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Microbial biomass and N mineralization in mixed plantations of broadleaves and nitrogen-fixing species

E. L. Pereira*, S. A. P. Santos, M. Arrobas and M. S. Patricio

Mountain Research Center (CIMO). School of Agriculture. Polytechnic Institute of Bragança. Campus Sta. Apolónia. Apdo. 1172. 5301-855 Bragança. Portugal

Abstract

Mixed stands with nitrogen fixing secondary species can improve the vigour and the stand environment of the targeted species. The aim of this study was to better understand the effect of the consociation of a N-fixing species (black locust) with a broadleaf quality timber production (wild cherry). The study was conducted in 11 year old plantations located in the Northeast of Portugal. The treatments considered were: pure black locust, pure wild cherry and mixture of wild cherry and black locust. Each plot had six lines with 12 trees and a buffer strip line. The samplings were collected on the plantation line within a radius of 50 to 100 cm from the tree. In each plot we measured soil N mineralization dynamic, soil microbial biomass carbon (MBC) and nitrogen (MBN), microbial quotient (MBC/Corg), metabolic quotient (qCO_2), microbial respiration and dehydrogenase activity. Results showed a positive impact of the black locust species on the supply of nitrogen to the soil. The net N-mineralization rates were, at the end of this study, about three times greater in the pure black locust than in the pure wild cherry and about two times greater in the mixture than in the pure wild cherry. MBC and cumulative soil respiration were higher in the mixture than in the pure cherry plantation soil which may reflect positive changes in the soil environment.

Key words: *Prunus avium*; *Robinia pseudoacacia*; accessory trees; soil respiration; dehydrogenase activity; metabolic quotient.

Resumen

La biomasa microbiana y la mineralización de N en plantaciones mixtas de frondosas y especies fijadoras de nitrógeno

Bosques mixtos con especies secundarias fijadoras de nitrógeno puede mejorar el vigor y el ambiente del rodal de las especies objetivo. El objetivo de este estudio era comprender mejor el efecto de la asociación de especies fijadoras de nitrógeno (algarrobo negro) con especies frondosas productoras de madera de calidad (cerezo silvestre). El estudio se realizó en plantaciones con una edad de 11 años situados en el nordeste de Portugal. El muestreo fue realizado en tres tipos de plantaciones: algarrobo negro, cerezo silvestre puro y una plantación mixta de ambas especies. Cada parcela tenía seis líneas con 12 árboles y una línea de borde. Los muestreos se recolectaron en la línea de plantación dentro de un radio de 50 a 100 cm del árbol. En cada parcela se midió la mineralización de nitrógeno en el suelo, el carbono en la biomasa microbiana (MBC), el nitrógeno (MBN), el cociente microbiano (MBC/Corg), el cociente metabólico (qCO_2) y la respiración microbiana. Los resultados mostraron un impacto positivo del algarrobo negro en el suministro de nitrógeno al suelo. Las tasas netas de mineralización de N fueron, al final del estudio, aproximadamente tres veces superiores en el algarrobo que en el cerezo y dos veces superiores en la plantación mixta que en cerezo. MBC y la respiración del suelo acumulada fue más elevada en las plantaciones mixtas que en el suelo de las plantaciones de cerezo puro que puede reflejar cambios positivos en el ambiente del suelo.

Palabras clave: *Prunus avium; Robinia pseudoacacia;* árboles accesorios; respiración del suelo; actividad deshidrogenasa; cociente metabólico.

* Corresponding author: epereira@ipb.pt
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Introduction

In Portugal, the forestation of set aside agricultural lands (EU reg. 2080/92) brought new challenges to agroforestry land use increasing the possibility of production of valuable broadleaves tree species. Wild cherry (*Prunus avium* L.) is one of these promising species for high quality wood production. Cherry wood is used especially in the veneer and furniture industry. Nowadays, there is an increasing interest in the wood of this species which can attain high prices in the markets. In Portugal, until the nineties the wild cherry was practically ignored in afforestations. Its existence was limited to some trees spread in forests, generally growing in mixed stands. After 1992, the afforestation area with wild cherry was greatly increased due to the European Union (EU) subventions. Actually, the oldest stands, mostly pure, are about sixteen years old and many of them are experiencing a high rate of mortality, gum pockets and low vigour. These problems are not generally observed in the situation where cherry grows naturally in mixtures under similar site conditions. Thus, we think the stand environment, the cultural practices and the genetic factors are major issues both for vigour of the species and quality of the timber.

Mixed stands with nitrogen fixing secondary species planted in abandoned agricultural lands are a cultural intensification process that can improve the vigour and the stand environment of the targeted species. Kelty (2006) stated that an adequate mixture favours the ecological soil properties, the site fertility and the resistance to the biotic and abiotic factors. Also, the productive potential increases in mixed stands, in relations to pure stands, mainly due to the greater availability of nitrogen in the soil provided by the accessory species, since nitrogen availability is frequently the most limiting factor for plant growth in temperate forests. Buresti and Frattegiani (1994) and Hellmann *et al.* (2011) referred that the presence of nitrogen fixing secondary species [*e.g. Alnus cordata* (Loisel.) Desf., *Robinia pseudoacacia* L., *Elaeagnus angustifolia* L. or *Acacia* longifolia (Andrews) Willd.], among other aspects, improves soil fertility by enriching the system with N derived from atmospheric N_2-fixation. Many times, the high productivity of wood in mixed stands has been attributed to the improvement of mineral nutrition (Kelty, 1992; Frivold and Kolström, 1999; Binkley, 2003; Tani *et al.*, 2006). Moreover, in mixed compositions of compatible species, mechanisms of competitive reduction or facilitation processes occur, reflecting different levels of exploitation by the species at the territorial level (Vandermeer, 1989; Kelty, 1992).

Nutrient availability and productivity of agroecosystems mainly depend on the size and activity of the soil microbial biomass (Friedel *et al.*, 1996) that is responsible for regulating nutrient cycling, and acts as a highly labile source of plant-available nutrients (Kara and Bolat, 2008). There is increasing evidence that microbial properties could be used as potential indicators of impacts of forest management practices on soil (Mendham *et al.*, 2002; Li *et al.*, 2004). In fact, several authors consider that the ratio of the soil microbial biomass carbon to soil organic carbon, soil microbial biomass nitrogen, the metabolic quotient and soil enzyme activities are reliable indicators of changes in soil management compared to total organic C and N, which are unresponsive over short periods (Powlson *et al.*, 1987; Mendham *et al.*, 2002; Li *et al.*, 2004; Caldwell, 2005).

The aim of this study was to better understand the effects of a nitrogen fixing secondary species, black locust, on the consociation with wild cherry. Black locust was used as a nitrogen fixing model species and the results obtained can be extrapolated to other species since some of the effects seem to be consistent across many sites and species (Binkley, 2003). To reach this objective, we measured soil microbial biomass carbon (MBC) and nitrogen (MBN) as well as the dynamic of nitrogen mineralization. We also determined the ratio of MBC to soil organic carbon (MBC/C_{org}), soil respiration and dehydrogenase activity, related with microbial activity in the soil, and the metabolic quotient (qCO_2) as a measure of microbial efficiency. Thus, we hypothesized that these biological indicators, which are considered sensitive indicators in understanding the response of soil microflora to alterations in soil environmental conditions, as well as N mineralization dynamics are higher in the mixed plantation than in the pure wild cherry.

Material and methods

Study area

The present study was conducted in 11 year old plantations located in Uva-Vimioso (41° 34' 12'' N; 6° 30' 7'' W; altitude 750 m; slope < 5°), Northeast of Portugal. The mean annual temperature is 12°C and the mean annual precipitation is 555 mm. The soil was classified as Dystric Leptosols (FAO, 1998).

Table 1. Mean diameter breast height (DBH), mean height and mean soil characteristics in the upper 10 cm soil layer in pure cherry (PP), pure black locust (PR) and mixed cherry × black locust (MPR) plantations

Treatments	Mean DBH (cm)	Mean height (m)	Bulk density (Mg cm^{-3})	Organic C (g kg^{-1})	Total N (g kg^{-1})	C:N	pH (in H_2O)
PP	3.50 (0.15)	3.40 (0.09)	1.19^a (0.05)	12.15^a (2.18)	0.96^a (0.03)	12.56^a (2.15)	5.56^a (0.09)
PR	4.35 (0.13)	4.77 (0.09)	1.04^a (0.52)	11.23^a (1.45)	1.00^a (0.03)	11.18^a (0.65)	5.62^a (0.21)
MPR	3.93* (0.18)	3.80* (0.11)	1.03^a (0.08)	13.38^a (1.44)	1.08^a (0.05)	12.27^a (0.94)	5.64^a (0.06)

Values are means with standard errors given in parentheses. Values in the same column followed by the same letter are not significantly different at $p < 0.05$ according to LSD test. * Dendrometric parameters for wild cherry.

The experimental design included the following treatments: pure wild cherry, *Prunus avium* (PP); a pure secondary N-fixing species, the black locust, *Robinia pseudoacacia* (PR), and a mixture of wild cherry and black locust (MPR) alternately lined (intimate mixture). Planting density was 1,667 tree ha^{-1} with 3 × 2 m spacing. Each plot had six lines with 12 trees and a buffer strip line. In each plot, four randomly sampling points were considered for material collecting. The samplings were collected on the plantation line within a radius of 50 to 100 cm from the wild cherry tree in the mixture and the respective species in the pure treatments. General characteristics of the stands are presented in Table 1.

Soil sampling and analyses

The dynamics of nitrogen mineralization was studied *in situ* in the experimental plantations from February to September 2008, using a method adapted from Raison *et al.* (1987). Four replicate cores per site were periodically sampled. After sampling, the samples were sieved and frozen at –18°C until processed. Mineral-N was extracted from each core sample with 2 M KC1 solution (1:5, w/v) for 1 h. NO_3-N was determined in extracts by the method of sulfanilamide/α–naphthylamine, after its reduction in cadmium column and NH_4-N by Berthelot reaction. All determinations were done in an autoanalyzer (Skalar San-Plus) by molecular absorption spectrophotometry.

Net nitrogen mineralization for a time interval was calculated from the difference between the contents of inorganic nitrogen in the initial and incubated samples (Raison *et al.*, 1987; Uri *et al.*, 2003). Therefore, for each time interval $\Delta t_i = t_{i+1} - t_i$

$$\Delta N_{min} = \Delta NH_4^+ - N_i + \Delta NO_3^- - N_i \quad [1]$$

was measured, where ΔN_{min} is the net increment in available inorganic nitrogen, $\Delta NH_4^+ - N_i$ the net ammonification, and $\Delta NO_3^- - N_i$ the net nitrification in the time interval Δt_i.

$$\Delta c(NH_4^+ - N)_i = c(NH_4^+ - N)_{i+1} - c(NH_4^+ - N)_i \quad [2]$$

$$\Delta c(NO_3^- - N)_i = c(NO_3^- - N)_{i+1} - c(NO_3^- - N)_i \quad [3]$$

where $c(NH_4^+ - N)_i$ is the mean concentration of ammonium nitrogen in the initial samples, $c(NH_4^+ - N)_{i+1}$ is the mean concentration of ammonium nitrogen in the incubated samples at the end of the incubation period, $c(NO_3^- - N)_i$ is the mean concentration of nitrate nitrogen in the initial samples, and $c(NO_3^- - N)_{i+1}$ is the mean concentration of nitrate nitrogen in the incubated samples at the end of the incubation period. Net ammonification [4] and net nitrification [5] were calculated as follows:

$$\Delta NH_4^+ - N_i = k\Delta c(NH_4^+ - N)_i \quad [4]$$

$$\Delta NO_3^- - N_i = k\Delta c(NO_3^- - N)_i \quad [5]$$

where k is the weight of a soil layer per hectare.

Soil microbial biomass C (MBC) and N (MBN) were determined on fresh soil samples collected in April and June 2008, using the chloroform fumigation-extraction method (Vance *et al.*, 1987). The samples were collected by bulking random cores taken with a 4 cm diameter Dutch auger of 10 cm depth which were then sieved moist to < 2 mm. Plant material, stones and visible organisms were removed by hand. Field moist soils were stored at 4°C until processed. Twenty-five grams of mineral soil was fumigated with ethanol-free chloroform, incubated for 24 hours in the dark, at 25°C. Fumigated samples and controls (not fumigated) were extracted with 100 mL of 0.5 M K_2SO_4 (extractant-to-soil ratio of 4:1) for 1 hour on a reciprocating shaker and filtered through Whatman Nº 42 filter papers. The extracts were frozen at –18°C until further analysis. Organic C and total soluble N were determined by near infrared detection (NIRD) and by chemiluminescence detection after combustion at 850°C in an elemental

analyzer (Formac, Skalar). Soil MBC was calculated as the difference in extractable C contents between fumigated and control samples divided by a K_{EC} factor of 0.38 (Vance *et al.*, 1987). The K_{EC} factor was used to account for the efficiency of extraction for MBC. All results are expressed on an oven-dry (105°C) weight basis.

Soil respiration was measured in a laboratory incubation experiment for 42 days from samples collected in April. A moist sample of 50 g was placed in a 0.750 L glass jar and the soil moisture was adjusted to about 60% of water holding capacity with distilled water. Five containers, without soil, were considered as blanks. Then a plastic vial containing 40 mL 1 M NaOH was placed in the jar and replaced with a fresh NaOH at 3, 15, 30 and 42 days after the start of the incubation. A vial containing 40 mL of distilled water was placed in the jar to prevent soil desiccation. Upon replacing the NaOH solution, the jar was opened and samples were re-aerated to supply adequate oxygen. The evolved CO_2 was trapped in NaOH and the excess alkali was titrated with 0.24 M HCl after precipitating the carbonate with 1.5 M $BaCl_2$ solution. Soil respiration was calculated as the accumulative CO_2-C released from the soil.

The qCO_2 was calculated by dividing basal respiration (the average CO_2-C respired during 78 h, mg CO_2-C h^{-1}) by microbial biomass C and it was expressed as mg CO_2-C mg^{-1} C_{mic} per day.

Dehydrogenase activity (DHA) was determined by the method adapted from Solaiman (2007) using 1 mL of a 0.1 M Tris/2,3,5-triphenyltetrazolium chloride (INT) substrate solution per 5 g of soil (dry weight equivalent). INT was reduced to a red-colored iodonitrotetrazolium formazan (INTF) that was detected using a spectrophotometer (464 nm) after incubation (24 h at 40°C).

Statistics analyses

One-way analysis of variance was performed to test MBC, MBN, MBC/C_{org}, qCO_2, soil respiration and dehydrogenase activity as well as the dynamic of nitrogen mineralization by period and by treatment, using the SPSS version 13.0 for Windows. The assumptions of ANOVA were previously verified. Least significant differences test (LSD) was done for mean multiple comparisons when significant differences were observed.

Results

Soil physical and chemical properties

In the present study, physical and chemical properties in soils collected from the three experimental plantations showed that these treatments were very similar since there were no significant differences ($p > 0.05$) in soil bulk density, organic C, total N contents, C/N ratio and pH values determined in the different treatments (Table 1).

Dynamics of soil mineral nitrogen

The mean concentrations of soil mineral nitrogen in the initial samples, collected from February to September, varied between treatments during the study period for the upper 10 cm layer. In general, the highest concentrations of mineral N, $NO_3^- - N$ and NH_4^+-N in soil were observed in PR treatments (Fig. 1). Only in September, the mean concentrations of soil mineral N and $NO_3^- - N$ in PR (20.36 and 11.30 mg kg^{-1}, respectively) and MPR (14.89 and 10.69 mg kg^{-1}, respectively) were significantly higher ($p < 0.05$) than in PP (5.04 and 2.84 mg kg^{-1}, respectively). In the other sampling months no significant differences were observed among treatments. Concerning NH_4^+-N, in the same month, significant differences were observed between PR and the other treatments.

The concentration of mean NH_4^+-N during the months under study varied significantly ($p < 0.05$) only in the PP treatment between February and the summer months (June, July and September). In regards to $NO_3^- - N$, no significant differences were observed. However, in the MPR treatment the mean $NO_3^- - N$ concentration obtained in September was significantly higher ($p < 0.05$) than that observed in the previous months. The concentration of mean mineral N in the PP treatment decreased during the months under study. However, the differences were significant only between the months of February and both July and September. In the PR treatment, a significant difference was observed only between the months of April and July in relations to September. In contrast, in the MPR treatment, the mean mineral N concentration was similar during the study period.

Considering the daily net N mineralization and the daily net N nitrification, significant differences ($p < 0.05$) were observed in the periods of February-April and July-September between PP and PR treatments (Fig. 2). However, for the daily net N ammonification,

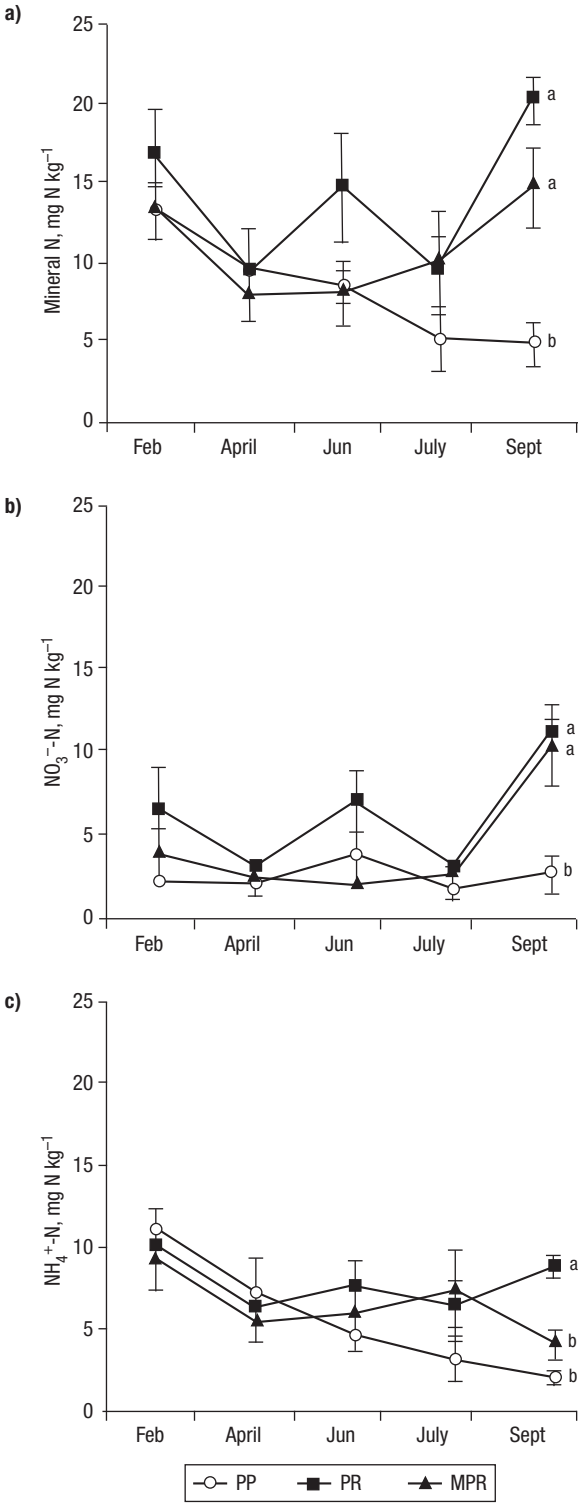

Figure 1. Mean concentrations of mineral N (NO_3^- – N + NH_4^+ – N,) (a), NO_3^- – N (b) and NH_4^+ – N (c) in the 0-10 cm mineral soil in pure treatments of black locust (PR); pure cherry (PP) and mixed cherry × black locust (MPR). Vertical bars are standard errors (n = 4). In September, different letters indicate significant difference among treatments ($p < 0.05$).

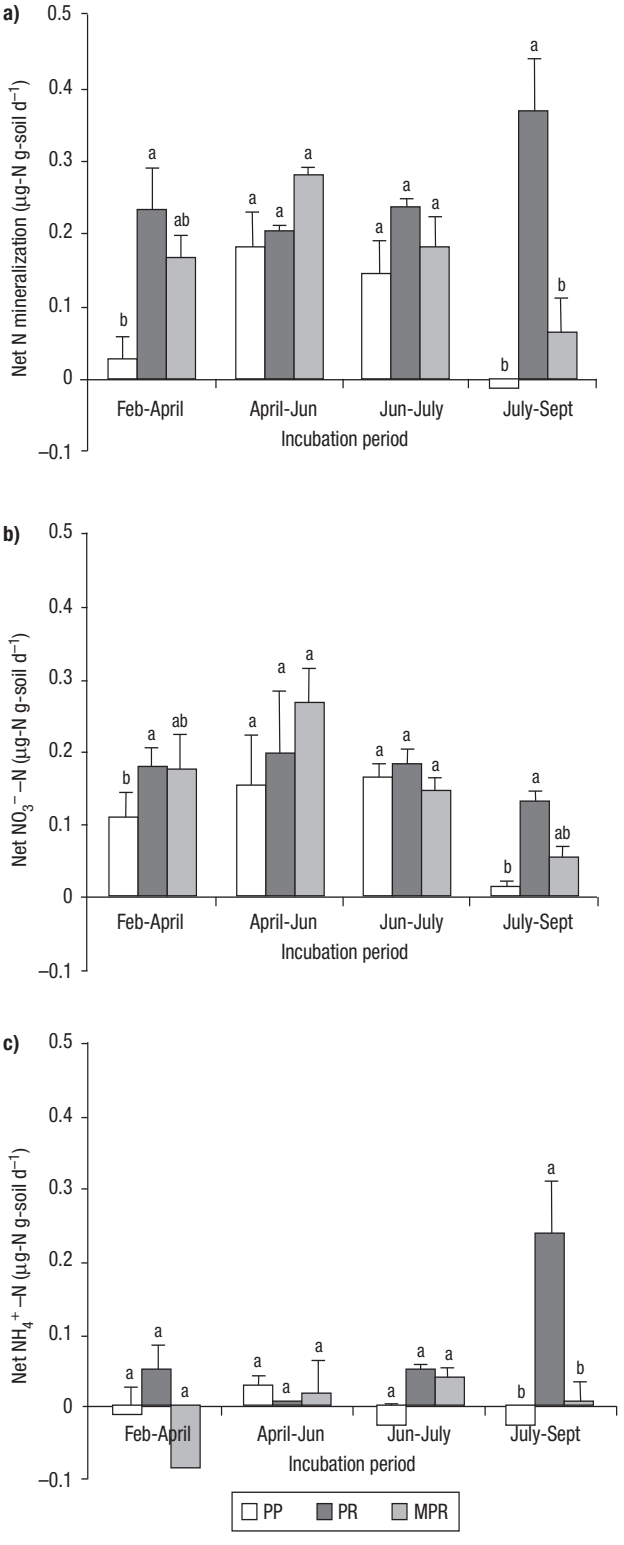

Figure 2. Average daily of net N mineralization (a), net nitrification (b) and ammonification (c) (µg-N g-soil^{-1} day^{-1}) in the upper 10 cm layer. Vertical bars are standard errors (n = 4); Different letters, in same incubation period, indicate significant differences among treatments ($p < 0.05$).

Table 2. Microbial biomass C (MBC), MBC/C_{org}, and metabolic quotient (qCO_2) determined in soils collected in pure cherry (PP), pure black locust (PR) and mixed cherry × black locust (MPR) plantations in the upper 10 cm soil layer, April and June 2008

Treatments	MBC (μg g^{-1})	MBC/C_{org} (mg g^{-1})	qCO_2 ($mgCO_2$-Cmg^{-1})	MBC (μg g^{-1})	MBC/C_{org} (mg g^{-1})
	April 2008			June 2008	
PP	353.80[b] (18.29)	32.58[a] (6.43)	0.08[a] (0.05)	343.3[a] (68.41)	30.30[a] (3.84)
PR	460.87[ab] (39.03)	41.92[a] (3.18)	0.251[a] (0.06)	359.8[a] (34.39)	33.58[a] (4.13)
MPR	533.60[a] (34.14)	41.61[a] (5.66)	0.177[a] (0.03)	436.6[a] (20.55)	34.38[a] (5.66)

Each value represents mean (n = 4); standard errors of means are included in parenthesis. Values in the same column followed by the same letter are not significantly different at $p < 0.05$ according to LSD test.

only in July-September significant differences were observed between PR and both PP and MPR. In the PP treatment, nitrogen was immobilized instead of being mineralized since the negative values for the daily net N ammonification indicate microbial immobilization. In general, the average daily net N nitrification rates were higher than net ammnofication in all the studied periods, except for July-September in PR treatment. The greatest peak of net ammonification occurred in July-September in PR treatment, being significantly different from PP and MPR treatments.

In general, the PR plantation presented significantly higher N mineralization than the PP and MPR plantations.

Microbial biomass carbon and nitrogen, respiration and dehydrogenase activity

In April, microbial biomass C was significantly higher in MPR than in PP plantations, with averages of 533.6 and 353.8 μg g^{-1}, respectively (Table 2). In June, the pattern observed for microbial biomass C was similar to that determined in April (Table 2), however no difference was observed. In April, the ratio MBC/C_{org} ranged from 32.6 to 41.9 mg g^{-1} in PP and PR, respectively, and in June from 30.3 to 34.4 mg g^{-1}, in PP and MPR, respectively. MBC and MBC/C_{org} slightly decreased from April to June in all treatments, but no differences were observed ($p > 0.05$). The qCO_2 did not differ significantly among treatments although a higher value was calculated for PR treatments (Table 2).

Microbial biomass nitrogen (MBN) and MBN/TN showed similar trends to those observed for microbial biomass C and MBC/C_{org}. However, no statistical differences ($p > 0.05$) were observed among plantations for any parameter (Table 3).

The cumulative microbial soil respiration patterns of incubated soils are shown in Figure 3. The cumulative soil respiration was significantly higher ($p < 0.05$) in MPR, at 15, 30 and 42 days of incubation, than in the pure treatments.

Considering dehydrogenase activity, this enzyme reached higher values in PP plantation in all the months studied (Fig. 4). However, no statistical differences were observed among plantations.

Table 3. Microbial biomass N (MBN) and microbial biomass/total N ratio (MBN/TN) determined in soils collected in pure cherry (PP), pure black locust (PR) and mixed cherry × black locust (MPR) plantations in the upper 10 cm soil layer, April and June 2008

Treatments	MBN (μg g^{-1})	MBN/TN (mg g^{-1})	MBN (μg g^{-1})	MBN/TN (mg g^{-1})
	April 2008		June 2008	
PP	38.94 (7.42)[a]	41.10 (8.61)[a]	30.06 (5.16)[a]	31.20 (5.34)[a]
PR	51.63 (4.70)[a]	51.57 (2.26)[a]	38.42 (4.18)[a]	38.78 (4.30)[a]
MPR	59.88 (7.33)[a]	55.73 (7.17)[a]	44.95 (5.20)[a]	41.63 (4.56)[a]

Each value represents mean (n = 4); standard errors of means are included in parenthesis. Values in the same column followed by the same letter are not significantly different at $p < 0.05$ according to LSD test.

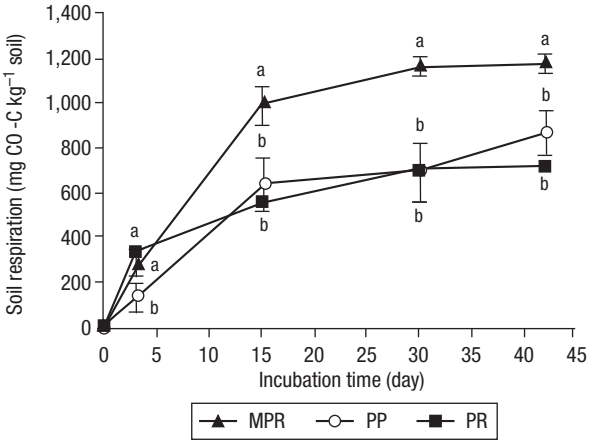

Figure 3. Microbial respiration of surface (0-10 cm) soils sampled from three experimental plantation sites during 42 days of laboratory incubation at 25°C. Each point represents mean (n = 4); error bars indicate standard errors. Different letter, in same incubation period, indicate significant differences among treatments ($p < 0.05$).

Discussion

Although the exotic black locust acts as an invasive species, in the present study, it was used as a model nitrogen-fixing species planted in consociation with the wild cherry in order to understand its impact in soil quality. Physical and chemical soil properties registered in bulk soil were very similar in all the experimental treatments. In these eleven year old mixed plantations, it seems that the black locust still has no significant effects on those soil properties. In this study, the C/N ranged between 11.2 in PR to 12.5 in PP, which is favorable for nitrification, according to Uri *et al.* (2003). Low C/N ratios and organic matter contents were also found by Fonseca *et al.* (2002) in one young stand of black locust in the Northeast of Portugal when compared to other species (*Cupressus lusitanica* Miller and several land shrubs). The authors attributed these results to the higher rate of leaves decomposition (k = 0.81).

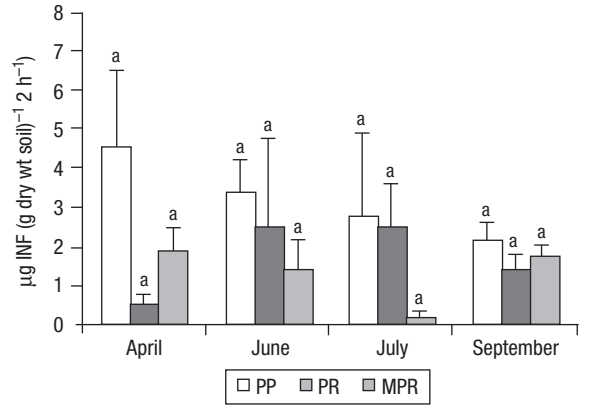

Figure 4. Dehydrogenase activity (mean + SE) measured in soil from pure cherry, pure black locust and mixed MPR treatments. Vertical bars are standard errors (n = 4); Similar letters, in same period, indicate no significant difference among treatments ($p < 0.05$).

The concentrations of soil mineral N, nitrate nitrogen and ammonium nitrogen in PR were considerably higher than in the PP plantation which was significantly higher only in September. In all treatments, the concentration of soil NH_4^+-N was considerably higher than the concentration of soil $NO_3^- - N$, except in September (Fig. 1b and c). A higher level of NH_4^+-N compared with that of $NO_3^- - N$ may indicate that nitrogen was assimilated by plants mainly in the form of nitrate. Also, NH_4^+-N is a less mobile ion than $NO_3^- - N$ since NH_4^+ has strong affinity for negatively charged clay and organic matter particles (Groffman, 2000). The decrease of the concentration of mineral nitrogen in PP plantation, from February to September, was likely caused by its intensive uptake by plants during this period. The significant increase in the soil mineral nitrogen concentration in PR in September could be related to the release of readily mineralizable N as well as its reduced uptake by the black locust. This pattern was found in previous studies made by Uri *et al.* (2003).

Net nitrogen mineralization was positive in all cases, except in July-September in PP (Fig. 2). The negative values of net nitrogen mineralization observed in July-September can be explained by N immobilization (Maag and Vinther, 1996; Uri *et al.*, 2003; Trap *et al.*, 2009) which leads to the reduction of the availability of nitrogen for plants. At the end of this study, the net nitrogen mineralization rates were about three times greater in PR than in PP and about two times greater in MPR than in the PP. Elevated net nitrogen mineralization rates in PR plantations could be associated with an abundance of high nitrogen and low lignin in the leaf litter. This evidence was also observed by Rice *et al.* (2004), who found high levels of nitrogen (average 26 g kg^{-1} for brown leaflets) and low lignin (average 16.0%) in leaf litter from black locust stands. The cumulative amounts of net nitrogen mineralization from February to September were 15.0, 41.2 and 23.3 kg N ha^{-1}, in PP, PR and MPR, respectively. These results show that the nitrogen-fixing black locust species can supplement nitrogen pools and increase rates of

nitrogen cycling and availability (Vitousek and Walker, 1989). The lower net nitrogen mineralization in PP could be a consequence of less favorable soil conditions or a lower leaf litter N concentration (12.3 g kg^{-1}, unpublished data), contributing to lower microbial biomass (Table 2).

The microbial dynamic pool depends on the nitrogen contents and organic residues in soils (Wardle, 1992), increasing or decreasing in the presence of the organic label or recalcitrant compounds such as lignin and tannins (Slapokas and Granhall, 1991). In our study, microbial biomass C was significantly higher in MPR than in the PR and PP plantations. These results could be related to the higher levels of nitrogen and lower levels of lignin in leaf litter provided by the black locust, as well as the mixture of the leaves of the two species in the litterfall. Indeed, Rice *et al.* (2004) found that the black locust supplements soil nitrogen pools, increasing nitrogen return in litterfall, and enhancing soil nitrogen mineralization rates when it invades nutrient poor mixed pine-oak ecosystems.

Up to the present time, the black locust has had no impact on the increase of microbial C efficiency, indicated by the dehydrogenase activity and microbial qCO_2, since there have been no significant differences.

In relations to the cumulative soil respiration, the results showed that the MPR treatment released significantly higher C-CO_2 than PP and PR (Fig. 3) which were in agreement with the greatest amounts of microbial biomass observed in the MPR during the analyzed period (Table 2). The increases of both soil respiration and MBC in the MPR plantation were probably due to the higher annual input of decomposable organic matter than in the other plantations. It may also be possible that the plant activity in the mixture (root respiration and exudates production) exerted a great influence on soil metabolic activity. However, more studies are required to further understand the effect of the mixture in the quantity and quality of litterfall, as well as the role of the living roots in the microbial activity.

Conclusions

Eleven years after setting up the plantation, we observed the highest quantities of net nitrogen mineralization in the black locust pure soil plantation, indicating that it had a positive impact on the supply of nitrogen to the soil. At the end of this study, the net N-mineralization rates were, about three times greater in the pure black locust than in the pure wild cherry and about two times greater in the mixture than in the pure wild cherry. However, N mineralized in mixed plantations of wild cherry × black locust cannot be considered different from the pure cherry plantation. Nevertheless, there are increases in the amounts of MBC and soil respiration in the mixed plantation. Since these parameters are considered sensitive indicators for positive changes in the soil environment and, a long-term, in site productivity, we expect these trends to become more pronounced over time, resulting in better growth, vigor and resistance to diseases of the wild cherry when consociated with black locust. Although Patrício *et al.* (2010) have already observed better growth, for the wild cherry when consociated with black locust, in the same plantations, more studies are needed to confirm assumptions such as vigor and resistance to diseases, as well as the processes of facilitation and complementarity in order to better understand the benefits of this consociation.

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