Seasonal evolution of soil respiration in a mixed forestry plantation of walnuts \((Juglans \times \textit{intermedia} \text{ Carr.})\) and alders \((Alnus \textit{cordata} \text{(Loisel.) Duby})\) in Domaine de Restinclières (France)
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Árbol, buen árbol, que tras la borrasca
te erguiste en desnudez y desaliento,
sobre una gran alfombra de hojarasca
que movía indiferente el viento...

Hoy he visto en tus ramas la primera
hoja verde, mojada de rocío,
como un regalo de la primavera,
buen árbol del estío.

Y en esa verde punta
que está brotando en ti de no sé dónde,
hay algo que en silencio me pregunta
o silenciosamente me responde.

Sí, buen árbol; ya he visto como truecas
el fango en flor, y sé lo que me dices;
yá sé que con tus propias hojas secas
se han nutrido de nuevo tus raíces.

Y así también un día,
este amor que murió calladamente,
renacerá de mi melancolía
en otro amor, igual y diferente.

No; tu augurio risueño,
tu instinto vegetal no se equivoca:
Soñaré en otra almohada el mismo sueño,
y daré el mismo beso en otra boca.

Y, en cordial semejanza,
buen árbol, quizá pronto te recuerde,
cuando brote en mi vida una esperanza
que se parezca un poco a tu hoja verde...

Antonio Machado
Dedicado a Campana.

Porque te llevo dentro,

porque tú me guías.
RESUMEN

La intensificación ecológica de cultivos se propone como una solución a la creciente demanda de recursos agrarios y forestales en contraposición a los monocultivos intensivos. La introducción de cultivos mixtos como mezclas entre especies no fijadoras de nitrógeno y especies fijadoras de nitrógeno pretende aumentar el rendimiento como resultado de una mejora de la cantidad de nitrógeno y fósforo disponible en el suelo. Las relaciones entre cultivos han sido poco estudiadas a pesar de las ventajas que la diversidad de especies confiere a estos sistemas, tales como el aumento de la productividad, resistencia y adaptación a condiciones adversas y sostenibilidad ecológica. Los bosques y las plantaciones forestales pueden desarrollar un papel muy importante como secuestradores de carbono en sus tejidos, sobre todo en su madera, lo que supone una inmovilización del carbono a largo plazo. Un parámetro que simplifica el cálculo de la cantidad de carbono incorporado en el suelo por una plantación, es el TBCA (total belowground carbon allocation), por el cual, para períodos menores a un año y plantaciones en periodo de madurez, se calcula como la resta del flujo de carbono saliente del suelo (respiración del suelo) menos la hojarasca incorporada anualmente por el árbol. La respiración del suelo depende de una gran variedad de factores, tales como la temperatura y la humedad del suelo, así como la fertilidad de este y la presencia y tipo de vegetación, entre otros.

El presente estudio se ha realizado en una plantación forestal mixta de nogales híbridos (*Juglans × intermedia* Carr.) para madera con alisos (*Alnus cordata* (Loisel.) Duby.), especie fijadora de nitrógeno a través del actinomiceto *Frankia alni* ((Woronin, 1866) Von Tubeuf 1895). La zona de ensayo se situó en el parque natural de Restinclières, en las proximidades de Montpellier (Sur de Francia).

La respiración del suelo varió enormemente a lo largo del año, influída fundamentalmente por la temperatura del suelo. La humedad del suelo no influyó significativamente en la respuesta de la respiración, ya que se mantuvo constante durante el periodo de medidas en niveles altos, por lo que no hubo condiciones de estrés hídrico. La distancia desde donde se tomaron las medidas respecto a cada nogal de control fue un factor altamente influyente en la respiración del suelo. Por lo general ha habido una tendencia a la disminución de la respiración del suelo cuanto mayor es la distancia al árbol. También se analizó la respuesta de la respiración del suelo en función del manejo de fertilizante (50 kg N·ha$^{-1}$·año$^{-1}$ desde 1999 hasta 2010) y de la presencia de aliso al nogal de referencia. En general, ninguno de estos dos tratamientos influyó de manera significativa en la respiración, a pesar de que estudios realizados por otros autores constatan una inhibición en la tasa de respiración de suelo en condiciones de fertilización y en un aumento de nitrógeno disponible. Sin embargo los tratamientos sin fertilización o sin presencia de aliso registraron las mayores tasas de respiración en aquellas muestras en las que se obtuvieron diferencias significativas.

La falta de diferencias significativas entre tratamientos puede haberse debido a la alta variabilidad que han presentado las medidas de respiración de suelo en cada tratamiento. Por último, se ha observado una fluctuación asíncrona entre la respiración del suelo y la caída de la hojarasca durante el periodo de senescencia de las hojas. Esto posiblemente se haya debido a la ralentización en la emisión de exudados radicales por el árbol que se produce en el periodo de senescencia foliar, los cuales son responsables de gran parte de la actividad microbiana.

Palabras clave: Cultivos mixtos, *Juglans × intermedia*, *Alnus cordata*, Respiración del suelo.
ABSTRACT

The ecological intensification of crops is proposed as a solution to the growing demand of agricultural and forest resources, in opposition to intensive monocultures. The introduction of mixed cultures as mixtures between nitrogen fixing species and non-nitrogen fixing species intended to increase crop yield as a result of an improvement of the available nitrogen and phosphorus in soil. Relationship between crops have received little attention despite the wide range of advantages that confers species diversity to these systems, such as increased productivity, resilience to disruption and ecological sustainability. Forests and forestry plantations can develop an important role in storing carbon in their tissues, especially in wood which become into durable product. A simplifying parameter to analyze the amount allocated carbon by plantation is the TBCA (total belowground carbon allocation), whereby, for short periods and mature plantations, is admitted as the subtraction between soil carbon efflux and litterfall. Soil respiration depends on a wide range of factors, such as soil temperature and soil water content, soil fertility, presence and type of vegetation, among others.

The studied orchard is a mixed forestry plantation of hybrid walnuts (*Juglans × intermedia* Carr.) for wood and alders (*Alnus cordata* (Loisel.) Duby.), a nitrogen fixing specie through the actinomycete *Frankia alni* ((Woronin, 1866) Von Tubeuf 1895). The study area is sited at Restinclières, a green area near Montpellier (South of France).

In the present work, soil respiration varied greatly throughout the year, mainly influenced by soil temperature. Soil water content did not significantly influence the response of soil respiration as it was constant during the measurement period and under no water stress conditions. Distance between nearest walnut and measurement was also a highly influential factor in soil respiration. Generally there was a decreasing trend in soil respiration when the distance to the nearest tree increased. It was also analyzed the response of soil respiration according to alder presence and fertilizer management (50 kg N-ha$^{-1}$·año$^{-1}$ from 1999 to 2010). None of these treatments significantly influenced soil respiration, although previous studies noticed an inhibition in rates of soil respiration under fertilized conditions and high rates of available nitrogen. However, treatments without fertilization and without alder presence obtained higher respiration rates in those cases with significant differences. The lack of significant differences between treatments may be due to the high coefficient of variation experienced by soil respiration measurements. Finally an asynchronous fluctuation was observed between soil respiration and litterfall during senescence period. This is possibly due to the slowdown in the emission of exudates by roots during senescence period, which are largely related to microbial activity.

Key words: Mixed cultures, *Juglans × intermedia*, *Alnus cordata*, Soil respiration.
LIST OF TABLES

Table 1: Historic climatic data (1971-2010) from Montpellier- Fréjorgues station.................................................................................................................................12

Table 2: Physical characteristics of soil. Field A4 of Domaine de Restinclières. Soil analyse made in 2002........................................................................................................13

Table 3: Chemical characteristics of soil. Field A4 of Domaine de Restinclières. Soil analyse made in 2002........................................................................................................14

Table 4: Variance analyse of the influence of different factors in soil carbon flux. ....27

Table 5: Carbon content (g C·m\(^{-2}\)) corresponding to walnut and alder litterfall........34
LIST OF FIGURES

Figure 1: Processes and induced properties in multispecies systems.................................2

Figure 2: Conceptual model of the components and responses of CO₂ efflux from soil..3

Figure 3: Analogy for mass balance approach to estimate TBCA........................................4

Figure 4: Botanical characteristics of *Juglans × intermedia* Carr. .....................................8

Figure 5: Botanical characteristics of *Alnus cordata* (Loisel.) Duby. ................................9

Figure 6: Satellite picture of the Domaine de Restinclières.............................................11

Figure 7: Annual evolution of rainfall and temperature of the study period......................12

Figure 8: Plan for experimental design of soil respiration................................................14

Figure 9: Forestry plantation of walnuts and alders.........................................................15

Figure 10: LI-COR 8100 with the chamber placed on a collar.........................................16

Figure 11: CS616-L Water Content Reflectometer and CR1000 Measurement and Control Datalogger (Campbell Scientific, Ltd., Shepshed, UK).................................17

Figure 12: Picture of trays in one of the plot studied.......................................................18

Figure 13: Plan for experimental design of litter traps.....................................................19

Figure 14: Annual variation in soil carbon flux, soil temperature and volumetric soil water content .........................................................................................................................22

Figure 15: Relationships between soil carbon flux and soil temperature, and between soil carbon flux and soil water content.............................................................23

Figure 16: Annual flux variation according to distance to walnuts.................................24

Figure 17: Flux evolution versus distance in fertilized and not fertilized treatments....25

Figure 18: Flux evolution versus distance to walnuts according to alder presence.......26

Figure 19: Seasonal flux distribution..............................................................................28

Figure 20: Seasonal flux variation by distance to walnuts.............................................29

Figure 21: Daily flux variations according to distance to walnuts along year seasons...30
Figure 22: Seasonal flux variation versus distance to walnut according to fertilization management.................................................................32

Figure 23: Seasonal flux variation versus distance to walnut according to alder presence...............................................................................................................33

Figure 24: Autumn trend in soil flux respiration and litterfall both in g C ·m$^{-2}$·day$^{-1}$......35
1. INTRODUCTION

1.1. THE IMPORTANCE OF MIXING PLANT SPECIES IN CROPPING SYSTEMS

The ecological intensification in forestry plantations studies how to increase wood production by tree, like a result of an availability improvement of nitrogen and phosphor in soil. Mixtures of nitrogen non-fixing trees with nitrogen fixing trees or nitrogen fixing herbaceous species are proposed as alternatives to forestry plantation in monoculture.

Multispecies cropping systems are an application of ecological principles of biodiversity and plant interaction (Fig. 1). Species diversity is one of ecology's most respected hypotheses (Frank and Naughton, 1991). Biomass productivity in natural ecosystems is achieved through a high genetic diversity of plants involving different complementary functional groups. Multispecies systems are assumed to have potential advantages in productivity, stability of outputs, resilience to disruption and ecological sustainability (Vandermeer, 1989). In opposition there are the intensive agricultural systems in which predominate monocultures: crops in which plants and its genetic are reduced. External inputs are largely supplied in these systems to compensate the absence of the mutually beneficial functions and natural subsidies that lend stability and sustainability to natural systems. Several agronomists recently proposed that traditional multispecies systems could be used as models for designing sustainable cropping systems (Gliessman, 2001; Altieri, 2002). These traditional multispecies systems that have been largely cultivated would be maize/beans, groundnut/cotton in Africa; cereals/herbaceous legumes and grasses in Europe; shadow crops (coffee, cocoa or pineapple) between coconuts in Central America and tropical home gardens in humid tropics (gardens with lots of services for self-sufficiency). In Spain the mayor examples are mixtures of vineyard and olives, cereals and olives and holm oak and pasture. This last one is called dehesa, and it is an example of integrated farming. It constitutes an agroforestry system which combines the production of pastures for animal feed and the presence of trees which give several services as animal feed by its acorns or wood products. Nowadays it has a high environmental value added (Hernández, 2003).
In France, mixed plantations can be frequently found in *Juglans* sp. or *Prunus* sp. associated with perennial cultures of forage alfalfa (*Medicago sativa* L.) or sainfoin (*Onobrychis* sp.). But in Europe, nitrogen fixing trees are not very common, that is why mixed perennial trees plantations are not found easily. The only genus of native nitrogen fixing species in Europe is *Alnus* sp. (alders).

Despite of their increasing interest, mixed forestry plantations with nitrogen fixing species are not widely used. The *Intens&Fix* project is encompassed in studies which compare their interests and weaknesses with monoculture forestry plantations.

### 1.2. CARBON SINK IN FORESTRY PLANTATIONS

It is generally accepted that forests can play a critical role in capturing and storing large amounts of carbon from the atmosphere. By the process of growing, trees absorb atmospheric CO$_2$ and they store it as cellulose (photosynthesis). Nevertheless, when these trees are used (e.g. for wood) or when they reach maturity, death of specimens and natural processes of decomposition return CO$_2$ to the atmosphere at the same rate that they removed it, due to which there is not a net increase of stored carbon. The use of plantations for absorbing CO$_2$ is more effective when it is a short term
solution or when the final product is wood that becomes into durable products, this immobilize CO$_2$ as cellulose for larger periods (Montagnini, 1998). Wood plantations exert a positive influx while trees are growing and they can contribute to reduce the atmospheric CO$_2$ tax, especially if wood is used properly. Heliophilous trees of quick growing have immediate effects, but with a mix of no heliophilous trees, shrubs or herbaceous species which increase total yield of a site, in this case, mix would be a more effective sink of CO$_2$ (Fig. 2).

Figure 2. Conceptual model of the components and responses of CO$_2$ efflux from soil. Both the autotrophic and heterotrophic components of soil respiration are strongly controlled by substrate availability-phloem transport of carbohydrate supply for root and mycorrhizal respiration, and dead organic material for microbial respiration (Ryan and Law, 2005).

1.3. TOTAL BELOWGROUND CARBON ALLOCATION

Total belowground carbon allocation (TBCA) is defined by Giardina et al. (2005) as the quantity of carbon allocated belowground by plants to produce coarse and fine roots, root respiration, and root exudates and mycorrhizae. Raich and Nadelhoffer (1989) originally proposed a mass-balance approach to quantify the total quantity of carbon allocated belowground by trees on an annual time step (Fig. 3). The scheme is as follows: Plants send fixed C to roots. This C must either be respired by microbes or roots (measured as soil-surface CO$_2$ efflux or $F_S$) or stored in soil as organic matter, in the litter layer, or in living and dead roots:

$$TBCA = F_S - F_{AL} + \Delta[C_S + C_R + C_L]$$

Where $C_S$ is carbon content of mineral soil, $C_R$ is carbon content of root (coarse and fine) biomass, and $C_L$ is carbon content of the litter layer. This approach to estimate TBCA assumes that losses of C to leaching or erosion ($F_E$) are negligible, which is true for most forests on level topography (Giardina and Ryan, 2002).
Introduction

If C storage in soil, roots, or the litter layer does not change over the measurement period of interest, and leaching and erosion losses are negligible, then conservation of mass dictates that TBCA must equal $F_S$ minus $C$ inputs from aboveground litter ($F_{AL}$):

$$TBCA = F_S - F_{AL}$$

Figure 3. Analogy for mass balance approach to estimate TBCA. The flux of water into the tub from the underwater faucet (TBCA) can be calculated for any period of time by measuring the flux of water out of the tub ($F_S + F_E$), the flux into the tub form the faucet above water ($F_A$ or $F_{AL}$), and any change in water stored in the tub over the period $\Delta(C_S + C_R + C_L)$. Through conservation of mass, the flux of water from the underwater faucet equals outputs minus inputs plus storage change. Source: Giardina and Ryan (2002).

1.4. SOIL RESPIRATION

Giardina et al. (2005) described CO$_2$ efflux ($F_S$) by the following equation:

$$F_S = F_R + F_M + F_{AL} + F_{BL} + F_{SOC}$$

Where $F_R$ is the flux of CO$_2$ from respiring roots, $F_M$ is the flux of CO$_2$ from respiring mycorrhizae, $F_{AL}$ and $F_{BL}$ are fluxes of CO$_2$ from decomposing above and belowground litter (including root and mycorrhizal exudation and turnover), and $F_{SOC}$ is the flux of CO$_2$ from decomposing organic C stored in mineral soil (microbial biomass, low-quality remains and by-products of litter decomposition). $F_R$ represents CO$_2$ of autotrophic origin while $F_M$, $F_{AL}$, $F_{BL}$ and $F_{SOC}$ represent CO$_2$ released by heterotrophic organisms, though $F_M$ can be also described as autotrophic (Gower et al., 2001).

Quantifying the individual components of soil surface CO$_2$ efflux is challenging because belowground C processes are intimately associated with the soil matrix. Roots, mycorrhizae and soil are intimately connected, so these studies may not accurately represent belowground processes as they would occur in undisturbed soil (Högberg et al., 2001).

Belowground (roots, microbes) and aboveground (leaf and branch litterfall) components of soil surface CO$_2$ efflux may not respond similarly to changes in the
environment (Giardina et al., 2004). Where more than one variable is changing (soil temperature, soil water content, nutrient supply), ecosystem responses to these multiple changes may be quite complex.

Factors affecting soil respiration

Several factors and mechanisms are involved in the formation and distribution of CO$_2$ in soils (Wood et al., 1984). CO$_2$ production in soils is related to biological activities such as root respiration and decomposition of soil organic matter (SOM) by microbes (Amudson et al., 1990). One of the main factors controlling soil respiration is soil temperature. For example, soil CO$_2$ production rate and emission flux increase by between 1.5 and 3 times for every 10°C increase in temperature from 0°C to 50°C (Parada et al., 1983). Soil moisture also affects CO$_2$ production and distribution through its influence on gas diffusion and microbial activity. Increasing soil moisture between the permanent wilting point and 60 to 80% saturation increases the rate of CO$_2$ emission from soil (Salomon et al., 1987). Other important factor to take into account is energy supply in soil (active carbon), which regulates microbial activity or soil respiration, since heterotrophic components in soil respiration are dominant. Dissolved organic carbon (DOC) has been used as an indicator of C available to soil microbes.

The distance between collected measurements and closest trunk tree is an important factor that modulates soil respiration. Plants affect soil respiration through several ways:

1. By altering the soil moisture regime through the interception of precipitation and the extraction of soil water via transpiration (Rulter and Morton, 1977)
2. By influencing soil microclimate and structure (Raich and Tufekcioglu, 2000)
3. By providing the principal carbon source to decomposer microorganisms through dead plant material, thus determining both litter quantity and quality (Swift et al., 1979)
4. The fact that a strong fraction of soil respiration derives directly from the vegetation via root or rhizosphere respiration (Högberg et al., 2001)

Soil respiration also varies with vegetation type. Different plant communities frequently demonstrate differences in soil respiration rates (Lundergårdh, 1972). Such findings indicate that vegetation type is an important determinant of soil respiration rate, and therefore that changes in vegetation have the potential to modify the responses of soils to environmental change.

Mechanism of mixed plantation is based on the differences in physical or phenological characteristics of component species, the interactions between certain trees species
can lead to an increased capture of the limiting growth resource (Kelty, 1989). Greater capture of the limiting resource would be accompanied by an increased ability to utilize non limiting resources and the system as a whole would then accrue a total biomass, greater than the cumulative production of those species if they were grown separately on equal land area (Cannnell, 1996). However, very often, there is an overlap between the needs and physical structures of two or more species. When this occurs, species will compete for resources. In mixed-species systems, this is more the rule than the exception (Ong et al., 2004).

1.5. LITTERFALL

A significant fraction of soil respiration is derived from aboveground litterfall carbon, even though this fraction has a high variation across seasons. Raich and Nadelhoffer (1989) found that aboveground litter (F_AL) contributed 23% of soil CO₂ respiration at low efflux rates (400 g C·m⁻²·yr⁻¹) to 31% of soil CO₂ respiration at high efflux rates (1500 g C·m⁻²·yr⁻¹).

These authors also found a global relationship between annual litterfall (F_A) and soil surface CO₂ efflux (F_S) and between F_A and TBCA (estimated as the difference between soil surface CO₂ efflux and annual litterfall) and developed empirical models of the relationships (linear equations which relate F_S and F_A). These relationships apply only to forests where a balance has been reached between litterfall, TBCA, soil carbon and organic matter turnover, such as mature forests and not recently disturbed forests or first rotation plantations, and always for annual periods. That is why Giardina and Ryan (2002) and Forrester (2006) concluded that litterfall was a poor predictor of TBCA, because their plantations were in a non-steady state.

Litterfall and soil respiration relationship across one phenologic year

Plant litterfall and soil respiration are processes whose intensity is asynchronous throughout the year and yet they are highly related processes.

There are two important belowground processes associated with plant litterfall: reduction in plant nutrient uptake and retranslocation of nutrients and carbohydrates from leaves to stems and roots, which often occur before leaves fall. Changes in plant nutrient uptake or carbon and nutrient retranslocation could trigger temporal changes in soil microbial biomass, and thus in soil respiration. A root trenching experiment that terminated both plant uptake and root exudates production showed a drastic decrease in soil microbial activity as indicated by reduced soil CO₂ production (Boone et al., 1998). Ruan et al. (2003) reported an increase in soil respiration one month before litterfall. This phenomenon might be explained if microbial growth was either controlled by the availability of soil nutrients or belowground carbon, both of which could be altered by the processes of retranslocation of carbohydrate production and
competition for nutrients that are associated with plant litterfall during the annual cycles.

Asynchronous uptake of nutrients by plants and soil microbes has been recognized as a mechanism for retaining nutrients and maintaining ecosystem productivity in temperate deciduous forest with strong temperature seasonality. Microbial biomass and microbial nitrogen and phosphor contents increase during the dry season, whereas microbes release nitrogen and phosphor at the beginning of the wet season when plant growth and demand for nutrients are high (Singh et al., 1989). The immobilization of soil nitrogen and phosphor into microbial biomass during dry season when plant uptake is low prevents soil nutrient accumulation to high levels that are subject to nutrient loss through gaseous emission or water leaching when wet season arrives.

1.6. WALNUTS

Hybrids walnuts (*Juglans × intermedia* Carr.) are a natural hybrid between common walnut (*J. regia* L.) and black walnut (*J. nigra* L.). They can produce eatable nuts, but its principal propose of culture is wood harvest. Walnut wood in France is the most precious produced wood. It has a semi-hard wood, fine grained, easy to work with and very aesthetic. The wood production in France has been decreasing since the 19th century, when it was gathered about 100 000 m³ · year⁻¹ (Centre Régional de la Propriété Forestière d’Auvergne, 1995). Nowadays annual national production in France would be about 20 000 m³. Production average would be higher than 1 m³·ha⁻¹·year⁻¹ of wood that have reached the size of slicing with a density of 80 walnuts·ha⁻¹. Oldest French plantations have an age of 40 years and they are estimated to logging every 25 years.

The botany of hybrid walnut is very similar to that of common walnut (*Juglans regia* L.) and black walnut (*J.nigra* L.). These trees could measure 20-30 m height and 2 m in trunk diameter. Stem bark is deeply longitudinally fissured, with a color pale gray. Leaves are deciduous, imparipinate with 5-9 foliols of 6 to 15 cm. Foliols are oval of 6-12 cm long and 3-6 cm wide. They are glabrous and dull green colored. The cup has a compressed spherical shape. Its flowers are monoecious, the male flowers are inserted into the axils of the previous year twigs, which are devoid of leaves in flowering time. The female flowers are grouped in terminal spikes on the wood of the same year. Since it is a natural hybrid, it has no seeds, what gives it an advantage in wood formation.
Introduction

Figure 4. Botanical characteristics of *Juglans × intermedia* Carr. Botanical details (A) and shape in a mature non-commercial walnut (B).

Hybrid walnuts have shown an increase in vegetative vigor than its “parents”. It grows quicker than *J.regia* or *J.nigra* and it has a greater disease resistance. It can resist heavy frost (until -25°C), being even less sensitive to frost than its parents. It combines the good qualities of common walnut and black walnut in wood production. It requires an annual rainfall of about 900-1350 mm (Navés, 2003) (average annual rainfall at Restinclières is about 950 mm).

They can accept carbonates in soil and also pH of about 8, although they prefer soils near neutrality. They develop best in deep healthy soils without a trace of water stagnation, with a good water reserve and a balanced silty-clay texture. It grows, like *J.regia* and *J.nigra*, in cool temperate humid climate of Europe (Munchazaraz, 2001).

Commercial plantations of hybrids walnuts for wood have a density of plantation of about 160-210 trees·ha\(^{-1}\). It is usual to perform thinning because trees start to hinder each other and like this the best trees can be chosen. It can be expected an annual average growth of 3 cm of the tree trunk circumference. Green pruning is a widely used technique at June or the start of July in which it is cut the year’s growth to avoid it continuing to grow but to allow it to feed the tree.

The root system is more extended superficially than the aboveground part, although its proportion in biomass is lower 1/2.5 (Catlin, 1998). Roots can be extended from a distance to trunk of above 20 meters when they reach its greater development. Lateral roots do not develop following a specific shape, therefore its distribution in soil is not uniform. Its density is very low if it is compared with herbaceous plants root system (Munchazaraz, 2001). Walnut roots could reach at 4 meters deep when soils are well
drained and have a sandy texture although 75% of roots are between 0.7 and 1 meter from the surface (Catlin, 1998).

1.7. ALDERS

Italian alders (*Alnus cordata* (Loisel.) Duby), also called Corsica alder, is a tree native of the south of Italy, Sardinia and Corsica. It has a normal size of 10-15 m, with a maximum of 20 m. Its shape is slender with narrow crown and branches inserted at an acute angle. Its colour is a glossy dark green, and they are deciduous trees. Its bark is smooth with large lenticels. Its leaves have a brilliant green, the foliole is pointed and its base is heart-shaped, 5-12 cm long. Male flowers are slender cylindrical catkins, with a reddish colour, and a height of 2-4 cm long. Female catkins are ovoid and shorter, 0.5 cm. These flowers are similar to some conifer cones. Pollination occurs in early spring, before the leaves emerge. Fruits are really small globular cones (20-25 mm) with two flattened winged seeds. This seeds disperse through the winter, leaving the “cones” on the tree for the rest of the year.

![Figure 5. Botanical characteristics of *Alnus cordata* (Loisel.) Duby. Male catkins (A), Female catkins (B), slender shape (C) and leaves (D).](image)

Its principal characteristic is its capacity of nitrogen fixation, due to its symbiotic relationship with *Frankia alni* ((Woronin, 1866) Von Tubeuf 1895), which occurs in the entire genus *Alnus* sp. This bacterium is a filamentous actinomycete that is found in light brown root nodules of the host plant, which may be 15 cm long. The relationship between tree and bacterium is classic example of a mutually beneficial symbiosis. The alders provide the bacteria with sugars and a variety of minerals which it produces through photosynthesis, and the bacteria provide the host with a usable supply of nitrogen.

When bacterium enter the plant through the root hairs, it start to stimulate the production of large quantities of hormones by the plant, which force the root cells to proliferate faster than normal, causing nodules where bacteria population can be
Introduction

founded. The nodules are perennial an increase in size each year. The nodules are where the nitrogen fixation is done. *Frankia* sp., like *Rhizobium* sp., possesses nitrogenases enzymes that make the transformation of the nitrogen gas in the air (N$_2$) into ammonium (NH$_4^+$) which will be converted into amino acids. This reaction can only be made in a low oxygen environment. That is why the process is often dependent on hemoglobin compounds found in the nodules, which kidnap O$_2$ molecules and create the microenvironment that the nitrogenase enzyme requires. Most of the N fixed by the actinorhizal plants enters the nutrient cycle through the decomposition of fallen leaves, twigs, branches and fine roots. This process is much slower than that which occurs in agricultural situations, where leguminous cover crops are plowed into the soil at the end of a single growing season (Del Tredici, 1996). The presence of nodules of *Frankia* sp., also reduces trees diseases and increases phosphor absorption (Molina et al. 2008).

1.8. OBJECTIVES

There is an increasing global demand in wood products which in the future will not be fulfilled by natural forests because of threats of deforestation or soil degradation (e.g. the advance of the agricultural frontier). Forestry plantations will provide an increasing part of the world’s wood necessities. Nevertheless their expansion is limited due to competition with other forms of land use. That is why an increased productivity of these systems must be obtained in lands that can not necessarily support such an increase, and therefore losses of nitrogen (N) and phosphorus (P) related to biomass exports generally would not be compensated by fertilization. New methods of sustainable management of forestry plantations should be implemented.

The project *Intens&Fix* aims to promote ecological intensification of forestry plantations by the association of nitrogen-fixing species. The objective is to sustainably increase the production of forestry plantations and the availability of N and P in soil.

The objective of the present work was to analyze the annual variation of soil respiration within a mixed forestry plantation. Differences in soil respiration within fertilizer management and alder presence have been examined to see if soil carbon efflux is more or less efficient between treatments. Seasonal differences in soil respiration have been related to soil temperature and soil water content. It has also been analyzed the quantity of walnut and alder litterfall and its relationship with soil respiration.
2. MATERIAL AND METHODS

2. 1. EXPERIMENTAL SITE

2.1.1. Location

The plantation (Fig. 6) was situated in the Domaine de Restinclières, in the department of L’Hérault, France (43°42’26’’N, 3°51’34’’W). It is a green area with recreational and instructional purposes, of which several experimental study areas are ceded by the region authorities to the INRA (Institute National de la Recherche Agronomique).

![Figure 6. Satellite picture of the Domaine de Restinclières. Study site circled in red.](image)

2.1.2. Climatic characteristics

Restinclières has a typical Mediterranean weather (Table 1). With few precipitation days (less than 100 days for year) some of which are of high intensity, especially in autumn from September to December in what is called the épisode cévenol. During this period flooding events are frequent in low areas near the river Lez (average of two or three times each year), which crosses through the area of Restinclières. Soil water reserve is filled in this period for almost all year. Instead summer is dry, with few rainfall in August related to storms. There is a dry season of four months, from May to July, in which average rainfall is about 35 mm. Winters are also dry and mild. Water table in 2010 varied from 0.7 meters depth (February) to 2.5 meters (August).
Material and methods

Table 1. Historic climatic data (1971-2010) from Montpellier-Fréjorgues station.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Average T° (°C)</th>
<th>Average high (°C)</th>
<th>Average low (°C)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>72.3</td>
<td>6.6</td>
<td>11.1</td>
<td>2.2</td>
<td>75</td>
</tr>
<tr>
<td>February</td>
<td>72.3</td>
<td>7.8</td>
<td>12.4</td>
<td>3.3</td>
<td>73</td>
</tr>
<tr>
<td>March</td>
<td>55</td>
<td>9.8</td>
<td>14.7</td>
<td>4.9</td>
<td>68</td>
</tr>
<tr>
<td>April</td>
<td>54.9</td>
<td>12.6</td>
<td>17.5</td>
<td>7.8</td>
<td>68</td>
</tr>
<tr>
<td>May</td>
<td>52.1</td>
<td>16.1</td>
<td>21.1</td>
<td>11.2</td>
<td>70</td>
</tr>
<tr>
<td>June</td>
<td>33</td>
<td>19.9</td>
<td>25.3</td>
<td>14.6</td>
<td>66</td>
</tr>
<tr>
<td>July</td>
<td>20</td>
<td>22.8</td>
<td>28.4</td>
<td>17.1</td>
<td>63</td>
</tr>
<tr>
<td>August</td>
<td>41.7</td>
<td>22.2</td>
<td>27.7</td>
<td>16.7</td>
<td>66</td>
</tr>
<tr>
<td>September</td>
<td>62.3</td>
<td>19.4</td>
<td>24.7</td>
<td>14.2</td>
<td>72</td>
</tr>
<tr>
<td>October</td>
<td>109.5</td>
<td>15.4</td>
<td>20.2</td>
<td>10.6</td>
<td>77</td>
</tr>
<tr>
<td>November</td>
<td>62.8</td>
<td>10.3</td>
<td>14.7</td>
<td>5.9</td>
<td>75</td>
</tr>
<tr>
<td>December</td>
<td>63.3</td>
<td>7.2</td>
<td>11.7</td>
<td>2.8</td>
<td>76</td>
</tr>
</tbody>
</table>

The period of study (from September 2011 to May 2012) was characterized for an extremely rainy November (270 mm of rainfall recorded during this month, 47% of the total rainfall during the year of study), while for a normal year the rainiest month would be October. The 1/11/2011 a rainfall of 75.3 mm in a 24 hours period was recorded (which represents 120% of the average rainfall recorded for a normal year throughout November) (Fig. 7). The period from December 2011 to April 2012 was especially dry with a monthly average rainfall of 3 mm against the normal year monthly average rainfall of 65 mm during this period.

![Annual evolution of rainfall and temperature of the study period (2011-2012). Data from Montpellier-Fréjorgues station.](image)
2. 2. THE ORCHARD

2.2.1. Description

The experimental orchard was in the field A4 of *Restinclières*, a forestry plantation of hybrids walnuts (*Juglans × intermedia* Carr.) with Italian alders (*Alnus cordata* (Loisel.) Duby), planted in 1995. At the orchard, the elevation was 67 m above sea level and the annual average rainfall was about 950 mm. The annual average air temperature was 14.5 °C and the annual average air humidity was 59%. The soil was an alluvial silty-loam soil with a deep of above 4 meters.

Soil management was very simple at the orchard. The vegetal cover was not seeded and was left in the ground for all over the year without being incorporated to the soil. Mechanical weeding is performed every three years to limit weed growth. Nevertheless the fight against weeds is hard. Neither irrigation nor phytosanitary treatments were applied. Fertilizers were manually applied in the proximity of the tree trunks.

Plantation density was of 408 trees·ha\(^{-1}\) at a spacing of 3.5 x 7 meters. The percentage of walnuts and alders was 50%. The average diameter at breast height in the beginning of measurements was 16 cm for walnuts and 12.5 for alders. Both walnuts and alders begin senesce at the end of September.

2.2.2. Soil characteristics

A basic analysis of the soil characteristics was made in 2002. The soil profile is very uniform, with a silty texture (silt content between 60-65%). Values for field capacity and wilting point are typical for silty-loam soil (Urbano, 2008) (Table 2).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Clay (%)</th>
<th>Fine silt (%)</th>
<th>Coarse silt (%)</th>
<th>Fine sand (%)</th>
<th>Coarse sand (%)</th>
<th>Soil texture USDA</th>
<th>Field Capacity (3.0 pF) (%)</th>
<th>Wilting point (4.2 pF) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>22.40</td>
<td>44.70</td>
<td>15.90</td>
<td>8.90</td>
<td>8.10</td>
<td>Silt Loam</td>
<td>20.75</td>
<td>10.68</td>
</tr>
<tr>
<td>20-40</td>
<td>22.70</td>
<td>43.50</td>
<td>16.10</td>
<td>9.20</td>
<td>8.50</td>
<td>Silt Loam</td>
<td>19.60</td>
<td>10.04</td>
</tr>
<tr>
<td>40-60</td>
<td>23.60</td>
<td>44.50</td>
<td>16.30</td>
<td>8.90</td>
<td>6.70</td>
<td>Silt Loam</td>
<td>19.32</td>
<td>9.90</td>
</tr>
<tr>
<td>60-80</td>
<td>26.50</td>
<td>48.60</td>
<td>14.80</td>
<td>6.50</td>
<td>3.50</td>
<td>Silt Loam</td>
<td>25.84</td>
<td>12.27</td>
</tr>
<tr>
<td>80-100</td>
<td>26.90</td>
<td>51.10</td>
<td>14.70</td>
<td>6.00</td>
<td>3.30</td>
<td>Silt Loam</td>
<td>26.84</td>
<td>13.31</td>
</tr>
<tr>
<td>100-140</td>
<td>26.80</td>
<td>47.80</td>
<td>15.10</td>
<td>7.50</td>
<td>2.80</td>
<td>Silt Loam</td>
<td>25.60</td>
<td>12.66</td>
</tr>
</tbody>
</table>

The more remarkable characteristic in this soil is the very high content in total carbonates, more than 50%. This is a typical characteristic of soils from Montpellier region (*L’Hérault*) where dolostones (sedimentary carbonate rock) are predominant. Therefore soil pH tends to be moderately alkaline (< 8.4) for the first (probably because an acidification from organic matter) and last horizon, but strongly alkaline (>8.4) for the rest of horizons (Table 3).
Material and methods


<table>
<thead>
<tr>
<th>Profondeur</th>
<th>pH</th>
<th>Total carbonates (%)</th>
<th>Organic matter (%)</th>
<th>Organic Carbon (%)</th>
<th>Total nitrogen (%)</th>
<th>C/N</th>
<th>CEC METSON (me/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>8.15</td>
<td>64.13</td>
<td>3.28</td>
<td>1.90</td>
<td>1.38</td>
<td>13.79</td>
<td>11.96</td>
</tr>
<tr>
<td>20-40</td>
<td>8.45</td>
<td>65.36</td>
<td>2.42</td>
<td>1.40</td>
<td>1.12</td>
<td>12.53</td>
<td>11.16</td>
</tr>
<tr>
<td>40-60</td>
<td>8.48</td>
<td>67.36</td>
<td>1.24</td>
<td>0.72</td>
<td>0.68</td>
<td>10.55</td>
<td>10.55</td>
</tr>
<tr>
<td>60-80</td>
<td>8.42</td>
<td>66.73</td>
<td>1.47</td>
<td>0.85</td>
<td>0.70</td>
<td>12.08</td>
<td>11.71</td>
</tr>
<tr>
<td>80-100</td>
<td>8.43</td>
<td>67.39</td>
<td>1.37</td>
<td>0.79</td>
<td>0.76</td>
<td>10.39</td>
<td>12.73</td>
</tr>
<tr>
<td>100-140</td>
<td>8.22</td>
<td>66.06</td>
<td>1.00</td>
<td>0.58</td>
<td>0.78</td>
<td>7.48</td>
<td>12.30</td>
</tr>
</tbody>
</table>

Organic matter percentage is acceptable in the first two horizons, but rather low in the rest of them. Organic matter decrease with depth because the consequently decrease in oxygen. C/N ratio indicates equilibrium between mineralization and humification when values are from 8 to 12. Due to the humification process of the plant litter, organic matter is higher at the two upper horizons. Soil CEC (Cation exchange capacity) is acceptable (>10 me/100g) but still rather low for all horizons (Table 3).

2.3. MEASUREMENTS

A split plot with three blocks was used as experimental design. The whole-plot factor was fertilization (F) and no fertilization (N) management (50 kg N·ha\(^{-1}\) input from 1999 until 2010). The subplot factor was alder and no alder presence (Fig. 9).

2.3.1. Soil respiration

Soil respiration was measured at 12 different locations, 6 measuring alder influence in walnut (collars 1 to 6), and 6 measures without alder presence (collars 10 to 15) (Fig. 8). It was used the soil respiration chamber LI-COR 8100 (Lincoln NE, USA).

![Figure 8. Plan for experimental design of soil respiration. Distances from reference walnut are signaled in legend. Same pattern for walnut without alder presence.](image-url)
Figure 9. Forestry plantation of walnuts (O) and alders (X). Six different experimental plots in which measurements were taken (circled in red). N means not fertilized plots and F means fertilized plots.
Material and methods

Soil collars made of PVC, with 10 cm in diameter and 10 cm length, were inserted 5 cm into the soil. Once inserted, the collars were left in place throughout the course of the experiment (Fig. 10). Soil respiration was measured about once every fifteen days from September 2011 to May 2012. Measurements were not taken from 11/10 to 2/12 (52 days between measures) because a period of torrential rains (épisode cévenol) occurred, during this period the soil was flooded and there was no soil respiration. Before every measurement, green sprouts were plucked, as well as arthropods, to avoid distortions in soil respiration measurements. Each sampling day measurements were taken randomly in each point. Thereby 72 daily readings were made over 5 hours of sampling. A single measurement takes a sampling time of 90 seconds.

![Figure 10. LI-COR 8100 with the chamber placed on a collar. Yellow case is analyzer control unit (ACU). Computer model is one special for measures in the field.](image)

LI-8100 is a Dynamic Closer Chamber equipment, which means that air is mixed inside the measure system, and then it pass into the IRGA (InfraRed Gas Analyzer). The chamber is placed into a collar, then it closes, and a one minute and thirty seconds measurement begins. LI-8100 IRGA takes measurements of absolute humidity and CO₂. The software that extrapolates the CO₂ flux (µmol C · m⁻² · s⁻¹) uses measured CO₂ density, along with the dry air and the water vapour flux, to compute the CO₂ flux according to the change in the molar fraction with respect to dry air (Bocanegra, 2007). Each measure is computed with FV8100 software, which gives additional computations based on the slope of the exponential fit of carbon concentration in dry air according to measured time. The IRGA generates an infra-red light which impacts into the air sample. CO₂ has an absorption band very high in the infra-red spectrum, so IRGA quantifies CO₂ molecules depending in the radiation that arrives to a sensor in ppm. For extrapolating the flux function it use an exponential approach.
2.3.2. Soil temperature and water content

Both soil temperature and soil water content were measured with water content reflectometers Campbell Scientific CS616 (Campbell Scientific, Ltd., Shepshed, UK) (Fig. 11). CS616 measures moisture content by sensing changes in the dielectric constant of the material. Free water has a dielectric constant about 20 times greater than that of mineral matter, so any water increases the observed dielectric constant. It has two stainless steel rods 30 cm long, 3.3 mm in diameter spaced 3.2 cm apart (Fig. 11A). It has a multivibrator circuit which generates electric waves and its oscillation frequency depends on the dielectric constant of the media surrounding the probe rods. The higher moisture content (larger dielectric constant), the lower operating frequency.

Probes were installed at 10 cm depths into the soil. They take instantaneous measurements at 1 hour intervals. For our data analyses hourly average were taken from the day of soil respiration measurement. From measure day 02/12/2011 to 23/03/2012, 5 reflectometers were installed in N1, N2, N3, F2 and F1-F3 (these last ones shared reflectometer). Nevertheless since 20/04/2012 only two reflectometers remained in field (they were relocated in others fields because they were needed for others experiments), one controlling blocks N1, N2, F1 and F3, and the other one controlling blocks N3 and F2. Measurements were collected with a computer from the data loggers, with an independent battery, installed near the reflectometers (Fig. 11B). In order to export the data CS616 data logger software has to be installed into a computer.

![Figure 11. CS616-L Water Content Reflectometer (A) and CR1000 Measurement and Control Datalogger (B) (Campbell Scientific, Ltd., Shepshed, UK).](image)

2.3.3. Litterfall

Litterfall was collected to measure the organic C inputs from aboveground. Six squared litter traps were placed by block, three in walnut with alder presence, and three in walnut without alder presence. Each one has a surface area of 0.25 m², and has been
placed at different distances from chosen walnuts (1, 2 and 3 meters) (Fig. 12 and 13). Trays have been collected weekly all over fall time, from 13/09 to 20/10. Last day results were not taken into account because all litterfall was collected in the previous days.

![Figure 12. Picture of trays in one of the plot studied.](image)

Only fallen leaves from walnuts and alders were trapped. Not any fallen branch or fruit was taken into account since we assumed that its mineralization and consequent nutrient contribution was slower. Leaves are directly associated with the processes of plant nutrient uptake or nutrient/carbon retranslocation, not as bark tree or other tree litter. We assumed a carbon concentration for aboveground tree biomass of 50% (Giardina, 2002). All dry matter was converted to its weight in carbon and it was added daily. The collected litterfall data were divided by the number of days between collections in order to obtain the quantity of carbon restored by squared meter and day.

We also assumed that litter decomposition did not occur while litterfall was into the traps, because the environmental humidity was not high enough, and the lapsing time between trappings was short. Litter decomposes rapidly in wet tropical environment, therefore works placed in these environments correct measured input with a constant factor. This correction is also used in works which collect monthly instead of weekly (Giardina, 2002).

After being trapped, aboveground litterfall was dried at 60°C during 48 hours, and then walnut and alder leaves were weighed separately.
2.4. DATA ANALYSE

For soil flux respiration, litterfall, soil humidity and soil temperature measurements, several tests of analysis of variance (ANOVA) and Pearson test correlation were performed using R software (2.15.0 version). Data follow a normal distribution.

It was analyzed the influence in soil respiration of six factors: fertilized management, alder presence, distance of point of measurement to walnut, date of measurement, soil temperature and soil water content.

Litterfall was analyzed according to fertilized management, alder presence and date of measurement. ANOVA test was performed to analyzed effects of alder presence in alder litterfall between fertilized or not fertilized treatments.
3. RESULTS AND DISCUSSION

3.1. SOIL FLUX RESPIRATION

Soil respiration varies substantially over site and time, as a result of several factors, including soil temperature, soil water content, distance from points of measurements to trees, fertilizer management and alder presence in the proximities of measurement points.

3.1.1. Annual variation of soil respiration and relationship with soil temperature and soil water content.

Along the considerate period, soil flux varied between 1.6 µmol C · m$^{-2}$ · s$^{-1}$ (16/02) and 4.1 µmol C · m$^{-2}$ · s$^{-1}$ (13/09), with an average value of 2.9 C · m$^{-2}$ · s$^{-1}$ (Fig. 14A); soil temperature varied between 16°C (15/05) and 0.6°C (16/02), with an average value of 9°C (Fig. 14B); and soil water content varied between 32% (2/12) and 28% (20/04), with an average value of 30% (Fig. 14C).

Influence of date was highly significant on soil flux respiration (Table 4) ($P < 2 \cdot 10^{-16}$). As it is shown in Fig. 14, soil flux respiration varied a lot throughout the year. Temporal trends in soil respiration were coincident with seasonal trends in soil temperature, which is confirmed in the significant relationship established between both parameters (Fig. 15, $R^2 = 0.904$). Soil temperature, with a Pearson’s coefficient of correlation of $P = 0.95$, was able to account for the major portion of the variance in soil flux respiration (Fig. 15A), while a low Pearson’s coefficient of correlation of $\rho = -0.06$ showed no correlation between soil water content and soil flux respiration in this study (Fig. 15B).

Kikang Bae et al. (2012) also reported a high significant seasonal difference in soil respiration. This seasonal variation was closely related to fluctuations in soil temperature, but was not related to soil water content in our work (Fig. 14). Similar responses were found in Kim (2008) and Kikang Bae et al. (2012). These last ones suggest that the lack of relationship between these variables could be due to the fact that the soil moisture was never under soil water stress conditions throughout the study period. During our study soil water content was almost constant, about 30%, always above field capacity values. Flood conditions were reached during the period of torrential rains (from 11/10 to 02/12), which led to anaerobic conditions that stopped the microbial activity. Due to the weather conditions soil respiration measurements were not collected during this period, preventing any analysis of the most likely negative correlation of the flooding event and its consequences on the microbial activity. Soil moisture affects the CO$_2$ production and distribution through its influence on gas diffusion and microbial activity (Yunsheng Lou, 2003). Following this author’s work, the low correlation obtained in our study may be due to not consider moisture related factors in data analyses. Soil moisture regimes depended on not only precipitation but also evaporation, which are very susceptible to others factors like soil
Results and discussion

properties, plant growth, vapour pressure deficit, etc. Also in our study soil moisture was measured at a depth of only 10 cm which was too shallow to match potential abilities of the plant root system and microbial organisms.

![Figure 14. Annual variation in soil carbon flux (A), soil temperature (B) and volumetric soil water content (C) at a depth of 10 cm. Summer (S), Autumn (A), Winter (W) and Spring (SP) periods are indicated for each parameter. Red arrow indicates period of torrential rains.](image)

Almagro (2009) observed a positive correlation ($P < 0.01$) between soil water content and soil respiration. However, in his study soil temperature was always above $16^\circ$C, and in our work temperature in the soil surface did not exceed $16^\circ$C. The results obtained by Almagro (2009) also showed that low soil water content strongly limited the response of soil respiration to soil temperature. The relationship between soil CO$_2$
Results and discussion

Efflux and soil temperature was strongly modulated by soil moisture, but in our case, soil moisture remained constant and therefore it did not modulate the soil respiration-soil temperature response.

![Graph A](image)

$$y = 1.44 e^{0.066x}$$

$R^2 = 0.91$ *

![Graph B](image)

Figure 15. Relationships between soil carbon flux and soil temperature (A), and between soil carbon flux and soil water content (B) in a mixed walnut – alder plantation. Significant differences: * $P < 0.05$.

Other works have also reported a significant exponential correlation between soil CO$_2$ flux and soil temperature (Kang Ni, 2012; Liu et al., 2009). Microbial activities as decomposition of soil organic matter are related to soil temperature. Thus, Parada et al. (1983) reported an increase in soil respiration between 1.5 and 3 times for every 10°C in temperature from 0 to 50°C. According to Pietikäinen et al. (2005) optimum temperatures for bacterial activity were between 25 and 30°C for agricultural and humus soils. The bacterial activities at the optimum temperatures were around 14 and 9 times above that at 0°C for the agricultural and humus soil, respectively. Above the optimum temperature the bacterial activity decreased, but some activity was observed
even at 45°C, indicating that the maximum temperature for growth of bacterial communities was above this temperature. Temperatures reached in our work varied between 0 and 16°C. Pietikäinen et al. (2005) reported that from 0 to 16°C there is a low increase of soil respiration, while soil temperatures above 16°C have a more pronounced increase. So we can conclude that in our work we are moving between soil temperatures in which an increase in soil temperature gives some increase in soil respiration (exponential relationship showed in Fig. 15A), but this pattern would change for higher temperatures in which the increase would probably be higher.

3.1.2. Influence of distance from points measurements to walnuts in soil respiration

**Figure 16. Annual flux variation according to distance from measurement points to walnuts.**

Error bars represent standard deviation of means. Different letters indicate significant differences between treatments.

The influence of measurement distance in soil respiration was highly significant despite the great variability between measurements (Table 4, $P < 2 \cdot 10^{-16}$). Standard deviation of these measurements varies from ±1.05 to ±1.5 µmol C · m$^{-2}$ · s$^{-1}$ and the mean coefficient of variation (CV) was 44% (Fig. 16).

There was a trend in which flux respiration decreased from a measurement distance of 1.75 m onwards. The highest respiration measurements were obtained at distance 1.75 m (3.4 µmol C · m$^{-2}$ · s$^{-1}$), while the lowest ones were found in distance 3.2 m (2.4 µmol C · m$^{-2}$ · s$^{-1}$). From distance 2.6 m soil respiration diminished significantly regarding soil respiration obtained at 1.75 m.
Results and discussion

Flux respiration was similar for distances 3.2 and 3.65 m (Fig. 16). We can infer that from 3.2 m flux is stabilized so there is not an influence of distance to walnut in flux respiration, maybe because tree roots still do not get that far.

Soil respiration is the result of a large set of factors, such as soil moisture, physical soil properties that control gas diffusivity and nutrient availability and soil topography (Epron et al. 2006), which might cause the great data variability found in soil respiration in our work.

Moreover, data variability is higher in shortest distances, with CV of 46% and 41% respectively at 1.3 and 1.75 m. Martin and Bolstad (2009) reported that in a spatial scale of less than 1 meter, there is an important source of variability, but 20% less than variability obtained at a scale of 100 m or more.

On the other hand, as said by Martin and Bolstad (2009), differences in soil respiration between samplings positions makes the mean annual soil respiration easily under or over estimated.

Influence of distance in fertilizer management

Soil flux evolution according to distance from point of measurement to walnuts was similar in fertilized and not fertilized treatments (Fig. 17). This is pointed out in the variance analysis (Table 4), in which the relationship between the averages of both parameters was not significant different ($P = 0.64$). Nevertheless fertilizer in our field was only incorporated from 1999 to 2010, so its effects are maybe blurred since it has been two years since the last treatment. This could explain our lack of significant difference for fertilization factor. The interaction between fertilization and distance was significantly different ($p < 2 \times 10^{-7}$, Table 4).
Average for not fertilized plots varied from 3.7 to 2.4 µmol C · m$^{-2}$ · s$^{-1}$. Fertilized management readings went from 3.3 to 2.3 µmol C · m$^{-2}$ · s$^{-1}$.

Giardina et al. (2004) found a decrease of 22% in the quantity of current assimilates that moved through the rhizosphere in fertilized plots, which relates to a 17% reduction in TBCA (Total Belowground Carbon Allocation). Together these changes led to 18% reduction in soil surface CO$_2$ efflux. Fertilization management in Giardina et al. (2004) was intense since they applied N, P, K, Ca, S, Mg and micronutrients.

Lower trends in flux respiration for fertilized treatments (Fig. 17) than for not fertilized could be explained by a decrease in root growth activity and biomass with greater nutrient availability, whereby soil respiration should decrease with fertilization (Raich and Nadelhoffer, 1989; Kim, 2008). This could explain, even if there is not a significant difference, why respiration in fertilized treatment was always lower than in not fertilized one, except for distance 1.3 m.

*Influence of distance with or without alder presence*

**Figure 18.** Flux evolution versus distance to walnuts according to alder presence. Significant differences: ** $P < 0.01$.

The alder presence did not influence soil respiration from 1.75 m to walnuts (Table 4, $P > 0.3$). However, at 1.3 m soil respiration with alder presence was significantly higher than without alder ($p < 0.002$), despite their CV was quite high (32%).

In both alder and no alder presence, the soil flux evolution decreased according to the increase of distance to walnuts. Except at 1.3 and 3.2 m from walnuts, flux without alder presence was higher than with alder presence (Fig. 18).
Values reached in alder presence varied from 3.6 (at 1.3 m) to 2.3 µmol C · m\(^{-2}\) · s\(^{-1}\) (at 3.65 m) and for no alder presence from 3.5 (at 1.75 m) to 2.3 µmol C · m\(^{-2}\) · s\(^{-1}\) (at 3.2 m).

Alder trees are known to fix nitrogen by *Frankia* sp. in ammonium form (NH\(_4^+\)). Higher available nitrogen makes higher production of aboveground biomass, but lower belowground biomass (Forrester et al., 2006). The explanation of no significant observed difference between alder and no alder presence could be that nitrogen is not a limiting factor in this soil. However, flux tends to be higher without alder presence even if there is no significant difference. N addition could inhibit soil CO\(_2\) emission because a decrease in microbial biomass and activity (Lee and Jose, 2003).

Alder presence induces higher production of walnut aboveground biomass, this must cause a higher litterfall production too (table 5), and thus, more biomass should be incorporated to the soil.

Table 4. Variance analyse of the influence of different factors in soil carbon flux. Where F is fertilized, A is alder presence, D is distance to walnut and d is date of measurement. Interactions between them were also analyzed. \(\chi^2\) is Chi Square value, Df are degrees of freedom and \(P\) is the reached probability. Significance codes: Significant differences: *** \(P < 0.001\); * \(P < 0.05\).

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>(\chi^2)</th>
<th>Df</th>
<th>(P)</th>
</tr>
</thead>
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<td>1</td>
<td>0.64</td>
</tr>
<tr>
<td>A</td>
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<td>1</td>
<td>0.332</td>
</tr>
<tr>
<td>D</td>
<td>136.09</td>
<td>5</td>
<td>&lt; 2 · 10(^{-16}) ***</td>
</tr>
<tr>
<td>d</td>
<td>710.21</td>
<td>12</td>
<td>&lt; 2 · 10(^{-16}) ***</td>
</tr>
<tr>
<td>FxA</td>
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<td>1</td>
<td>0.847</td>
</tr>
<tr>
<td>FxD</td>
<td>39.34</td>
<td>5</td>
<td>2.0 · 10(^{-07}) ***</td>
</tr>
<tr>
<td>Fxd</td>
<td>11.69</td>
<td>5</td>
<td>9.3 · 10(^{-08}) ***</td>
</tr>
<tr>
<td>AxD</td>
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<td>12</td>
<td>0.471</td>
</tr>
<tr>
<td>Axd</td>
<td>5.89</td>
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<td>0.921</td>
</tr>
<tr>
<td>Dxd</td>
<td>60.29</td>
<td>60</td>
<td>0.465</td>
</tr>
<tr>
<td>FxAxD</td>
<td>14.93</td>
<td>5</td>
<td>0.011 *</td>
</tr>
<tr>
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<td>FxAxDxd</td>
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<td>60</td>
<td>0.988</td>
</tr>
</tbody>
</table>
3.1.3. Seasonal influence in soil respiration.

The seasonal influence in soil respiration was highly significant \( P < 2.2 \cdot 10^{-16} \). The lowest flux proportion was reached in winter (14%), while the highest one was in summer (33%).

The lowest coefficient of variation was for summer measures \( CV = 30\% \), while the highest one was reached for winter measures \( CV = 47\% \). Average temperature for autumn time was 9.7°C, for winter time was 4°C and 13.2°C for spring time (soil temperature for summer time was not measured).

Seasonal variation has been reported in most of the works relating soil respiration to temporal variability (Lou et al., 2003; Almagro, 2009; Liu et al., 2009). Moreover, this seasonal variation is directly related to soil temperature (Fig. 14). It is known that active root growth and increasing microbial respiration are both associated with high soil temperature (around 30°C) and suitable precipitations (in summer and spring). This is why soil respiration in winter and autumn were likely related to the root and microbial respiration depression by low temperature (< 10°C) (Fig. 14 and 19). There is a higher root respiration in growing plant period (spring and summer). Furthermore additional nutrients are returned to the soil through root exudates in this period, which benefit the microbial respiration in the rhizosphere (Lou et al., 2003).

Figure 19. Seasonal flux distribution. Error bars are standard deviation of means. Percentages of total annual carbon flux, in Summer (S), Autumn (A), Winter (W) and Spring (SP).
Seasonal influence according to distance to walnuts.

Soil respiration rates varied in summer from 3.6 to 4.8 µmol C \cdot m^{-2} \cdot s^{-1}, with average CV = 28% (Fig. 20A). In autumn, soil respiration rates varied from 2.3 to 2.9 µmol C \cdot m^{-2} \cdot s^{-1} (Fig. 20B). In winter, with the lowest ratios in the year, soil respiration rates varied from 1.4 to 2 µmol C \cdot m^{-2} \cdot s^{-1}. In this period, the average soil temperature was 4.6\degree C and the average soil water content 31% (Fig. 20C). Finally, in spring soil respiration rates varied from 4.6 to 3.3 µmol C \cdot m^{-2} \cdot s^{-1}. In this case, average soil temperature was 14\degree C and the average soil water content 30% (Fig. 20D).

Most marked trends of negative relationship between soil respiration and distance from the nearest tree stem were showed in summer and spring time (Fig. 20). Epron et al. (2006) suggest that changes in soil respiration in short distances to nearest tree stem (from few centimeters to 10 meters) are influenced by biotic factors (organic detritus, microbial populations, etc.). However it is noticeable that these variations are lower in autumn and in winter. This could be because the principal motor for these biotic factors is an abiotic factor: soil temperature. If soil temperature is around 10 \degree C or less, it does not trigger the biotic processes which make spatial variability in soil (microbial activity is minimal and roots do not develop). Also it can be explained...
because roots are more active in summer and spring, which makes more pronounced heterogeneity in soil respiration (Fig. 19 and 20).

Stoyan et al. (2000) has reported that presence of pockets or aggregates within the soil gets more significant as the soil dries, what could affect the heterogeneity in soil respiration. Our highest CV was reached in winter (38%) which was extremely dry in the period of study (only 9 mm fallen from December to March).

**Daily flux evolution according to distance to walnuts**

![Daily flux evolution according to distance to walnuts](image)

**Figure 21.** Daily flux variations according to distance to walnuts along year seasons.

In Fig. 21 it can be seen how in every sampling day soil respiration decreases with distance to walnuts. This trend is more accused in summer and spring measurements than in autumn or winter measures, like it can be seen also in Fig. 20, Fig. 22 and Fig. 23.

In summer, on the 13/09 flux varied from 4.7 (at 1.75 m) to 3.6 µmol C · m$^{-2}$ · s$^{-1}$ (at 3.65 m). In autumn the maximum flux was 4.3 µmol C · m$^{-2}$ · s$^{-1}$ (02/12) and the minimum was 2.05 µmol C · m$^{-2}$ · s$^{-1}$ (12/12). Seasonal average of soil temperature was 10 °C. In winter, the maximum flux reached 2.5 µmol C · m$^{-2}$ · s$^{-1}$ and the minimum 1.3 µmol C · m$^{-2}$ · s$^{-1}$ (both 23-24/01/2012) and seasonal average of soil temperature was 4°C. Finally, the maximum flux in spring reached 4.8 µmol C · m$^{-2}$ · s$^{-1}$ (15/05) and the
Results and discussion

minimum 2.3 µmol C · m$^{-2}$ · s$^{-1}$ (20/04), with a seasonal average of soil temperature of 14°C.

Average of daily variation of flux was 1.1 µmol C · m$^{-2}$ · s$^{-1}$ in summer, 1.2 µmol C · m$^{-2}$ · s$^{-1}$ in autumn, 0.9 µmol C · m$^{-2}$ · s$^{-1}$ in winter and 1.4 µmol C · m$^{-2}$ · s$^{-1}$ in spring.

Saiz et al. (2006) also reported that the lowest soil respiration rate was observed during winter (1.3 µmol C · m$^{-2}$ · s$^{-1}$ in our case) and the highest one was during summer (4.8 µmol C · m$^{-2}$ · s$^{-1}$ in our case). In all the measurement days there was a strong negative relationship between soil respiration and the distance to the nearest tree trunk. Generally, soil respiration was highest close to the trunk. Saiz et al. (2006) also obtained a significant relationship between distance to trees and soil respiration in Sitka spruce stands, except in the most mature of them. Nevertheless, we have worked with mature trees (17 years) and relationship was highly significant.

As it was showed in Fig. 14, temperature and humidity variation influenced the seasonal trend observed in the spatial variability of soil respiration. Therefore, metabolic activities will be more or less favored depending on the specific environmental conditions present at a particular location.

Spatial patterns remained remarkably stable throughout the year, which agrees with results saw by Søe and Buchmann (2005) who reported that the spatial patterns in soil respiration are associated with stable characteristics of the underlying processes.

Seasonal influence according to fertilizer management

There were not significant differences between fertilized and not fertilized treatments, except in winter at distance 2.6 m ($P < 0.04$). In spring at distance 1.75 m it was reached a significance of $p < 0.07$, where there was higher soil respiration in not fertilized than in fertilized management.

In summer, soil flux respiration varied from 5 to 3.3 µmol C · m$^{-2}$ · s$^{-1}$ in not fertilized management, while for fertilized plots varied from 4.8 to 3.5 µmol C · m$^{-2}$ · s$^{-1}$ (CV = 30%) (Fig. 22A). In autumn, soil flux respiration varied from 3 to 2.2 µmol C · m$^{-2}$ · s$^{-1}$ in both treatments. (CV = 34%) (Fig. 22B). In winter, soil flux respiration varied from 2.1 to 1.5 µmol C · m$^{-2}$ · s$^{-1}$ in not fertilized management, while for fertilized plots varied from 2 to 1 µmol C · m$^{-2}$ · s$^{-1}$ (CV = 38%) (Fig. 22C). In spring, soil flux respiration varied from 5.2 to 3.5 µmol C · m$^{-2}$ · s$^{-1}$ in not fertilized management, while for fertilized plots varied from 4.6 to 3.1 µmol C · m$^{-2}$ · s$^{-1}$ (CV = 30%) (Fig. 22D).
Results and discussion

Figure 22. Seasonal flux variation versus distance to walnut according to fertilization management. One representative day of measure was chosen for each season. Significant differences: * $P < 0.05$.

Seasonal influence according to alder presence

Only significant difference was found at 2.5 m in spring ($p < 0.008$), in which alder presence diminished soil respiration regarding no alder presence (Fig. 23D). In summer, distance 2.6 m significance was $P < 0.07$ (Fig. 23A) (CV = 11 %), and in winter, distance 3.65 m significance was $P < 0.09$ (Fig. 23C).

In summer the maximum soil flux respiration was 5 and minimum 3.3 µmol C · m$^{-2}$ · s$^{-1}$ without alder presence, while in alder presence was 4.9 and 3.6 µmol C · m$^{-2}$ · s$^{-1}$ (Fig. 23A). In autumn soil flux respiration reached its maximum of 3.2 and its minimum of 2.2 µmol C · m$^{-2}$ · s$^{-1}$ without alder presence, while in alder presence varied from 2.8 to 2.2 µmol C · m$^{-2}$ · s$^{-1}$ (Fig. 23B). In winter soil flux respiration varied from 2.1 to 1.3 µmol C · m$^{-2}$ · s$^{-1}$ without alder presence, while in alder presence varied from 2.4 to 1.5 µmol C · m$^{-2}$ · s$^{-1}$ (Fig. 23C). In spring (Fig. 23D), the maximum soil flux respiration was 4.6 and minimum 3.2 µmol C · m$^{-2}$ · s$^{-1}$ without alder presence, while in alder presence was 4.9 and 2.9 µmol C · m$^{-2}$ · s$^{-1}$.
Results and discussion

Figure 23. Seasonal flux variation versus distance to walnut according to alder presence. One characteristic day of measurement was chosen for each season. Significance codes: ** $P < 0.01$.

In our work, the highest values in soil respiration are found in not fertilized treatment or without alder presence blocks whenever there is a significance difference between treatments (Fig. 22 and 23). This is in accordance with Raich and Nadelhoffer (1989) who described that root growth activity and biomass decrease with greater nutrient availability, so soil respiration should decrease with a higher nutrient availability (we assume that alder makes nitrogen more available through its fixation) or under fertilizer application. Inhibition of microbial activity could be more pronounced at spring when microbial activity is tender and could be more influenced by fertilization or alder presence (Fig. 22D).

3.2. LITTERFALL

3.2.1. Daily trapped litterfall

Leaf drop period took place from 13/09 to 18/10. Walnut total litterfall was higher than alder litterfall, having 2200 g $\cdot$ m$^{-2}$ and 109 g $\cdot$ m$^{-2}$ of dry matter respectively, which means that 95 % of the litterfall collected came from walnuts.
Table 5. Carbon content (g C · m\(^{-2}\)) corresponding to walnut and alder litterfall. F NA is fertilized without alder presence; F A is fertilized with alder presence; N NA is not fertilized without alder presence; N A is not fertilized with alder presence. The showed data is the total daily collected litterfall, separating walnut from alder leaves and converted in carbon content (50% of leaves dry matter).

<table>
<thead>
<tr>
<th>Date</th>
<th>Walnut</th>
<th>F NA</th>
<th>13/09/2011</th>
<th>14.08 ± 3.48</th>
<th>1.77 ± 0.41</th>
<th>1.49 ± 0.26</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alder</td>
<td>F A</td>
<td>3.65 ± 0.59</td>
<td>2.16 ± 0.56</td>
<td>0.51 ± 0.17</td>
<td>4.05 ± 0.67</td>
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<td>Walnut</td>
<td>N NA</td>
<td>2.89 ± 0.36</td>
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<tr>
<td></td>
<td>Alder</td>
<td>N A</td>
<td>0.32 ± 0.11</td>
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<td>4.05 ± 0.67</td>
</tr>
<tr>
<td>27/09/2011</td>
<td>Walnut</td>
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<td>9.31 ± 0.69</td>
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<td>11.08 ± 1.66</td>
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<tr>
<td></td>
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<td>44.38 ± 4.48</td>
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<td>21.02 ± 2.59</td>
<td>41.82 ± 5.51</td>
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<td></td>
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<td>0.66 ± 0.15</td>
<td>2.64 ± 0.32</td>
<td>0.12 ± 0.04</td>
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</tr>
<tr>
<td>11/10/2011</td>
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<td>55.95 ± 3.99</td>
<td>102.35 ± 13.85</td>
<td>85.99 ± 11.96</td>
<td>73.59 ± 9.01</td>
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<td></td>
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<td>3.87 ± 0.45</td>
<td>2.73 ± 0.46</td>
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<tr>
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<td>130.68 ± 6.38</td>
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<td>6.16 ± 0.74</td>
<td>1.08 ± 0.29</td>
<td>7.09 ± 0.99</td>
</tr>
</tbody>
</table>

Maximum rate for alder litterfall fell in 11/10, registering a 1.5 % of total annual litterfall. The minimum rate for walnut litterfall fell in 19/09, registering 1.2 % of total annual litterfall and the maximum fell in 18/10 registering 45 % of total annual litterfall.

Alder presence was not significant different in fertilized management, while it was significant in not fertilized management (P < 0.02). The lack of significant differences in collected litterfall between traps near or far an alder (except in not fertilized management) could be due to the experimental design. Alder leaves were all over the field and the trap places gave not an idea of alder presence because trees were not isolated between treatments. Other studies like Ruan et al. (2003) covered canopy trees with mesh netting of about 4 m\(^2\), collecting litterfall from there without interferences between trees or treatments.

Sommerville et al. (2004) have demonstrated the interactive effects of mixed-litter quality and soil fertility, increasing litter quality, faster decomposition and greater mineralization rates. Nevertheless, Quested et al. (2005) have confirmed that the decomposition environment, rather than species-specific litter quality, could determine litter-mixing interactions, and that decomposition was greater in soils with higher fertility.
3.2.2. Relationship between soil flux respiration and trapped litterfall.

In Fig. 24 it can be seen a decrease in soil respiration (red curve) until 11/10 and then there is a recovery more than a month later. Litterfall (blue curve) has fell with lower speed from 13/09 to 27/09. From this last date there is a more beset slope until the stop of the leaf drop period. The decrease in soil respiration was of 1.5 g C · m$^{-2}$ · day$^{-1}$, while the increase in leaf litterfall of 2 g C · m$^{-2}$ · day$^{-1}$.

![Autumn trend in soil respiration and litterfall](image)

**Figure 24. Autumn trend in soil flux respiration (red) and litterfall (blue) both in g C · m$^{-2}$ · day$^{-1}$.**

Högberg et al (2001) observed two peaks in microbial activity, one before peak in plant litterfall and the other one a month after. These peaks could explain our high rates in soil respiration after and before maximum in plant litterfall (Fig. 24). Before leaf and fruit senescence, trees often retranslocate nutrients and carbohydrates to stems and roots. An increase in root exudates could result in elevated microbial growth in soils, as microbial biomass variations are driven by root exudates variations (Högberg et al, 2001). However Ruan et al. (2003) reported that soil microbial activity fluctuated throughout the year of study with the same magnitude for the control and litter-exclusion treatments, so litter exclusion did not alter the fluctuation of soil microbial activity.

During the senescence period, it is known that there is a reduction in plant nutrient uptake, and consequently an increase in soil nutrient availability. This could induce an increase of microbial population (and so an increase in soil respiration) because of the decrease in competition for nutrients between plants and soil microbes.
Trapped litterfall in our study only concerned leaves, which are directly associated with the processes of plant nutrient uptake or nutrient/carbon retranslocation, not as bark tree or other tree litter which could overestimate total belowground carbon incorporated to soil.

It must be notice that aboveground carbon inputs by its own not influence soil respiration, as is confirmed by Högberg et al. (2001). Belowground processes like a mayor nutrient availability, exudates driven from roots to soil, among others, which could be related to aboveground events, are the ones which drive soil respiration.
4. CONCLUSIONS

Soil respiration was highly influenced by numerous factors, which require further research.

- There is a high significant influence of soil temperature in soil respiration rates. This factor is mostly driven by the response of microbial activity above a determined soil temperature.

- During our year of study, soil water content did not influence soil respiration since soil water was constant over the season. Nevertheless if we had compared within more than one phenologic year with different rates in soil moisture, we probably would have inferred the relationship between soil water content and soil respiration.

- Mostly, alder presence has not modified the response of soil respiration. Neither does fertilizer management. However, treatments without fertilization and without alder presence obtained higher respiration rates in those cases with significant differences (except in Fig. 18 distance 1.3 m).

- To better analyze alder presence, it would be appropriate to study nitrogen availability that alder generates between treatments (alder presence and fertilization management). It is important to know the projection area of roots from each tree to know where they are overlapped. This is possible with a Rhizotron equip, which can also analyze the annual growth rate of roots. Fertilization was not intensive (50 kg N·ha\(^{-1}\) annually) and it was not incorporated since 2010. With a more recent and intense fertilization, containing phosphor or potassium, we would probably have another response in soil respiration between treatments.

- Differences in weight of collected litterfall were not found between alder or not alder treatments. Seasonal trends of soil respiration and litterfall showed an asynchronous fluctuation between these two factors. To better analyze litterfall, it would have been desirable to have soil mineralization rates. This way the carbon quantity allocated annually in soil from aboveground can be known, and which proportion is respired by the soil. To analyze treatments it would be convenience to isolate tree top and analyze each walnut litterfall separately.
5. REFERENCES


References


