Spain origin. Native oak forest exhibited a significant difference with only the Toulon origin on soil carbon concentration. The soil carbon mass of the Maritime pine origins and oak ranged between 23.8 g/l and 27.2 g/l, and no significant differences were detected (Table 3).

Previous researchers (Yelenik *et al.*, 2007; Follstad Shah *et al.*, 2010; Castro-Díez, *et al.*, 2012) concluded that the introduction of exotic plants may alter the nutrient cycle of the system in some ways. For instance, they directly alter by modifying the quality and quantity of litter entering the soils beneath, or indirectly, by altering the physical-chemical site properties below their canopy. Our results indicated that total forest floor C concentration differed almost significantly among investigated plots. Vesterdal *et al.* (2008) presented similar results with our study. In this context, Arrabal *et al.* (2005) indicated different chemical composition (monoterpens, sesquiterpens, neutral diterpens and resin acids) of needles of different Spanish Maritime pine provenances-origins. Martins *et al.* (2009) assessed the nutritional status of 22 young contemporary Maritime pine plantations and most of plantations showed severe nutrient deficiencies in Northwest Spain. Correia *et al.* (2008) found significant differences on the carbon isotope composition among Maritime pine populations in Portugal, France and Spain. Bahrman *et al.*

(1994) separated of Maritime pines from seven different geographic origins in three groups according to their abundant proteins-terpens. These results mostly indicated that different origins of same tree species can also have different litter qualities and nutrient capacities which are significantly effective on decomposition and carbon content of forest floor and soil.

On the contrary our results showed that there were significant statistical differences among Maritime pine origins in terms of soil carbon concentration. Kim *et al.* (2010) reported that the soil C and N concentration and their content at a soil depth of 0-10 cm were not affected significantly by the plantation type. In forest ecosystems, soil organic carbon concentration and stock are highly variable over space (horizontally and vertically) and time (Lal, 2005).

Nitrogen

In terms of forest floor N concentration; both of Spain (1.23%) and Toulon (1.20%) origins can be grouped separately from the oak forest and other Maritime pine origins. Native oak forest presented no statistical significant difference compared to Corsica and Gironde. Toulon origin has the lowest forest floor N mass (6 g/m²) and showed a significant difference from other plots. Corsica origin with the highest forest floor N mass (24.2 g/m²) indicated a significant statistical difference from Gironde, Spain, and Toulon origins at $p < 0.05$ level, while they did not show any significant difference with the native oak forest (Table 2).

Generally, the soil nitrogen concentrations ranged from 0.19% to 0.21% and no significant statistical differences were found among investigated plots (Table 3). The soil nitrogen masses changed between 1.35 g/l and 1.60 g/l, and similar to soil nitrogen concentrations, there was not a statistical difference in comparison.

The N concentration and mass in forest floor of plots were statistically different despite no differences on soil N concentrations and masses. As cited in C section above; different origins of the same tree species can also have different litter qualities and nutrient capacities particularly effecting decomposition and N budget of forest floor. Phenolic compounds can be effective on nitrogen preventing the loss by altering the form of nitrogen released from litter (Northup *et al.*, 1995) or stimulating the microbial immobilization (Castells *et al.*, 2004; Kraus *et al.*, 2004; Steltzer and Bowman, 2005).

Similar to our results on soil nitrogen with no differences among investigated plots, Kim *et al.* (2010) reported that the soil N concentration and content were not significantly different among the three coniferous plantations in their study. In contrast to C remaining, the N remaining in decomposing larch needle litter showed higher N gains than in either of the pine needle litters because the N in larch needle litter is readily immobilized by microorganisms due to its high concentration compared with pine needle litter (Kim *et al.*, 2010). Added to that, Compton *et al.* (1998) stated that the land use or vegetation type had no significant impacts on soil N concentration. Also, many researchers (Sever and Makineci, 2009; Kantarci, 2000; Kurz *et al.*, 2000) emphasized that forest floor accumulation, low nitrogen release and very slow decomposition rate of Maritime pine forest floor because of its natural needle characteristics and structure. This might also be the reason for difference of soil nitrogen among investigated plots. However, some researchers (Lovett *et al.*, 2002; Vesterdal *et al.*, 2008) opposed to this result. They reported that the variability in soil C/N ratios and N retention can be closely linked with variation in tree species composition.

C/N ratio

In forest floor, the Gironde origin (54.8) with the high C/N ratio value showed statistically significant ($p < 0.05$) difference from the other origins and native oak forest, except the Corsica origin. The Spain origin has the lowest C/N ratio (42.1) that exhibited a statistically significant difference from other plots, but there was no indication of statistical difference with the Toulon origin. Native Sessile oak forest presented a significant difference from other Maritime pine origins except the Toulon and Corsica (Table 2).

Soil C/N ratios was changed between 16.1 and 20.4 among investigated plots. Soil C/N ratio of Corsica origin which has the lowest value (16.1) and Toulon origin which has the highest value (20.4) showed statistically significant difference, however, there was no statistical difference found between the other origins and native oak forest (Table 3).

Forest floor and soil N pools and C/N ratios are important parameters for assessment of tree species effects on ecosystem functioning (Vesterdal *et al.*, 2008). Our results on the forest floor C/N ratios of *Pinus pinaster* origins were in the range of 42 and 55.

Edmonds (1980), Berg (1986) and Joseâ Moro and Domingo (2000), informed that critical C/N ratios ranging from 19 to 109 in the conifers. When compared to literature, we detected that the obtained C/N ratios were lower than the reported. For instance, the initial C/N ratios for *Pinus pinaster* and *Pinus nigra* for other conifer species are higher than our results (Klemmedson, 1992; Joseâ Moro and Domingo 2000). The C and N distribution of the surface soil layer might reflect differences in the quality and quantity of litter fall inputs, litter decomposition dynamics, and the production and turnover of fine roots, which are the principal pathways for the return of C and N to the soil (Finzi *et al.*, 1998; Wang *et al.*, 2009; Kim *et al.*, 2010). On the other hand, there are many factors which were mentioned in different sections of the manuscript and are mainly effective on soil C/N ratios such as biological activity, nitrogen use efficiency of vegetation and soil organisms, mineralization, leaching or fixation.

Conclusion

In this study, C and N accumulation in surface soil and forest floor under different origins of maritime pine were investigated, 60 years after the establishment. Various origins of maritime pine showed significant statistical differences especially in terms of C and N concentration in forest floor. We monitored some statistical differences in surface soil for C concentration among the origins, however, we could not detect any statistical differences for soil nitrogen concentration. Natural oak forest exhibited statistical differences for both nitrogen and carbon concentration on forest floor and presented some statistical differences with some of the maritime pines in terms of the soil carbon concentration.

On the other hand, as a limitation of present study, the research was conducted on non-replicated experiment plots in a single local area, and one single origin of Maritime pine was in one experimental plot with the aim of keeping characteristics of research site as common among plots. Meanwhile, there are also some peculiar site characteristics of the research area. Therefore, the results are specific to this study. In addition, the measurements performed only once after 60 years the establishment of origins in the research area. The possible impacts on carbon and nitrogen balance of forest floor and soil during the consisting 60 years period are still uncertain.

We believe that, in the future, more detailed conclusions related with this subject can be obtained with to conduct long-term studies which have many replications of different origins of Maritime pine under various edaphic, climatic and site characteristics in Turkey. Not also Maritime pine is used frequently for afforestation as a fast growing exotic species but also it is still an important species for new plantations to create future forest ecosystems of Turkey.

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