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Influence of nitrogen and soil physical characteristics on belowground carbon flux dynamics of woody plants

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1. Introduction and aims

The carbon cycle

During the past decades, the link between climate change and the increase in the concentration of carbon dioxide (CO_2) in the atmosphere, one of the main factors leading to the so-called "greenhouse effect", has gained more and more evidences, pushing the scientific community to intensify the research on the carbon (C) cycle. Since 1958, the concentration of CO_2 in the atmosphere has growing at a rate of around 0.5-3 ppm per year (Keeling and Whorf 2001) likely due to human activities, such as the intensive use of fossil fuel for energy production, and land use change (Falkowski et al. 2000). Climate change is predicted to have significant effects on global average air and ocean temperatures, melting of snow and ice, and global average sea levels (IPCC 2007).

Most of the C on Earth resides in oceans (38.100 Gt C) (1 Gt = 1.000.000 tonnes), followed by soil (1500 Gt), atmosphere (762 Gt) and the vegetable component of terrestrial ecosystems (560 Gt) (Sabine et al. 2004). Carbon flows among these compartments mainly through red-ox processes, such as respiration and photosynthesis. Through photosynthesis about 122 Gt C per year are subtracted from the atmosphere. At the same time 60 Gt are released back to the atmosphere through autotrophic respiration of plants. Additional 60 Gt C are released into the atmosphere through heterotrophic soil respiration and 1 Gt C is lost to the atmosphere through the land use change and deforestation. The net balance of terrestrial ecosystems is therefore roughly 1 Gt C per year in favor of C-fixation. Additionally to that, 2 more Gt C yr^{-1} are sequestered by oceans. Every year, however, about 6 Gt C are released into the atmosphere through human activities, resulting in an net increase of the CO_2 concentration in the atmosphere.

The most important C flux from the soil is respiration (R_s), which includes autotrophic (R_a) and heterotrophic respiration (R_h). R_a is the respiration of roots and of rhizospheric microorganisms, including root symbionts like mycorrhizal fungi, while R_h is the respiration produced by microorganisms during the processes of decomposition of soil organic matter. R_a and R_h are closely linked together by the turnover of roots, the primary source of soil organic matter, which provides substrate for microbial decomposition (Cheng 1999).

Since the net C balance of terrestrial ecosystems is very close to the equilibrium point, it is important to understand the mechanisms and factors controlling soil respiration. Among those, an important role is played by soil chemical and physical characteristics, such as temperature, humidity and availability of nutrients. Unfortunately, processes generating belowground C-fluxes, namely those related to the activity of the plant root system, and to the decomposition of organic matter, are only partially understood.

C fluxes in orchard ecosystems

The annual C-balance of land ecosystems mainly depends on incoming and outgoing CO_2-fluxes between the ecosystem and the atmosphere. The main incoming CO_2-flux is represented by the C fixed through photosynthesis, the so called gross primary productivity (GPP). The main outgoing CO_2-flux is represented by plant respiration (autotrophic respiration, R_a) and soil microbial respiration (heterotrophic respiration, R_h). By subtracting R_a from the GPP, we obtain the net primary productivity (NPP) of the ecosystem, which represents the annual increase of C contained in plant biomass at ecosystem level. By further subtracting R_h from the NPP we obtain the net ecosystem productivity (NEP). C can also be "lost" in the case that biomass is removed from the ecosystem, or if CO_2 is released in the atmosphere through events like fire, or if compounds that contains C are leached to the groundwater. If those events are also considered and subtracted from the NEP, we obtain the net biome productivity (NBP), which finally determines whether an ecosystem can be classified as a sink or a source for atmospheric CO_2.

In contrast to forest and grassland ecosystems, agricultural ecosystems are often regarded as a net source of CO_2 (Schulze et al. 2010). Indeed, there are several reasons to explain this. First it has to be considered that plants grown in agricultural ecosystems are selected for particular characteristics, like a high partitioning of their NPP to plant organs of commercial value. Therefore, biomass contained in the harvested products usually represents a large fraction of NPP, that once removed from the ecosystem, accounts as a C loss. Second, agricultural ecosystems have to be managed through various practices, that implies the use of machineries, the consumption of fossil fuel and the production of CO_2. Moreover, it has to be considered that agricultural practices, like soil tillage, nutrient supply or irrigation, can have major influences on C-fluxes. And further, we have to consider the CO_2 that is produced during the production of fertilizers and all other facilities and machineries that are necessary to manage an agricultural ecosystem. However, in this context we must distinguish between annual (like corn, maize, soya) and perennial (like apple or grapes) crop systems. Most annual crops are regarded as a net source for CO_2 to the atmosphere, emitting on average 138 g C m^{-2} yr^{-1} (Ceschia et al. 2010), as a consequence of

the reasons listed before. In addition, annual crops do not cover the soil for long periods, which increases net annual carbon loss from the soil in the absence of photosynthesis. On the contrary perennial crops, like fruit orchards, particularly if managed with the presence of perennial herbaceous vegetations in the tree alleys, are potentially able to take advantage of the photosynthetic activities for most part of the year. On one hand, a large part of NPP produced by perennial crops is partitioned between woody plant organs (stem, branches, roots) and leaves. Recent studies report NPP-values ranging between 4 and 10 t C ha yr^{-1}, for 5 and 10 year old apple orchards respectively (Faqi et al. 2008; Panzacchi 2008). It has been estimated that the NPP annually removed with fruits ranges between 25-49% (Faqi et al. 2008; Panzacchi 2008) and that the remaining quota of C remains in the orchard. However, NPP of apple orchards is dependent on many factors, with tree age and planting density in particular, being the most important. On another hand, orchards can have permanent grassed alleys, that are periodically mowed and the mowed biomass is left in place, hence contributing to the C-fixation of the orchard. Furthermore, since practices like soil tillage are not common in orchards, there is a reduced disturbance to the soil and thus reduced CO_2-efflux from it. Orchards ecosystems might have the potential to be a sink for atmospheric C, but there is still poor evidence for it. In particular, there is the need for a better understanding about processes linked to belowground plant organs, like biomass allocation to roots, root turnover and root respiration, as well as soil microbial respiration, and how these processes are influenced by environmental parameters, like soil temperature, soil moisture and nutrient availability. All these C-fluxes, into- and from the soil will determine whether an orchard ecosystem is sustainable from a point of view of its C-footprint.

Root C dynamics

Part of the carbon fixed by trees during photosynthesis is allocated to the roots to meet the energy demand related to growth and nutrient absorption, and to cover the energy needed by the rhizospheric microbial community. Up to 60% of photosynthates produced during the day is transferred to roots (Van der Werf et al. 1992a; Lambers et al. 1998a) and 35% is lost through root respiration (Lambers et al. 2002). This amount may vary depending on the species, the growth rate (Poorter et al. 1991; Lambers et al. 1998b; Scheurwater et al. 1998 and 1999), the age, health, and nutritional status of the plant, as well as on the soil-climatic conditions (soil temperature and moisture, the availability of nutrients, the pH, and its texture) (Lambers 2005).

Among the factors related to the plant, the relative growth rate mostly affects the amount of photosynthates allocated to roots and their respiration rates. In plants with slow growth rates, the requirements of roots for C increase significantly, compared to plants with faster growth rates (Poorter et al. 1991). If, associated to low growth rate, there is also a limited availability of nutrients, the fraction of photosynthates used for root respiration further increases (Van der Werf et al. 1992b). In general, the fraction of carbohydrates allocated to the root system tends to decrease with the age of the plant (Van der Werf et al. 1988). Indeed, fine root respiration per unit weight is known to be higher for young than for old roots (Fig. 1.1).

The nutritional status of the plant also effects the respiration rate. In all plant organs respiration is related to N-concentration, given the importance of N in many metabolic processes (Ryan 1991). Reich et al. (2008) have shown that there is a positive relationship between the concentration of N in roots and their respiration rate by analyzing a database of 2510 measures of respiration rates and nitrogen concentration in various plant organs (leaves, stems, roots) among 287 different species.

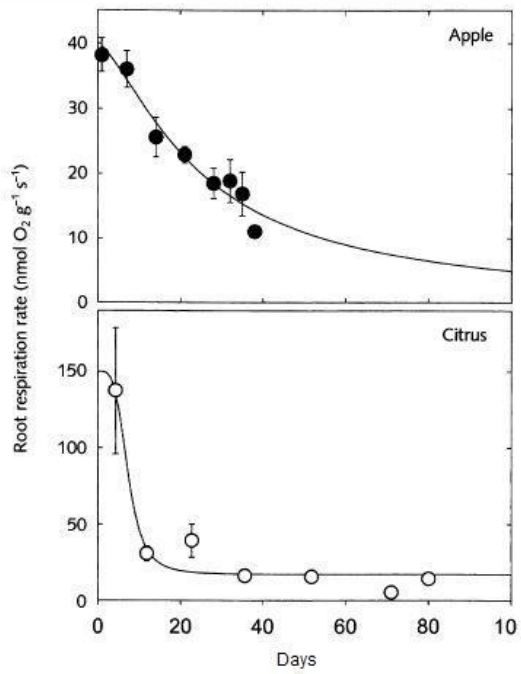

Fig. 1.1 - Fine root respiration as a function of age in the case of apple and citrus (from Bouma et al. 2001).

Among climatic factors, temperature and moisture mostly affect root carbon requirements and respiration rates. The relationship between soil temperature and root respiration is described by the factor Q_{10}, defined as the increase of respiration for every 10°C-rise of soil temperature. The Q_{10} is typically around a value of 2 (Atkin and Tjoelker 2003), normally ranging between 1.3 and 3. This value is strongly dependent on the interval of temperature. At lower temperatures the increase of respiration rates is steeper than at higher temperatures. In addition, sharp increases in respiration rates occur when the soil temperature changes rapidly (Fig. 1.2a), in the order of few hours (Bryla et al. 2001), while when soil temperature changes over longer periods (in the order of days), the response of respiration is less evident (Fig. 1.2a). This indicates an adaptation which most likely occurs in order to limit carbohydrate consumption (Eissenstat et al. 2006). Soil moisture represents the second most important parameter influencing root respiration. Bryla et al. (2001) found a decrease in root respiration with decreasing water availability, a response similar to that observed at low soil temperatures. This illustrates how roots reduce their carbohydrates consumption, if soil conditions are less than optimal to perform their functions (Fig. 1.2b).

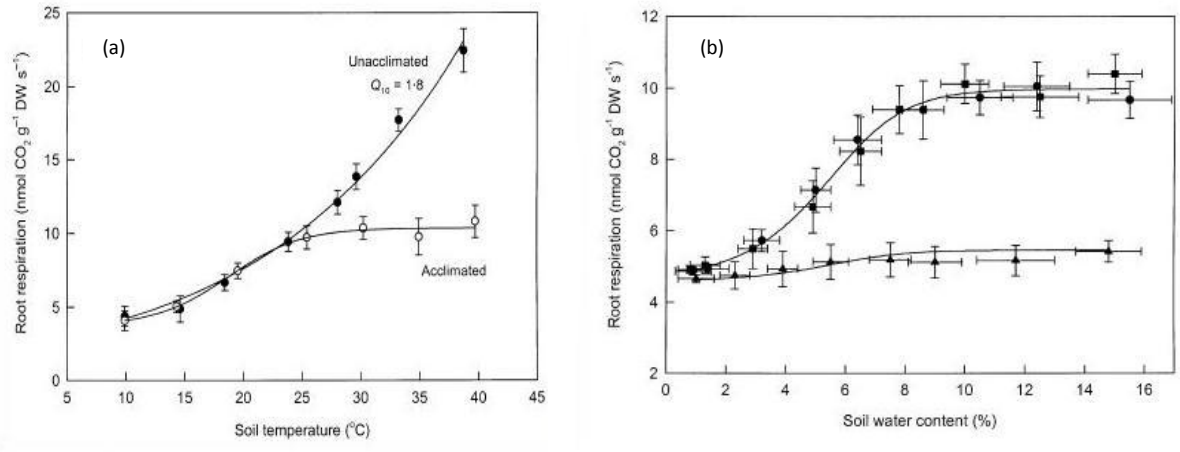

Fig. 1.2 - (a) Response of fine root respiration to soil temperature increase at hourly steps (●) and at intervals of 6 days (o). (b) Response of root respiration to changes in soil water content at different soil temperatures, in the case of orange trees (▲=15, ●=25, ■=35°C) (from Bryla et al. 2001).

The amount of C allocated to roots also increases significantly under conditions of limited nutrient availability in soil, especially in the case of phosphorous (P) and nitrogen (N), because plants need a more extended root system (Lambers et al. 1998b) and because the costs associated with the absorption of nutrients increase substantially (Van der Werf et al. 1992b). The absorption of N from the soil can account for up to 90% of the total energy needed by the roots to acquire nutrients. Especially if N is absorbed in the form of nitrate, because it has to be reduced to ammonium before assimilation, a process that requires additional energy. Another issue related to nutrient availability is that, especially N, stimulates the metabolism of fine roots, increasing their rate of respiration and their absorption capacity (Eissenstat 1992) and therefore the requirement for photosynthates.

The carbon cost associated with fine roots can be estimated by measuring the two most important components involved in this process. The first component is given by the C contained in the biomass of fine roots while the second component is represented by the amount of C respired as CO_2 produced by metabolic processes. Studies on apple showed that the fraction of dry matter contained in fine roots is only 2-3% of the dry weight of the tree (Fukuda et al. 1991). However, it is also necessary to take into account the fraction of C continuously released to the soil through the turnover of the roots. The turnover of fine roots is defined as the percentage of roots (fine) that dies over a period of time, compared

to the total amount of all the roots (fine), existing or newly formed during the same period. The turnover of fine roots is a significant component in the C-budget of plants. Through fine root turnover a considerable amount of organic matter and nutrients is transferred to the soil, in some cases even higher than that transferred to the soil by litterfall (Vogt et al. 1986; Scandellari et al. 2007). The process of turnover affects almost exclusively the pool of fine roots, and the smaller the diameter the higher are turnover rates (Wells and Eissenstat 2001). Turnover largely depends on the type of root, the species, and by environmental factors. Among these factors, the species has certainly a major effect. For example, the same type of fine root has an average lifespan of approximately 4 weeks in apple, and of 40 weeks in citrus. Moreover, turnover is positively correlated with increasing soil temperature, while with humidity the correlation is negative.

Fine root respiration includes the growth respiration and the maintenance respiration. The former takes place during the formation of the fine root, while the latter occurs during water and nutrients uptake. Studies on apple have shown that the root growth can take place throughout the growing season, but especially in late spring, during or immediately after flowering, and between late summer and early autumn (Rogers 1939; Head 1966 and 1967; Rogers and Head 1969). Processes of root growth and canopy growth tend to not overlap during the season, occurring at different moments, indicating that there is a competition for carbohydrates between above- and belowground plant organs (Priestley et al. 1976). In many deciduous fruit trees, fine root growth (< 2 mm) is an active process characterized by one or more events of greater magnitude (seasonal flushes), followed by periods of lower or totally depressed growth (Rogers 1939; Head 1966 and 1969a; Atkinson and Wilson 1980; Glenn and Welker 1993; Reid and Bowden 1995). In the case of apple, for example, it has been observed that root growth was reduced in winter, when soil temperature was between 2 and 7°C, and resumed when soil temperature exceeded 7°C (Rogers 1939). During the growth phase of the root, which in the case of the apple lasts about one week, the respiratory costs associated with this process can be quantified in 42.3 mmol of CO_2 per gram of dry matter produced (Eissenstat et al. 2001). The highest levels of fine root respiration are recorded just after the end of the growing phase when nutrient absorption capacity is also highest. Within a few weeks, the absorption capacity and the rate

of respiration decline. Eissenstat et al. (2001) calculated that, in apple trees, the carbon costs related to the maintenance of fine roots equals the cost of their production in about 30 days. The flow of C to roots has a major influence in the C-budget of trees, being necessary to a large extent for processes of root growth, root respiration and turnover of fine roots. The extent of respiratory processes related to growth and maintenance of roots and absorption of nutrients depends on the growth rate of the species and on soil-related factors such as temperature, moisture levels and nutrient availability. In general it can be said that root respiration reflects the environmental conditions of soil where the roots perform their functions and that respiration is of higher intensity in situations where soil conditions are favorable for absorption processes. The same principle can be applied in the case of root turnover, which is faster when plant growth is optimal. In this way the plant can invest resources to maintain a highly efficient root system by replacing older fine roots with fine roots of new formation.

Root turnover is relevant at an ecological and agronomical level. Large quantities of organic matter enter the soil through root turnover. Once decomposed by the microbial biomass, this organic matter becomes part of the soil carbon pool. In this way C is sequestered in the medium and long term contributing to the reduction of C-concentration in the atmosphere. In addition, the decomposition of dead roots improves soil fertility through the release of nutrients. This aspect is very important in the view of the sustainability of a productive ecosystem, such as orchards are, where the external supply of nutrients is usually very high and strategies to balance economic and ecological aspects becomes necessary.

Aims of the research

During this PhD, I focused my research activity on carbon cycling in managed tree ecosystems, with a special regard to soil respiration in apple orchards. The aim of my research was to study total soil respiration, to separate it in its two main components, root- and microbial respiration, and to define how their respiratory patterns are influenced by nitrogen availability and environmental parameters, such as soil temperature and soil moisture. I also performed an experiment using *Populus tremuloides* as a model plant. The aim was to study how fine root respiration is influenced by different levels of nitrogen availability, and how this response changes over the lifetime of a root.

The first experiment aimed to identify the role of environmental parameters and root density on soil respiration rates in an apple orchard, and to verify how soil respiration is affected by the history of nitrogen supply.

The second experiment was performed during my stage abroad taking advantage of the expertise on root studies present in the Root Ecology Laboratory of Prof. Eissenstat (Pennstate University). The experiment aimed to study whether fine root respiration is influenced by increasing availabilities of nitrogen and if nitrogen influences respiration over the lifetime of a root. Another aim was to test if the respiration of fine roots is influenced by uniform or localized nitrogen availability.

The third experiment aimed to separate soil respiration in its two main components, autotrophic- and microbial soil respiration, and to study if they are differentially affected by nitrogen supply.

2. Experiment one

Spatial and temporal effects of soil temperature and moisture and the relation to fine root density on root and soil respiration in a mature apple orchard

Introduction

There is an interest in understanding the drivers of the terrestrial carbon (C) budget on the global scale because of the effects of the increase of carbon dioxide (CO_2) concentration in the atmosphere on global warming. Soil represents the major sink for C in terrestrial ecosystems and the rate at which the soil C accumulates depends on the balance between C inputs from vegetation (directly through root exudates or indirectly through root turnover, leaf litter, etc.) and C losses due to the CO_2 efflux from the soil surface as a consequence of both autotrophic and heterotrophic respiration. In contrast to forest and grassland systems, agricultural systems are often regarded as a net source of atmospheric C (Schulze et al. 2010) for both direct causes (such as soil tillage and high partitioning of assimilated C to harvest products) and indirect causes (such as consequences of land use change and indirect CO_2 emissions associated with land management). Soil respiration (R_s) represents a significant fraction of the carbon leaving terrestrial ecosystems, as a result of both root-derived (R_a) and microbial (R_h) respiration. In spite of a relatively extensive literature about respiration of aboveground organs, little attention has been devoted to the study of root respiration in orchards.

Various environmental conditions have been found to affect seasonal variability of R_s, of which soil temperature and moisture are often most important (Tedeschi et al. 2006; Cook and Orchard 2008): both factors affect the rate at which soil organic matter (SOM) is decomposed by microbes, root growth and root metabolism. SOM quality and soil N availability may also control R_s by affecting the microbial growth and activity, while soil N may affect tree and root metabolism through an indirect effect on the partitioning of photosynthates and a direct effect on root respiration.

A range of methods have been used to partition the soil surface CO_2 efflux into R_a and R_h (e.g. Hanson et al. 2000; Kuzyakov 2006; Subke et al. 2006), each one having specific applications. Trenching and girdling are probably the most common methods used, but their results are difficult to interpret due to the significant disturbance they introduce into the system (Kuzyakov 2006). Isotopic approaches making use of 13C natural abundance

discrimination (Rochette and Flanagan 1997; Fu and Cheng 2002; 1997; Millard et al. 2008) cause no disturbance, relying on the different isotopic composition of C3 and C4 plants due to their different photosynthetic pathways. In the absence of a C3:C4 comparison this method has only been used once (Millard et al. 2010). An alternative approach to trenching or girdling is the root regression technique (Kuzyakov 2006; Rodeghiero and Cescatti 2006). This relies upon differing root densities in the soil, allowing soil surface efflux rate to be correlated with root density. A simple estimation of heterotrophic respiration can be obtained analytically as the y-intercept of the linear regression between soil-surface CO_2 efflux and root biomass. Extrapolation back to a zero root density should, in theory, give an estimate of R_h, which can be achieved without the disturbance caused by most other methods. Drip irrigated apple orchards potentially provide an excellent system in which to use this approach, as root density varies a lot over short distances (Sokalska et al. 2009).

The aim of this experiment was to identify the role of environmental parameters and root density on soil respiration rates in an apple orchard. In doing so, we have exploited variability in soil and tree conditions by measuring soil respiration in different periods of the year, positions and orchard plots with a different history of soil nutrient supply. The study also aimed to apply the root regression technique to partition R_s into R_a and R_h.

Materials and methods

Experimental conditions

The experiment was carried out in an apple orchard located at the Experimental Station of the Faculty of Agriculture of the University of Bologna in Northern Italy (44°33'N, 11°21'E; 32 m a.s.l.). The orchard soil was a silty clay loam (18% sand, 50% silt, 32% clay), with a pH of 7.3, organic matter content of 2.3% and with 1.05% organic C and 0.12% total N. The wilting point and the water field capacity were 0.18 and 0.41 m^3 m^{-3}, respectively. Trees of cv. Gala grafted on M9 rootstocks were planted in winter 1996/97 at 3.8 x 1.0 m spacing. The plot consisted of 30 rows, divided into blocks of six rows, each of them receiving a different nutrient supply regime since planting. For the measurements 8 tree rows were selected in 4 blocks: four rows (high fertilizer, HF) had been fertilized since 1997 with an annual application of 80 kg N ha^{-1}, 25 kg P ha^{-1} and 100 kg K ha^{-1}, while the remaining four rows (low fertilizer, LF) received half this amount of fertilizer. Nutrients were supplied through fertigation, split into several applications each year. The localized irrigation system used drippers along the tree rows (2.2 l h^{-1}) spaced every 40 cm. Soil underneath trees was kept weed-free using non-residual herbicides.

Soil respiration measurements

Measurements of R_s were taken on five separate occasions: December (5^{th}) 2006, May (16^{th}), August (7^{th} and 27^{th}) 2007 and April (1^{st}) 2008. Fruit harvest was carried out between August 7 and August 27, 2007. Measurements were made with an EGM 4 gas exchange system (PP Systems, Amesbury, Massachusetts, USA), equipped with a soil respiration chamber (SRC-1) and a soil temperature probe (STP-1); soil water content was measured with a Theta Probe ML2x (Delta-t Devices). Soil temperature was measured at 10 cm depth, while volumetric soil water content was measured at 0-10 cm depth. In addition, over the whole experimental period, soil temperature was constantly recorded by a weather station installed in the orchard (Addcon Telemetry GmbH). One week before each measurement, plastic collars (11 cm of diameter x 6 cm of height) were placed into the soil (at 2 cm depth)

between two adjacent trees, randomly selected in each tree row, along a line perpendicular to the tree row (Fig. 2.1). The central collar was placed at 30 cm from the nearest trunk along the row and the other 4 were placed at 30 and 60 cm from it, in opposite directions, perpendicular to the direction of the tree row. Leaf litter, which was only present in small amounts in December, was removed from the soil before placing the collars. The instantaneous measurement of R_s from each collar was obtained at four different times of the day, with each cycle of measurements starting at: 5:00, 10:00, 15:00 and 20:00. Each cycle lasted about two hours, during which R_s was measured on each individual collar (giving a total of 40 measurements). These instantaneous measurements of R_s from each collar were used to obtain the estimate of the daily average soil respiration. Similarly, instantaneous measurements of soil temperature and soil water content were integrated to obtain daily average values.

Soil collection and analysis

The day after each R_s measurement cycle, except for August 7th, one soil core of 40 cm depth and 47 mm diameter was collected using a soil column cylinder auger (Ejkelkamp, NL) from the centre of each collar (40 soil cores each period). After being removed, the core was divided into three parts according to depth: 0-5 cm, 5-20 cm and 20-40 cm. One sample of soil of approximately 40-45 g (wet weight) was collected from each layer where no roots were visually present, for subsequent organic C, total N and soil water content determinations. The samples were immediately weighed and dried at 35°C in a ventilated oven for 24 hours; a sub-sample for C and N analyses was collected and weighed. A second soil sample was weighed and fully dried at 105°C for 24 hours to obtain soil water content data. Organic soil C and total soil N concentrations were determined by an elemental analyzer (elemental analyzer EA1110, Carlo Erba instruments). Soil NH_4 and NO_3 concentrations were determined only on samples collected in May and August 2007 from central collars (position 0, Fig. 2.1) and were obtained through extraction in KCl (2M) and analyzed by an autoanalyzer (AxFlow AA3, Bran+Luebbe GmbH, Norderstedt, D). Average values of mineral nitrogen concentrations and soil water content were calculated for the 0-400 mm soil profile underneath the collars. To quantify root density, the remaining soil from

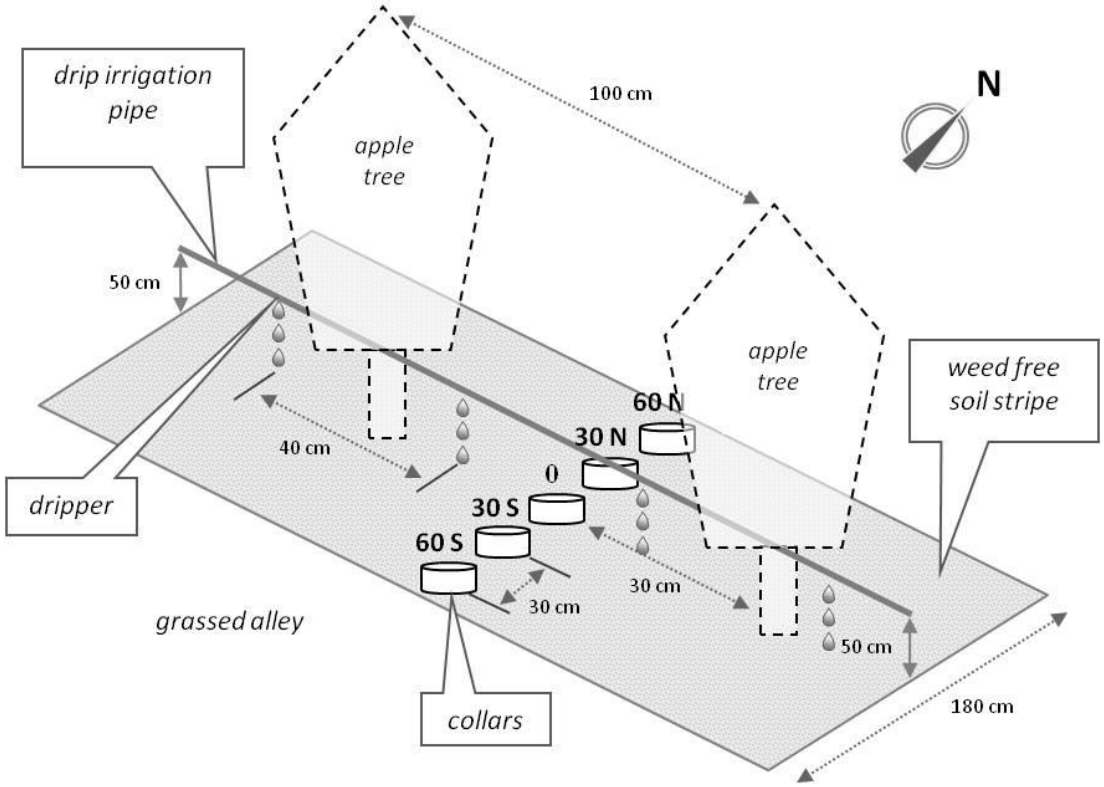

Fig. 2.1 - Scheme showing the location of collars where soil respiration was measured (not in scale).

each core was separated from roots. The first two layers of soil (0-50 mm and 50-200 mm) were pooled together but kept separated from the 200-400 mm soil layer. Each sample was immersed in a Na_2CO_3 solution (2 g l^{-1}) to facilitate deflocculation, shaken for 12 hours and then sieved under running water. Roots were carefully collected by tweezers and divided into three diameter classes: <2 mm (fine roots), from 2 to 5 mm (medium roots) and >5 mm (coarse roots). Roots were dried in a ventilated oven at 65°C until constant weight was reached, and their weight was determined. Samples of roots collected under the collars present along the tree rows were ground and analyzed for C and N concentration.

Treatment of data and Statistics

Seasonal data of soil CO_2 flux, total soil organic C and N, root density, soil temperature and moisture were analyzed by two-way analysis of variance (ANOVA) with nutrient supply and collar position as factors. Data of fine root N concentration, and NO_3 and NH_4 soil concentration obtained from collars in the central position (position 0) were subjected to one-way analysis of variance with nutrient supply as the only factor. Instantaneous measurement data of CO_2-flux were analyzed by multiple regression analysis using temperature and soil water content as independent variables.

Daily average R_s data (as dependent variable) were subjected to multiple regression analysis using temperature, soil water content, average soil profile water content, fine, medium and coarse root density and soil N and C concentration as independent variables.

We analyzed the scatterplots and the correlation between R_s and all the above variables to identify the potential regressors to include in the regression analysis. Then we performed a linear multivariate regression analysis using the stepwise regression method to determine the best fit linear model with the minimum set of independent variables. As scatterplots showed a non-linear behavior for some of the above variables we performed a non-linear multiple regression. Finally, as some of the variables (like temperature) were influenced by the season we also performed a multivariate linear and non-linear regression by season to understand whether the influence of the regressors on R_s applied across a year or was confined to a single season.

Results

Soil chemical and physical characteristics

Average soil temperatures differed in the four periods of measurements and ranged from 9°C in December 2006 to 28°C in August 2008 (Fig. 2.2A). Average temperature in May 2008 and April 2009 were 20 and 14°C, respectively. Collar position had no effect upon soil temperature in December, while in May and August 2007, collars located 30 and 60 cm south showed an average daily temperature more than 2°C higher than the others.

Soil water content (Fig. 2.2B) varied in the four periods of measurement and ranged from 17% (May 2007) to 33% (December 2006). Soil under collars differing for positions differed in soil water content, but this effect was not constant over the whole period: in May and August 2007, when trees were drip irrigated, collars in position 0 showed higher soil water content values, while lowest values corresponded to collars located south. In December 2006 and April 2008 soil water content was uniform among soil positions.

Total soil C and N concentrations were higher in the top 0-5 cm soil layer (14.1 g kg^{-1} and 1.6 g kg^{-1}, respectively) than in the 5-40 cm layer (9.8 g kg^{-1} and 1.1 g kg^{-1}, respectively). Both soil C and N concentration were unaffected by either fertilization regime or soil position (data not shown). Soil mineral N was unaffected by the fertilization levels and increased from May (8.3 mg kg^{-1}) to August (16.4 mg kg^{-1}).

Measurements of R_s

Values of R_s were lowest in December 2006 and highest in August 2007 (Fig. 2.2, D and E). The fertilization regime only affected R_s in December 2006 in positions 0 and 60 S, in which soil from HF collars had a 30-36% greater CO_2 efflux than LF collars (data not shown). Soil respiration differed according to the position of the collars: in December 2006 and in April 2008 the highest efflux was recorded from collars in position 0 (Fig. 2.2D), while in May and in August 2007 the highest CO_2 efflux was measured on collars located 30 S (Fig. 2.2, D and E). In general, soil positions with the lowest R_s were 30 N and 60 N. Diurnal variation of R_s occurred only in April 2008, when R_s significantly increased from 10:00 to 15:00 (Fig. 2.3A),

as a likely consequence of a temperature increase from 11.0°C to 16.5°C (Fig. 2.3B). Interestling, R_s decreased again from 15:00 to 20:00, even if soil temperature did not vary between these two times of the day.

Root density and root N concentration

Fertilization regime did not affect the density of roots of any size-classes (data not shown). There were significant differences in fine root density among the four measurement periods: highest values (Fig. 2.2C) were recorded in December 2006, while root density was similar in May and August 2007 and the April 2008 samplings (Fig. 2.2C). Fine root density changed according to collar position, being highest in the soil along the tree row (position 0) and in that below the collars in 30 S (May 2007 and April 2008). The lowest values of root density were recorded 60 cm from the centre of the tree row (Fig. 2.2C). Fine root N concentration (Tab. 2.1) averaged 8.7 g kg^{-1} and was unaffected by the fertilization regime.

Effects of root and environmental characteristics on R_s

Measurements of R_s were positively and linearly correlated with instantaneous measurements of soil temperature and negatively and linearly correlated with soil water content (Tab. 2.2, eq. 1 and 2 respectively). R_s measurements were related to soil temperature (oC) and soil water content (% in volume) by a multiple regression analysis (Tab. 2.2, eq. 3). When non-linear regression analysis was performed, an exponential equation produced the best fitting to experimental data (Tab. 2.2, eq. 4).

The variations of daily average soil CO_2 efflux were studied in relation to environmental factors and root biomass. When multiple linear regression analysis was applied to the whole dataset of daily soil CO_2-efflux, average soil temperature alone explained 26% (Tab. 2.2, eq. 5) of the variability of CO_2 efflux data; considering fine root density in addition to average soil temperature improved the goodness of fit of the model (Tab. 2.2, eq. 6). Non linear multiple regression analysis on the whole dataset again indicated soil temperature and fine root density as major determinants of daily average values of soil CO_2 efflux (Tab. 2.2, eq. 9).

The best fit of daily average R_s versus soil temperature using non linear regression was obtained by an exponential model (R^2=0.71, Fig. 2.4B). Surface soil water content also

related to R_s by a second order curve (Tab. 2.2, eq. 8; Fig. 2.4A). As surface soil temperature data were negatively correlated with surface soil water content data with r=-0.77 (P=0.01, not reported in tables), the models to predict daily average R_s using the two variables together were discarded.

As surface soil temperature was the major environmental parameter varying among the four measurements periods, we tested whether the effect of temperature on R_s was confirmed within each period or if other factors explained more of the variability in the data. In December 2006 fine root density and soil water content explained 48% of the R_s value (Tab. 2.3). In May and August 2007 soil temperature was the only factor affecting R_s, while in April 2008 R_s depended upon soil water content (Tab. 2.3).

To assess the heterotrophic-derived component of soil respiration, R_s was related to the density of roots of different size-classes. Only fine root density was related to R_s and this relationship was only significant in December 2006 (Tab. 2.4), when the rate of R_h was estimated being 0.096 g CO_2 m^{-2} h^{-1}.

Discussion

As soil respiration determines the major fluxes of CO_2 in many terrestrial ecosystems (Law et al. 1999; Bolstad et al. 2004; Borken et al. 2006; Euskirchen et al. 2006), it is of great importance to understand to which extent R_s is affected by environmental drivers and the activity of the trees (Knorr et al. 2005). In our study, R_s was measured under a relatively high range of soil temperatures, soil water contents and even a greater range of root densities (Tab. 2.1). In contrast to other studies which have quantified R_s across a range of sites with different characteristics, (e.g. Maekiranta et al. 2008; Martin et al. 2009), the variation in soil C and soil N concentration in the soil under our collars was relatively low (C.V. = 13 and 8% respectively, Tab. 2.1).

Instantaneous R_s data showed a large temporal and spatial variation due to the interactive effects of time of the day, the period of the year and the position of the collars in the soil, ranging from 0.06 g CO_2 m^{-2} h^{-1} in December 2006 to 1.49 g CO_2 m^{-2} h^{-1} in the south-exposed soil areas in August 2007 (data not shown). The fact that R_s was generally slower in the north exposed rings was probably due to their lower fine root density, cooler soil temperature and higher soil water content (Fig. 2.2, A, B and C). Our minimum values fall in the range reported by Davidson et al. (1998) for temperate mixed hardwood forest and by Law et al. (1999) for a ponderosa pine plantation. For apple trees, the only available data refers to the studies by Blanke (1996 and 1998) who measured soil respiration in the weed free strip underneath apple trees and concluded that maximum soil respiration can reach values as high as 3.8 g CO_2 m^{-2} h^{-1}, which, he concluded, exceed values of leaf photosynthesis in the corresponding periods.

The regression analysis applied to the whole dataset revealed that soil temperature was the environmental parameter accounting for most of the variance in R_s, with instantaneous measurements less correlated to soil temperature than were daily average values. This effect was likely explained by diurnal variation of soil temperatures, resulting in similar temperatures being recorded at different periods of the year at different times of the day. Temperature affects almost all aspects of respiration processes (Luo and Zhou 2006)

being either root-dependent or caused by the activity of heterotrophic soil organisms. Exponential models better explain the variation of soil respiration to soil temperature. As shown in Figure 2.4B, a Q_{10} of 2.4 for soil respiration was found in our experiment, which is slightly higher than that reported in a pine plantation under a similar range of temperature (Q_{10}=1.8), lower than the value of 3.1 reported by Boone et al. (1998), but equal to the estimated global median value reported by Raich and Schlesinger (1992). To estimate yearly soil CO_2 flux, we used its exponential relationship with soil temperature obtained by the weather station, which from 2006 to 2008 averaged 17.6°C. Simulation indicated a total flux of 2.5 kg CO_2 m^{-2} yr^{-1}, a value slightly higher than that reported by Liguori et al. (2009) in a citrus orchard soil. Scaling up our data to the orchard soil area underneath the weed-free strips in the centre of tree rows, where most apple roots resides, suggests that some 3.2 t C ha^{-1} were lost by soil respiration annually: this value is lower than that of above ground primary productivity of apple trees measured in the same experimental orchard in its sixth year from planting (Scandellari et al. 2010).

As a predictor, fine root density significantly improved the goodness of the prediction, when associated to temperature in non linear models, but did not improve the linear model (Tab. 2.2). The other parameters measured (medium- and large-sized root density, soil nitrogen concentration and the average of soil water content over the 0-40 cm profile) had no significant effect on R_s.

Soil water content negatively correlated to instantaneous values of R_s (Tab. 2.2, eq. 2) and, when associated to soil temperature, it increased the ability to predict soil respiration under multiple linear regression analysis compared to soil temperature alone. Xu and Qi (2001) have shown that the relationship between R_s and soil temperature is affected by soil water content, being significantly lower under soil water limitation. In our experiment, water limitations rarely occurred because the orchard was irrigated (Tab. 2.1). When we analyzed the factors affecting soil respiration within each single period of the year (Tab. 2.3), we found that soil temperature represented a significant predictor in May and August 2007. In contrast, in December 2006 fine roots and soil water content, together, explained almost half of the variability of soil respiration, while in April 2008 only soil water content related to soil respiration. In December and April soil temperature was relatively cool and showed

limited variations among collars (Fig. 2.2A). The inverse correlation between surface soil temperature and surface soil water content using the whole dataset, might suggest that the negative effect of soil water content on R_s was largely due to the positive effect of temperature. As the effects of soil water content were significant in periods when soil temperature showed a limited variation across the soil positions (Tab. 2.3 and Fig. 2.2A), data suggest that soil water content effects were independent from soil temperature. Soil respiration often increases with soil water content from values at wilting point until values of approximately 50-60% of water filled pore space are reached (Bryla et al. 2001), after which R_s decreases if the soil becomes wetter, due to a limitation in soil aeration and a depression of microbial activity (Linn and Doran 1984; Mielnick and Dugas 2000). Data in Figure 2.4A are in line with those reported by Mielnick and Dugas (2000) who found a negative trend of R_s at soil water content data above 25% of volumetric soil water content. Low R_s values in dry soils are likely associated with reduced root growth (Espeleta and Eissenstat 1998) and reduced ion-uptake (Eissenstat et al. 1999).

On an annual scale, the inability to use fine root density to predict R_s is likely because fine root metabolism varies during the season, possibly as a function of their N concentration. Bouma et al. (2001) have shown that fine roots reach their maximum respiration rates in their early developmental phases, which soon declines. The periods of more intense production of new fine roots have been studied using minirhizotrons but even in the same location data are not very consistent, showing either spring or summer peaks (Psarras et al. 2000; Eissenstat et al. 2006). The highest fine root density, at least from positions 60S and 0, were measured at the end of autumn (Fig. 2.2C), suggesting a cumulative root production over the season.

The history of nutrient supply to the orchard soil had a small effect on soil respiration only in December 2006. Previous studies carried out in the same experimental orchard indicated that nutrient supply stimulated tree growth and enhanced tree N status when the trees were young (Rombolà et al. 2000). However, when the trees reached the adult phase, different nutrient supplies only slightly affected leaf N concentration (averaging 2.5% and 2.3% in HF and LF trees) and had no effect on leaf P and K, fruit yield and vegetative growth (Malaguti et al. 2002). Total soil N might not really reflect the N availability for root uptake,

but, it is also possible that several years of localized application of two levels of N did not markedly modify either total soil N or soil mineral nitrogen. The literature on the effect of soil and root N availability on R_s draws different scenarios, depending on the time scale of the observations. In the short period after N addition, an initial increase of R_s, due to a priming effect on the microbial component and a consequent enhanced litter decomposition can be expected (Söderström et al. 1983; Fog 1988; Berg and Matzner 1997; Bowden et al. 2004), while increased long-term N availability tends to cause a decline in R_s due to both, the increase of recalcitrant soil organic matter compounds and the reduction of microbial activity (Fog 1988; Berg and Matzner 1997; Bowden et al. 2004; Burton et al. 2004). In a recent paper Migliavacca et al. (2010) suggested that on the whole an increased total N deposition in forests as well as in any anthropogenic managed ecosystem, tends to reduce soil respiration. This tendency has been also observed by others (Bowden et al. 2004) and can in part be explained by a reduction in mycorrhizal colonization and fungal development under nutrient rich soil conditions (Bryla and Eissenstat 2005). In trees, the long-term effect of soil N availability usually leads to a reduction in C allocation to the belowground plant organs in favor of aboveground organs (Vogt et al. 1990; Haynes and Gower 1995), but not necessarily to a reduction of total amount of photosynthates allocated to the roots. Roots with higher N availability usually show higher specific respiration rates (Burton et al. 1996, 2002; Ryan et al. 1996).

Assessing the relative contribution of the heterotrophic and autotrophic components of soil respiration is an important goal in ecological studies as each one can differentially respond to soil climate, nutrient availability and management practices (Maekiranta et al. 2008). With the exception of December 2006 and, to a less extent, of April 2008, daily average soil temperature varied among soil collars (Fig. 2.2A). Considering that temperature has a major effect on root activity and its respiration, it is not surprising that we could only detect a significant linear relationship between fine root density and R_s in December, when soil temperature values were relatively constant. Several other studies have used a regression between R_s and fine root biomass to estimate R_h, by calculating the y-intercept. In a ponderosa pine plantation, Xu et al. (2001) estimated that R_h contributed 53% of R_s during the growing season; while in tropical deciduous forest Behera et al. (1990) calculated that R_h

contributed 49% of R_s. In another study, Rodeghiero and Cescatti (2006) estimated that R_h accounted for 42-84% of annual R_s in a range of evergreen forests. When we applied the root regression technique to our December 2006 data (Fig. 2.4C) to assess the contribution of R_h, we found that it contributed to some 65% of total R_s, the remaining 35% being attributed to Ra. Root regression has been critically evaluated as an approach to measure R_h. Kuzyakov (2006) pointed out that, compared with other techniques, high numbers of replicates are needed; there can be overestimation of root-derived CO_2 when big and old roots are present and that frequently there is a poor correlation between root amount and total CO_2 with R_h estimates being biased by far extrapolation of the regression line. A clear limitation of the present study was our inability to estimate R_h when the trees were actively growing, when the overall contribution of R_h to R_s would likely have been smaller.

The increase of R_s values in August 2007 in the post-harvest period, as compared to pre-harvest period, is likely associated to fruit removal (Fig. 2.2E), as surface soil temperature and surface soil water content were respectively lower and higher in post-harvest compared to pre-harvest (Fig. 2.2, A and B). Fruits are a major sink for carbohydrates (Wardlaw 1990), and their removal results in a shift of carbohydrate allocation to the root system. This has been already reported in a study by Glenn and Walker (1993), who observed new fine root production immediately after fruit removal in mature peach trees. This suggests that the increase in CO_2 efflux after fruit removal is to assign to the autotrophic, rather than the heterotrophic component of R_s.

In conclusion, our study demonstrated that on annual scale, surface soil temperature was the most important environmental factor affecting respiration of the orchard soil. Surface soil water content showed an adverse effect on soil respiration and controlled a great part of the variability of soil respiration measured in December and April. The history of soil fertilization, soil C and N concentration, as well as root N and soil mineral N had negligible or no effect on soil respiration. Fine root density, but not medium- and large-sized root density, contributed to explain part of the yearly variability of soil respiration, and proved to be a good predictor of soil respiration in December; in this occasion only, autotrophic component of soil respiration could be estimated by the root regression technique and represented roughly 35% of total soil respiration.

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Tables and figures

Tab. 2.1 - Soil and root parameters when soil CO_2 efflux was measured across the four measurement periods.

Parameter	Average ± s.d.[1]	Min-max	CV[2] (%)	n
Soil respiration (g CO_2 m^{-2} h^{-1})	0.38 ± 0.25	0.06 - 1.49	66	720
Surface soil temperature (^oC)	18.6 ± 6.5	8.1 - 37.7	35	720
Surface soil water content (% in vol.) (average 0-10 cm depth)	25.9 ± 5.4	14.1 - 36.1	21	720
Soil water content (% in vol.) (average 0-40 cm depth)	23.9 ± 3.6	16.5 - 32.2	15	140
Soil C concentration (g kg^{-1})	10.5 ± 1.4	8.1 - 16.7	13	140
Soil N concentration (g kg^{-1})	1.2 ± 0.1	0.9 - 1.8	8	140
Soil mineral N concentration (g kg^{-1})	0.020 ± 0.009	0.007- 0.041	45	100
Fine root density (kg m^{-3})	1.12 ± 0.7	0.22 - 4.4	62	140
Medium-sized root density (kg m^{-3})	0.51 ± 0.58	0.04 - 2.8	115	140
Large-sized root density (kg m^{-3})	1.46 ± 2.81	0.054 - 15.2	193	140
Total root density (kg m^{-3})	3.09 ± 3.36	0.28 - 17.6	109	140
Fine root N concentration[3] (g kg^{-1})	8.7 ± 1.2	0.7 - 1.2	13	28

Except for surface soil temperature (recorded at 10 cm depth) and surface soil water content (average of 0-10 cm layer), the remaining parameters refer to the average of 0-40 cm soil layer, as recorded from the soil core.

[1] s.d. = standard deviation.

[2] CV = coefficient of variation.

[3] data refer only to collars in position 0.

Tab. 2.2 - Relationships between soil respiration (g CO_2 m^{-2} h^{-1}) and measured parameters.

Type of analysis	Dependent variable (y)	Independent variable(s)	R^2 and significance	Equation	(n)	Equation number
Simple linear regression	Hourly CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C)	0.32***[1]	y = -0.031 + 0.022x	(720)	1
		Soil water content (z, % in volume)	0.25***	y = 0.981 - 0.023z	(720)	2
Multiple linear regression (stepwise)	Hourly CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C) and Soil water content (z, % in volume)	0.34***	y = 0.32 + 0.016x - 0.010z	(720)	3
Non-linear simple regression	Hourly CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C)	0.29***	$y = 0.135e^{0.052x}$	(720)	4
Multiple linear regression (stepwise)	Daily CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C)	0.26***	y = -0.4771 + 0.0444x	(140)	5
		Soil temperature (x, ^{o}C) and fine root density (z, kg m^{-3})	0.32***	y = -0.503 + 0.0415x + 0.083z	(140)	6
Non-linear simple regression	Daily CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C)	0.71***	$y = 0.0629e^{0.09x}$	(140)	7
		Soil water content (z, % in volume)	0.50 ***	$y = -0.0846 + 0.0675z - 0.0183z^2$	(140)	8
Non-linear multiple regression	Daily CO_2 flux (g CO_2 m^{-2} h^{-1})	Soil temperature (x, ^{o}C) and fine root density (z, kg m^{-3})	0.67 ***	$y = -0.147 + 0.114e^{0.074x} + 0.083z - 0.00000071z^2$	(140)	9

[1] ***= significance 0.1% level of probability.

Tab. 2.3 - Multiple linear regression analysis applied to daily average soil CO_2 flux (g CO_2 m^{-2} h^{-1}) within each measurement period. (SWC=soil water content in % volume)

Period	R^2	Equation	n
Dec. 2006	0.48***[1]	y = 0.742 + 0.0417 (fine roots, kg m^{-3}) - 0.0196 (SWC, % vol.)	40
May 2007	0.22**	y = -0.4594 + 0.0447 (soil temperature, ^{o}C)	40
Aug. 2007	0.38***	y = -1.8566 + 0.1028 (soil temperature, ^{o}C)	40
Apr. 2008	0.23*	y = 0.8977 - 0.0217 (SWC, % vol.)	20

[1] *, ** and ***= significance at 5%, 1% and 0.1% level of probability, respectively.

Tab. 2.4 - Coefficients of determination, significance and equations for the linear regression analysis of data of daily average soil CO_2 flux (**y** in g CO_2 m^{-2} h^{-1}) versus fine root density (**x** in kg m^{-3}), in soil cores excavated at the centre of the collar after respiration measurements.

Period	R^2	Equation	n
Dec. 2006	0.35***[1]	y = 0.0965 + 0.0375x	40
May 2007	$0.05^{n.s.}$	y = 0.3682 + 0.075x	40
Aug. 2007	$0.09^{n.s.}$	y = 0.4129 + 0.104x	40
Apr. 2008	$0.05^{n.s.}$	y = 0.2186 + 0.0417x	20

[1] *n.s.* and ***= not significant effect and significance at 0.1% level of probability, respectively.

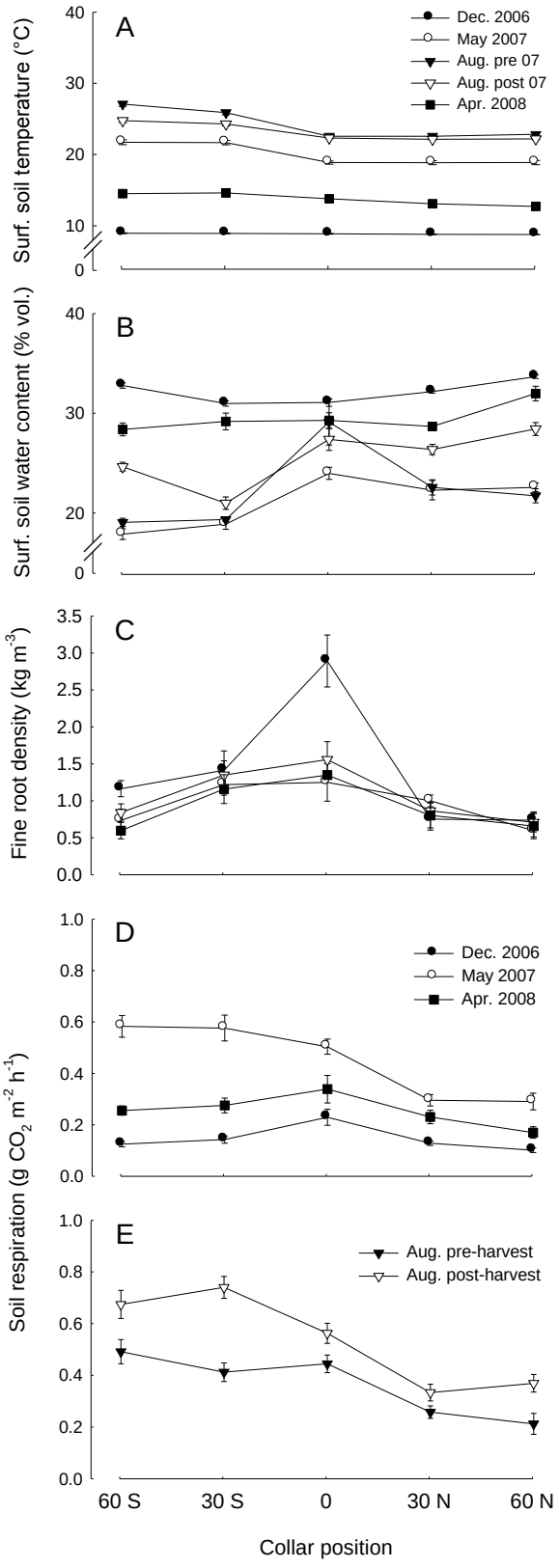


Fig. 2.2 - Effect of collar position on the variability of (A) surface soil temperature, (B) surface soil water content, (C) fine root density and (D and E) soil respiration. Values are the mean for each position (n=32, only fine root density n=8; bars are standard errors of the means). Data of August (E) refer to sampling before (-pre) and after (-post) harvesting of fruits. For collar position see Figure 2.1.

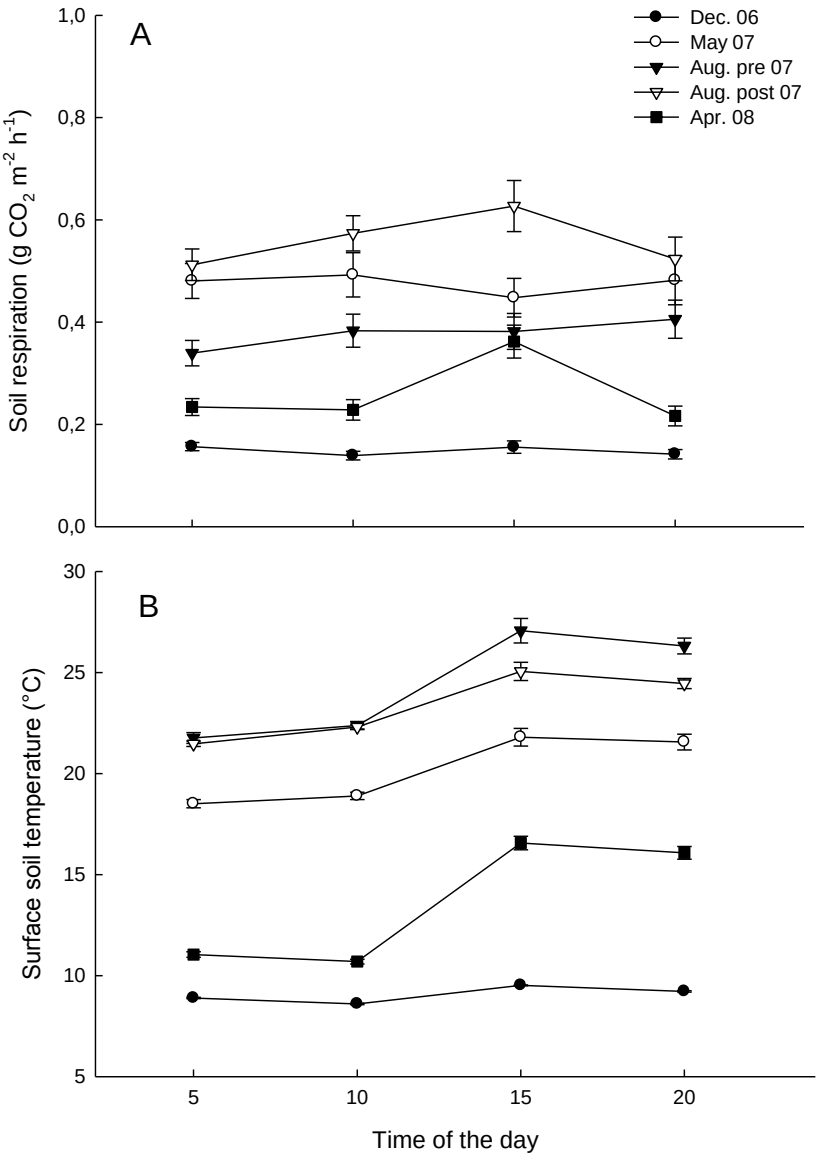


Fig. 2.3 - Daily pattern of soil respiration (A) and surface soil temperature (B), values are mean of all collar positions for each season (n=40, except for Apr. 2008 where n=20; bars are standard errors of the means).

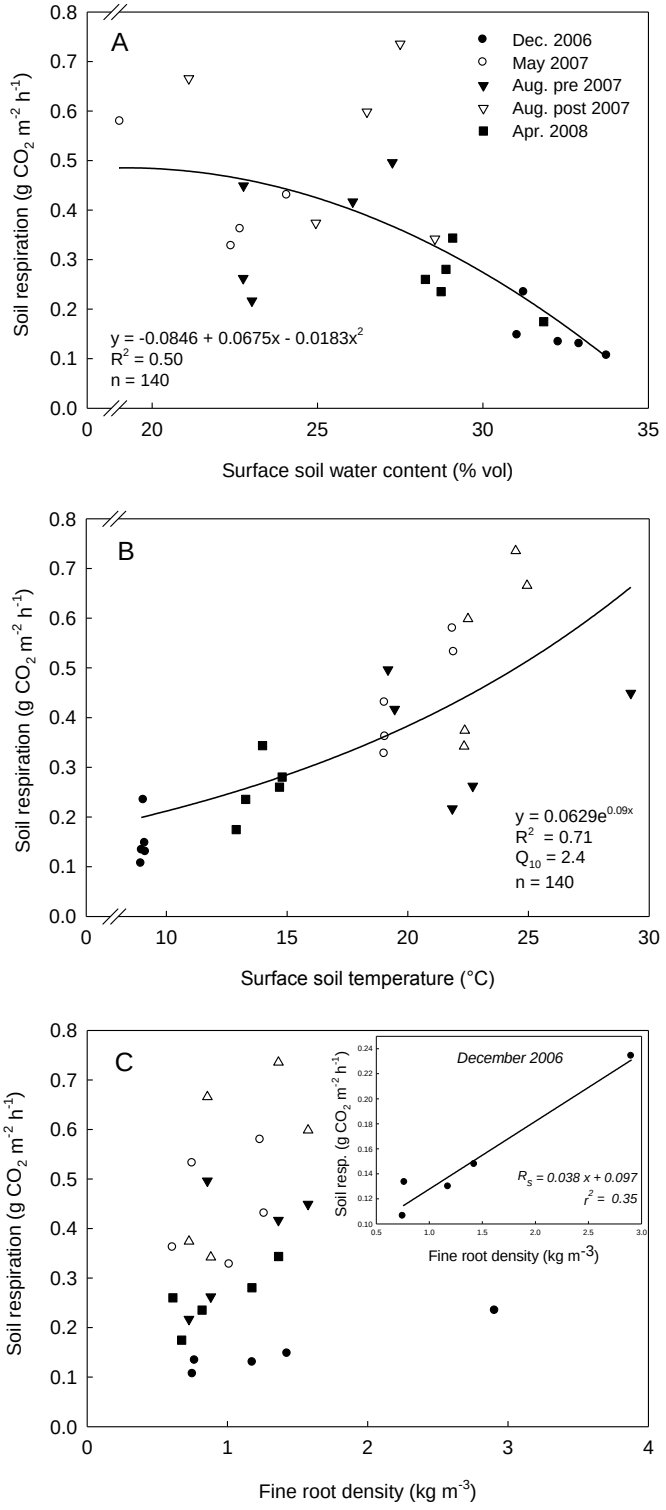


Fig. 2.4 - Soil respiration in relation to (A) surface soil water content, (B) surface soil temperature and (C) fine root density. Each point represents the mean value for each position (n=32, except for Apr. 2008 when n=16).

3. Experiment two

Factors affecting fine root respiration in *Populus tremuloides*: the negligible effect of nitrogen on age dependency

Introduction

Respiration plays a key role in plant metabolism, providing the energy for processes linked to growth, tissue maintenance, nutrient absorption and essentially all other plant organ functions. Respiration also contributes substantially to the plant carbon budget and, depending on different factors, can consume up to 75% of daily produced photosynthate (Van der Werf et al. 1992; Lambers et al. 1998). Plant carbon budgets, which depend on CO_2 sequestration (photosynthesis) and CO_2 production (respiration), are important in the context of carbon cycling and climate change, because of their large contribution to the CO_2-flux between terrestrial ecosystems and the atmosphere. Respiration of aboveground plant organs, especially leaves, has already been the object of extensive study (e.g., Reich et al. 1998a) and is known to be influenced by many physiological and environmental factors, including nutrient availability. Among the nutrients necessary for plant growth, nitrogen (N) in particular has a major influence on respiration (Bloom et al. 1992; Bouma et al. 1996), because it is required by plants for the synthesis and maintenance of storage and enzymatic proteins and because it is often concentrated in actively growing meristems. While the relationship of respiration to tissue N concentration in aboveground plant organs has been well studied (Oren et al. 2001; or C:N ratios, Throop et al. 2004), the relationship of respiration to N concentration in belowground plant organs has received less attention. In the last decade, root respiration (autotrophic respiration) has gained particular interest in the context of carbon cycling and climatic change, because together with soil microbial respiration (heterotrophic respiration) it is the main source for soil respiration (Hanson et al. 2000). Soil respiration represents the second largest C-flux after gross primary production (GPP) and can account for 60-90% of total ecosystem respiration in temperate forests (Valentini et al. 2000; Law et al. 2001). Since N availability in soils is predicted to become larger in the future, by higher N-depositions (Vitousek 1994; Sala et al. 2000), as well as higher mineralization of soil organic matter (SOM) due to warmer soils (Melillo et al. 2002), investigations about the influence N exerts on root respiration as well as on other components of soil respiration has become more important.

The effect of increased soil-N availability on soil respiration has been reported to be both positive and negative. Increases in soil respiration following N addition is commonly observed in young plantations, where N strongly enhanced photosynthesis until canopy closure is reached, leading to a stimulation of carbon allocated belowground (Janssens et al. 2010). In contrast, long-term studies conducted in mature stands indicate an overall reduction in CO_2-efflux (Burton et al. 2004; Janssens et al. 2010). The reduction of soil respiration in relation to soil N availability can be either the result of reduced microbial respiration or reduced root respiration, or a reduction of both. In the case of autotrophic respiration, the extent to which roots respire, largely depends on the type of root considered and the prevailing environmental conditions under which respiration is measured. Coarse lignified roots (diameter>5 mm) for example, respire slower (ca. 1/10) and at more constant rate over time than fine fibrous roots (Desrochers et al. 2002). On the other hand, fine root respiration rates exhibit greater variation with age, with the fastest respiration at young ages (Volder et al. 2005).

In the context of increased soil N availability predicted in the future, the contribution of fine root respiration to soil respiration may be affected in different ways:

1. Increased N availability may reduce overall carbon allocation to belowground organs leading to a lower root biomass fraction (W_R or root biomass/whole plant biomass; Brouwer 1983; Litton et al. 2007).
2. Increased N availability may reduce colonization of mycorrhizal fungi (Treseder 2004), which could lead to reduce specific rates of fine root respiration.
3. Increased N availability may lead to increased root N concentration, increased protein content and increased protein turnover, which would increase maintenance respiration (Ryan et al. 1996)
4. Increased N availability may lead to increased ion-uptake respiration, which can be a major fraction of total root respiration, especially if the N acquired is in the form of nitrate and the nitrate is reduced in the root (Bloom et al. 1992; Bouma et al. 1996).

A positive correlation between root respiration and N availability has been revealed in comparisons across different species and different sites (Burton et al. 2002). In most cases, these studies were conducted measuring root respiration on cohorts of excised roots, sampled from soil cores where excised root respiration was related with root N concentration. However, by using this approach, authors were probably not able to account for the effect of root age, or possibly root order, but only for their diameter classes. Another factor influencing fine root respiration according to N availability can be the patchy distribution of N in soil and its high variability spatially and temporally, especially if N is present as nitrate. Roots can respond in different ways in the presence of nutrient patches, including root proliferation in the nutrient-rich patch, and by increasing the uptake rate per unit root mass. Both these responses depend largely on plant species, nutrient availability and plant nutrient status (Robinson 1994) and can lead to higher carbon costs for the plant.

The aim of this study was to investigate the influence of N availability on belowground C allocation, including root biomass partitioning and fine root respiration (FFR), in the fast-growing tree, *Populus tremuloides*. In particular we wanted to study how N affects FFR as a function of root age and whether the age response is affected by the level of N supply or the spatial pattern (uniform or localized).

Materials and Methods

Experiment 1 (field experiment)

Experimental conditions and plant material

The influence of localized N availability on FFR was studied in the a field experiment conducted from June to September 2009, at a "Common garden" at the Russell E. Larson Experimental farm of The Pennsylvania State University (PA, USA; 40°40'N, 78°02'W; 350 m a.s.l.). The Common garden was established in 1996 and consists of a collection of 16 tree species (deciduous and evergreen). Each species is represented by 48 trees, randomly distributed in 8 blocks of 6 trees each, and planted at 2.5x2.5m distance. N availability in the upper 30 cm of soil was approximately 30 Kg N ha^{-1} (this value is estimated using 7.85 mg NH_4NO_3-N kg^{-1} $soil^{-1}$, measured through N-extraction in KCl). Trees used for this experiment were 13-year-old *Populus tremuloides* trees.

Experimental approach

Root respiration was measured on roots of known age, either exposed or not to a localized N-treatment using root access boxes similar to those used previously (Comas et al. 2000; Zadworny and Eissenstat 2011). We installed 8 root-boxes, 1 per block, between two adjacent poplar trees, at 90 cm distance from each trunk (8 root-boxes in total). Each box had two windows on two opposite sides, with each window consisting of two smaller ones (Pic. 1), in order to allow more root-samplings during the experiment. Windows were made of a thin transparent acetate-film, which allowed for observing and tracking root growth and root age prior to collection. When not in use, the windows were kept covered with foam insulation panels to avoid light penetration and to minimize temperature fluctuations. In addition, the boxes were covered and kept closed by a wood panel.

Root tracking and nitrogen treatments

From the end of June until mid September, new and existing roots were tracked every week, in order to assess root physiology at known root ages. The nitrogen treatment was applied every two weeks, using an equivalent of 40 Kg N ha^{-1} (dissolved in water) that was distributed on a small soil area (0.12 m^{-2}, 60x20 cm) behind one window of each root-box. The other window acted as control and received only water. Nitrogen was applied to the soil as ammonium-nitrate (NH_4-NO_3) 6 times (for a total of 240 Kg N ha^{-1}) at regular intervals.

Fine-root collection, respiration measurements and chemical analyses

On July 27th and on September 16th (57 and 106 days after root-box installation) fine roots were sampled for respiration estimates. Fine roots were collected by cutting the acetate film with a small scissors. On the first date, when 120 Kg N ha^{-1} had been already applied (3 applications of 40 Kg N ha^{-1} every two weeks), we sampled and measured respiration on roots whose age ranged from 2 to 33 days (5 age classes: 2, 9, 16, 23 and 33 days). On the second date, after 240 Kg N ha^{-1} had been applied, we measured respiration on roots whose age ranged from 7 to 81 days (12 age classes: 7, 14, 22, 29, 36, 42, 48, 50, 57, 64, 71 and 81 days). Fine roots of the same age class and grown on the same window were pooled together. Once collected they were immediately immersed in a buffer-solution (1 mM $CaSO_4$ + 5 mM MES-buffer, pH 5.5 using KOH) and transported to the nearby laboratory, where respiration measurements were performed within 2 hours after collection. Respiration rates were measured as oxygen (O_2) consumption (nmol O_2 g dry $weight^{-1}$ s^{-1}) at 20°C, using a Clark-type oxygen electrode (Hansatech Oxygraph, King's Lynn, UK) combined with a water-bath to allow constant temperature conditions. After respiration measurements fine roots were oven-dried at 65°C and their dry weight determined. Tissue N concentration was determined with an elemental analyzer (Flash-EA 2000, Thermo Fischer) for all roots used for the respiration measurements.

Experiment 2 (greenhouse pot experiment)

Experimental conditions and plant material

To assess how different levels of N supplied either in a homogeneous or patchy way affected FFR, we conducted an experiment in the greenhouse from July until mid October near State College, PA, USA. The experiment was conducted with 65, one-year-old, poplar seedlings (*Populus tremuloides*). Plants were first grown for 1 month in small pots (1-L volume), to let them emerge from their dormant phase. The substrate used for the experiment consisted of 1/3 (by volume) sieved soil (soil was taken at the common garden, where the field-experiment was conducted) combined with 2/3 fine sand. During their first month of growth plants received the same amount of a common fertilizer.

Experimental approach and treatment application

To test the respiratory response of fine roots exposed to different levels of N availability we used a split-root system. The root system of each plant was split between two adjacent pots (e.g., Eissenstat 1990). The pots used in this study were made out of plastic, had a squared section (12x12cm) and were 40 cm high (total volume of 5.5 L each). On the front side of each pot a window made of an acetate sheet was constructed, through which fine root growth was tracked every two weeks. When roots were not being tracked, windows were covered to avoid light penetration.

Three levels of N were applied to the soil at weekly intervals at rates of 21, 63 and 210 mg N L^{-1} and will be referred to as 1N, 3N and 10N, respectively. Nitrogen was applied as ammonium nitrate dissolved in a solution containing all other macro- and micro-nutrients. Nitrogen was applied either in a homogeneous way uniformly in both pots (both pots received the same N-treatment), or at different levels (different N-treatments between the two pots) (Fig. 3.4). Each time enough solution was distributed to completely saturate the soil. During the rest of the week plants received only water through drip irrigation.

Root sampling, respiration measurements and plant characterization

Fine root sampling and FFR measurements were performed on October 14th using the same methodology as the field experiment: only first and second order fine roots of the same pot and the same age were sampled and pooled together. Respiration was measured on roots, whose age ranged from 4 to 100 days, which were classified in 8 different age classes (4, 12, 19, 25, 56, 72, 89 and 100 days). Until the day of sampling, plants received 14 applications of the various fertilizer treatments (1N, 3N and 10N) at weekly intervals. After respiration determination, roots were oven-dried at 65°C and dry weight determined. Tissue N concentrations of all roots were determined separately as described previously. In addition, whole plants were harvest and divided into leaves, twigs and branches, stem and root system, oven dried and their weight determined. The dried root system of each plant was further subdivided in 4 root diameter classes (>0.5mm, 0.5-0.2mm, 0.2-0.1mm and <0.1mm).

Statistical analyses

Data were statistically analyzed by one-way ANOVA and the parameter of fine root age was used as covariate. All statistical analyses were performed with a significance level of 0.05 using Statgraphics Centurion XV. Data obtained from roots of the heterogeneous N distribution treatment of the greenhouse experiment were analyzed comparing separately from each other 3N and 10N treated plants, with their respective 1N combinations. All results from the combinations between 1N and 3N treated roots, namely 1N-(1N), 1N-(3N), 3N-(1N) and 3N-(3N) (in brackets it is shown the treatment applied to the neighbor pot) were analyzed together and then the results obtained from the 1N with the 10N treated roots were analyzed separately for all their combinations in the same way.

Results

In both experiments, FRR decreased logarithmically with root age (Fig. 3.1) and was unaffected by the N supply (Tab. 3.1 and 3.2). In the field experiment, N supply affected root N concentration, but did not affect FRR when estimated either on a biomass or N basis (Tab. 3.1).

In the experiment 2, when N was homogenously applied between pots, increasing N supply increased N concentration in root tissues (Tab. 3.2). When N was applied heterogeneously between pots, N concentration of roots exposed to low levels of N was positively influenced by the highest N availability in the adjacent pot (Fig. 3.2). In both experiments, N concentration decreased with age and the N regime affected the rate that N concentration decreased with age, being more severe for control than for N+ roots (Figs. 3.3 and 3.4, Tab. 3.1).

In the pot experiment, neither fine (diameter <0.1 mm) (Tab. 3.2) nor coarse (diameter classes >0.1mm and above) (not reported in tables) root biomass significantly differed among treatments. In addition, aboveground biomass also did not differ significantly among treatments.

Discussion

In both experiments, mass specific FRR at 20°C ranged from 1 to 32.7 nmol O_2 $g^{-1}s^{-1}$, with an overall mean of 7.0 nmol O_2 $g^{-1}s^{-1}$ (n=325). This value is very close to the FRR mean value for poplar trees found by Burton et al. (2002), in a study on the relation between FFR and N concentration in roots. In both field and greenhouse study, FRR rates fall in the same range of values (Fig. 3.1) and decrease with a log-trend as roots aged, as previously observed by others for grape (Comas et al. 2000; Volder et al. 2005), apple and citrus (Bouma et al. 2001). This trend was observed at all N levels (Fig. 3.1 and 3.5). In my experiment, mass specific FRR was not affected by N availability or by the way N was made available to the root system (uniform or localized N-supply), neither under field nor in the greenhouse conditions (Tab. 3.1 and 3.2). Similar results on a small influence of N on FRR were reported also by other authors for herbaceous (Wang et al. 2010) and for woody plants (Lu et al. 1997; Wang et al. 2010).

Fine root respiration rate sharply decreased within the first 3 weeks after fine roots were born, by 50 and 55% of initial values for field and greenhouse conditions respectively. The high FRR rates at the earliest stage of their lifetime, most likely reflects the initial growth and construction costs for root tissue (Eissenstat and Volder 2005) and comprises also costs related to nutrient absorption, which can account up to 70% of total respiratory energy of roots (Poorter et al. 1991). It has been shown, that fine root ion-uptake is highest during their first days of life and that uptake rates sharply declines with age (Volder et al. 2005; Volder et al. 2009). Therefore it seems reasonable to assume that the decline of FRR with age might be related to a reduced absorption activity and/or capacity for older roots. My initial hypothesis that respiration for ion uptake, would increase with increasing levels of N availability, was rejected. FRR decreased in a similar way at all N-levels. Since N was applied in the form of NO_3NH_4, I expected faster FRR at higher levels of N, due to higher costs for respiration related to the reduction of NO_3^- in the roots, but this was not observed. Min et al. (1998) observed that *Populus* is capable of a fast translocation of nitrate to leaves, where it

is subsequently reduced. In my case a translocation of excess nitrate to leaves might have contributed to keep FRR rates at lower levels, even in the presence of larger amounts of N.

When roots were older than 3-4 weeks the decline in respiration rates with increasing age became less pronounced, if compared to the decrease observed for young roots, while for roots older than 7 weeks the respiration-trend with increasing age showed to be almost flat (Fig. 3.1). This suggests that respiration of roots that are older than 3-4 weeks might already be representative of fine root maintenance respiration. Surprisingly the reduction of respiration rates at increasing root age was almost equal at all N-levels (Fig. 3.3). This result was in contrast to my initial hypothesis of faster maintenance respiration with increasing N, as a consequence of a higher protein content and protein turnover in fine roots.

As expected nitrogen addition significantly affected the N-concentration of roots (Tab. 3.1). In the field study this effect was only visible in the second sampling, when the entire N-rate was supplied. In experiment 2 differences between N-treatments were evident, especially in the case of uniform N distribution (Fig. 3.2). When plants received a heterogeneous N-distribution, roots exposed to low nitrogen treatment (1N) were affected only when neighbor-pots received the 10N-treatment, while the 3N-level was apparently too low to affect fine root N-concentration of 1N roots (Fig. 3.2). This result might be explained as a dilution of N within the plant, but is probably more a mechanism of active translocation of N. The translocation of N via phloem from shoots to roots has been shown to take place in herbaceous plants (Jeschke and Hartung 2000) as well as in trees (Grassi et al. 2003). The N translocation from shoots to roots take place during the phase of active growth of the plant, when through an accumulation of amino acids in roots, plants are able to regulate and reduce N uptake by roots (Padgett and Leonard 1996). This phenomenon might explain the higher N concentration in 1N roots when combined to 10N treatment (Fig. 3.2).

In both studies N concentration of roots gradually declined with root age, but to a lower extend if N was made available (Fig. 3.3 and 3.4). Interesting was the fact that newly born roots didn't show significant differences for their N-concentration, being equal at all N-levels and in both, field (Fig. 3.3) and greenhouse conditions. This was true until roots were 15 days old, after which N availability already influenced fine root tissue N-concentration.

To our knowledge, this experiment is the first addressing the hypothesis that N can affect respiration of roots of first and second order of a known age. In the past many studies on FRR in relation to N availability were performed comparing different species in different sites (according to natural soil N availability) and by classifying roots according to root diameter classes, rather than root order or root age classes. In many of these, authors have reported a positive linear correlation between FRR and N-concentration of fine roots (Pregitzer et al. 1998; Reich et al. 1998b; Burton et al. 2000; Tjoelker et al. 2005), but as some of them argue, this correlation could only be found when different species from different sites were compared, while by comparing FRR within species and site this correlation was only low. The within-species and -site variability for respiration rates likely depends on the difficulties in taking into account parameters like root order and function as well as root age. Moreover, the increase in N concentration in roots in our study might not represent, metabolically active N, such as that found in proteins associated with maintenance respiration. In our study we also found a positive, but weak (R^2=0.09), linear correlation between fine root N-concentration and FRR (data not shown). However, most of the variability in our FRR data is described by a logarithmic function with root age, with an R^2 of 0.47 among all data, regardless of experimental conditions and N-treatment (data not shown).

In conclusion we could not find a significant difference in fine root respiration in regard to different soil nitrogen availability (for both N-levels and N-distribution) or fine root nitrogen concentration. The main effect we observed on root respiration was related to root age, which also affected N-concentration of fine roots. N availability influenced only the decrease of root N-concentration with age, as well as overall plant N concentration. In addition we did not observe different patterns of biomass allocation among N-treatments. These results suggest that root respiration may not be correlated to N concentration where supply may be in excess. However, more investigations are needed in order to validate these findings.

Tables and figures

Tab. 3.1 - Respiration and root N concentration of fine roots of *Populus tremuloides* measured in the field-grown trees in central Pennsylvania, USA.
(n.s. = not significant, *** = *P*<0.001)

		1st sampling date			2nd sampling date		
Treatment		control	N+	*P*	control	N+	*P*
Root age (days)	*mean*	15	17		45	46	
Resp. per unit biomass (nmol O_2 g^{-1} dw s^{-1})	*mean*	11.20	9.13	*n.s.*	5.70	5.92	*n.s.*
	std. err.	*1.08*	*1.10*		*0.50*	*0.48*	
Resp. per unit N (nmol O_2 g^{-1} N s^{-1})	*mean*	683.5	533.0	*n.s.*	410.2	351.3	*n.s.*
	std. err.	*65.5*	*66.8*		*34.3*	*32.8*	
Root N concentration (%)	*mean*	1.66	1.71	*n.s.*	1.37	1.64	***
	std. err.	*0.06*	*0.07*		*0.04*	*0.03*	
					b	*a*	
Sample size (*n*)		26	25		54	59	

Tab. 3.2 - Mean values for fine root parameter as measured during the Greenhouse study. (n.s. = not significant, *** = p<0.001)

		Homogeneous N-supply between pots				Heterogeneous N-supply between pots					
Treatment		1N	3N	10N	*P*	1N(3N)	3N(1N)	*P*	1N(10N)	10N(1N)	*P*
Fine root age (days)	*mean*	63	53	54		59	60		50	48	
Resp. per unit biomass (nmol O_2 g^{-1} dw s^{-1})	*mean*	6.70	6.83	6.72	*n.s.*	5.45	6.52	*n.s.*	7.30	7.42	*n.s.*
	std. err.	*0.78*	*0.77*	*0.75*		*1.08*	*1.02*		*1.13*	*1.18*	
Resp. per unit N (nmol O_2 g^{-1} N s^{-1})	*mean*	354.8	308.0	244.3	*n.s.*	298.0	316.5	*n.s.*	347.2	293.7	*n.s.*
	std. err.	*33.2*	*32.5*	*32.0*	*(0.06)*	*52.0*	*48.8*		*50.7*	*52.7*	*(0.06)*
		a	*a*	*b*					*a*	*ab*	
Root N concentration (%)	*mean*	1.85	2.11	2.63	***	1.88	2.10		2.04	2.54	***
	std. err.	*0.06*	*0.07*	*0.06*		*0.10*	*0.07*		*0.07*	*0.11*	
		C	*b*	*a*		*b*	*a*		*b*	*a*	
Fine root biomass (g/pot)	*mean*	1.29	0.88	1.17	*n.s.*	0.88	0.85	*n.s.*	0.64	0.67	*n.s.*
	std. err.	*0.25*	*0.24*	*0.28*		*0.27*	*0.29*		*0.07*	*0.09*	
Sample size (*n*)[1]		33	34	35		15	17		14	13	

[1]Except fine root biomass

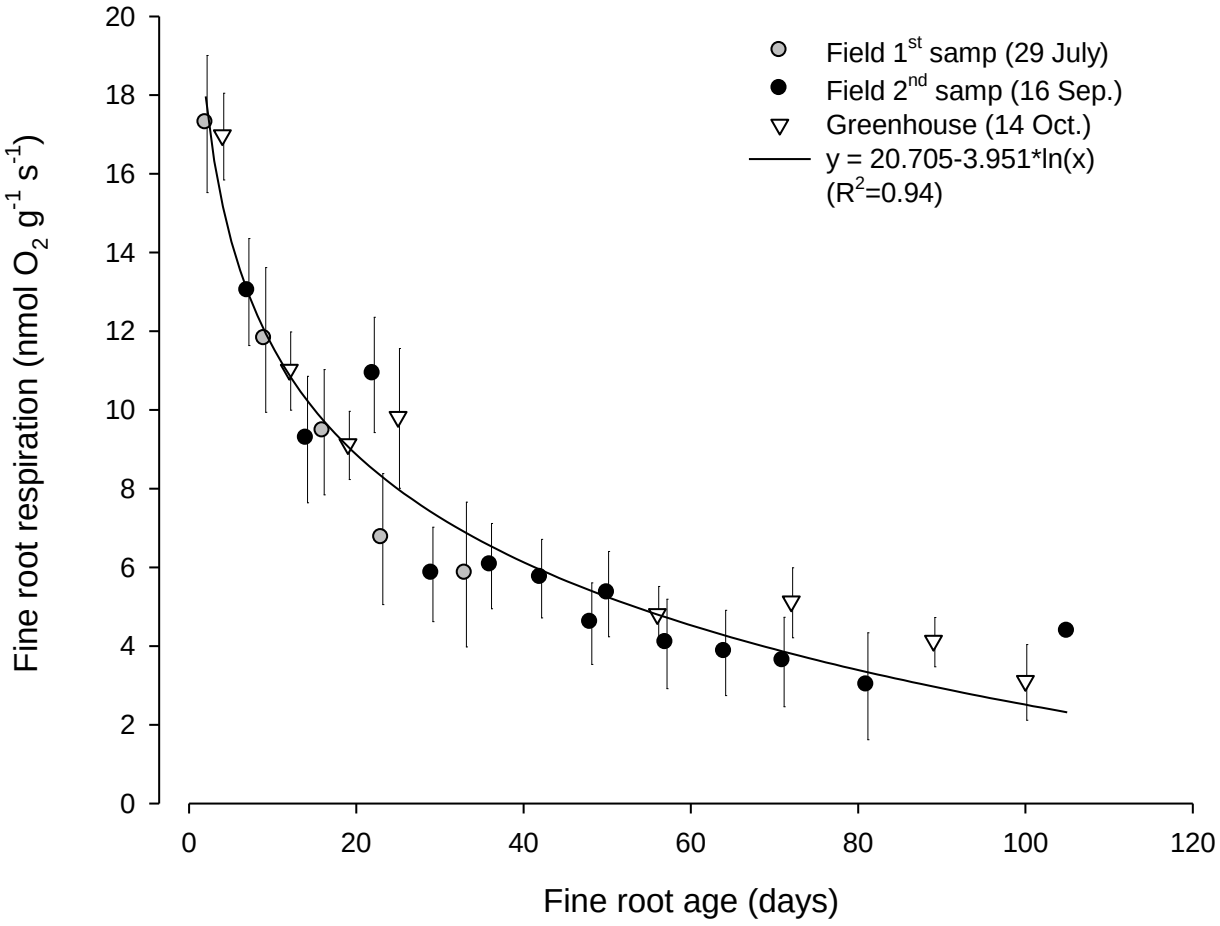
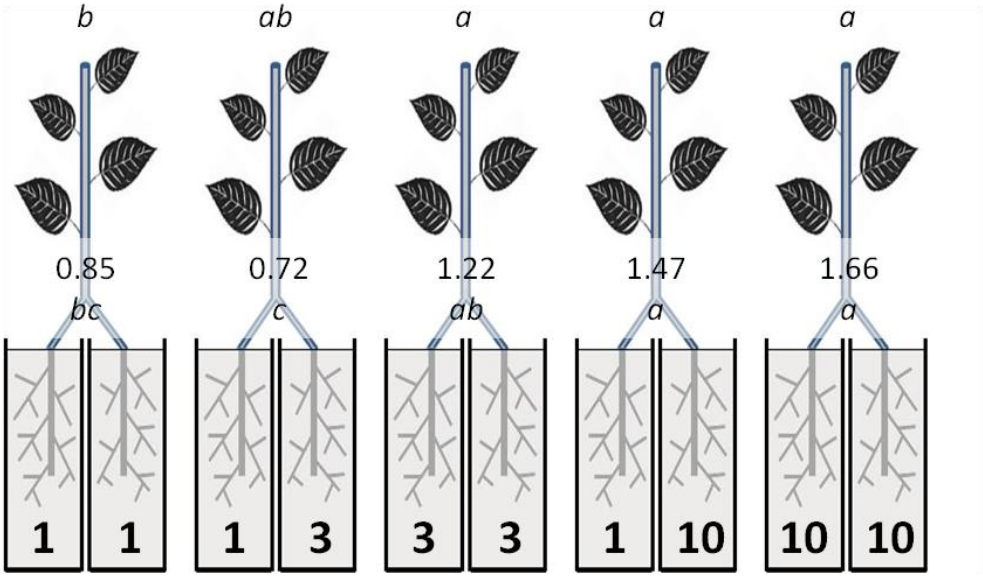


Fig. 3.1 - Mean FFR by age classes (all 3 sampling dates). No distinction between N treatments (average ± standard error).



Root-N%	(1.85)	(1.88)(2.10)	(2.11)	(2.04)(2.54)	(2.63)
homogeneous	*c*		*b*		*a*
patchy (1 vs 3)	*b*	*b* *a*	*a*		
patchy (1 vs 10)	*c*			*b* *a*	*a*

Fig. 3.2 - Nitrogen (N) concentration in leaves, stems and roots as affected by N supply. Different letters in rows indicate statistically significant differences in N concentrations of the various organs.

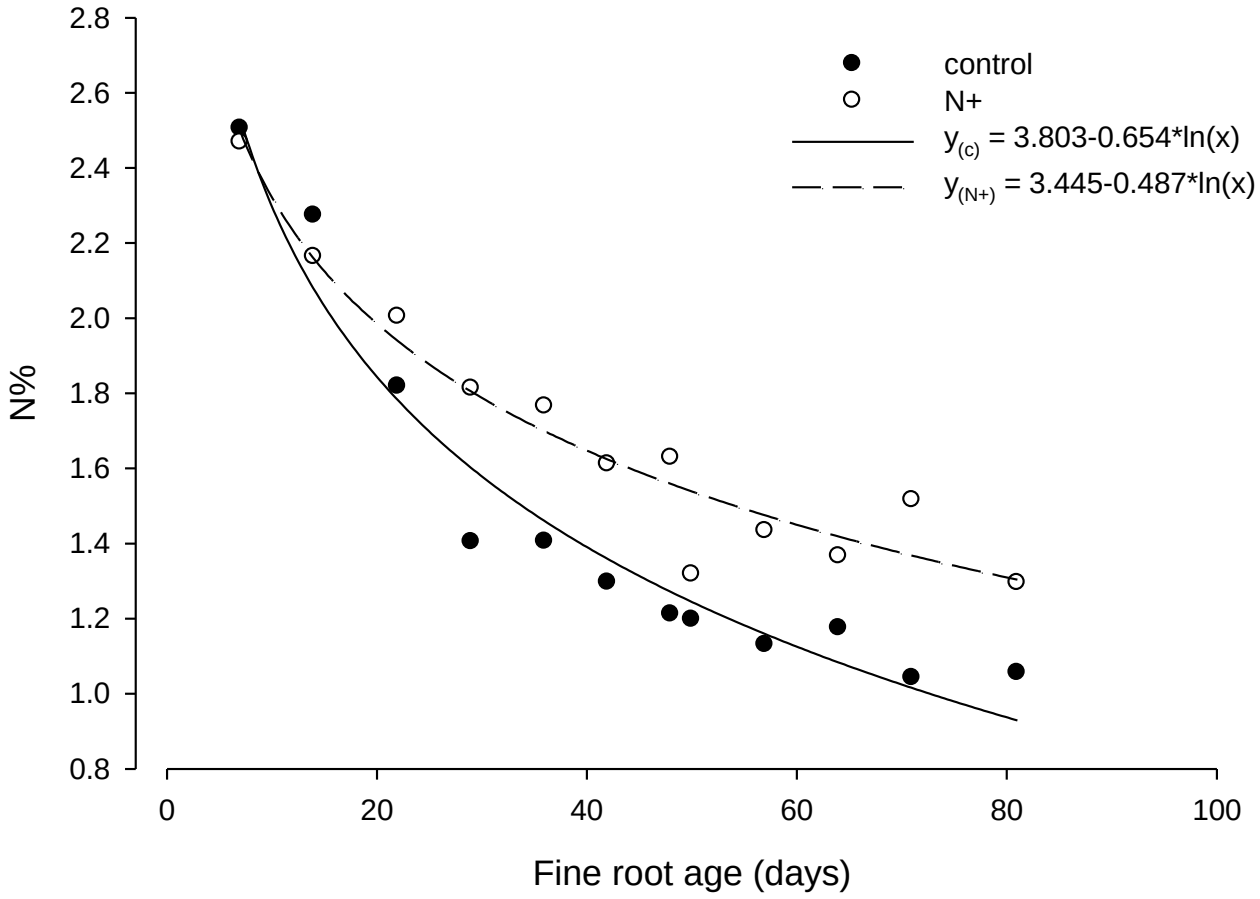


Fig. 3.3 - The influence of localized fertilization on fine root N concentration in relation to root age in *Populus tremuloides* (2nd field sampling). (Correlation lines are significantly different at $p<0.001$).

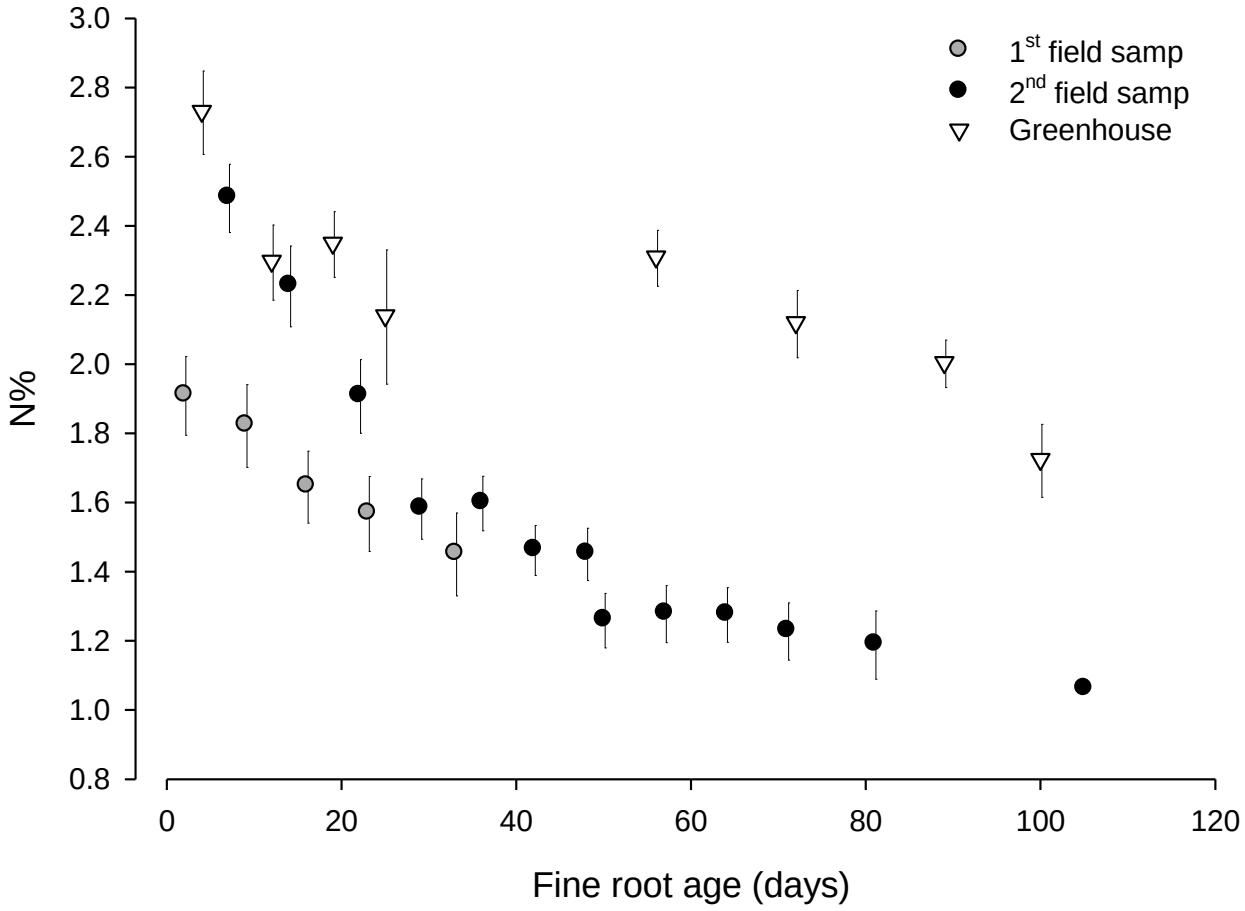


Fig. 3.4 - The influence of root age on root N concentration in the greenhouse and field experiments in *Populus tremuloides* (average ± standard error).

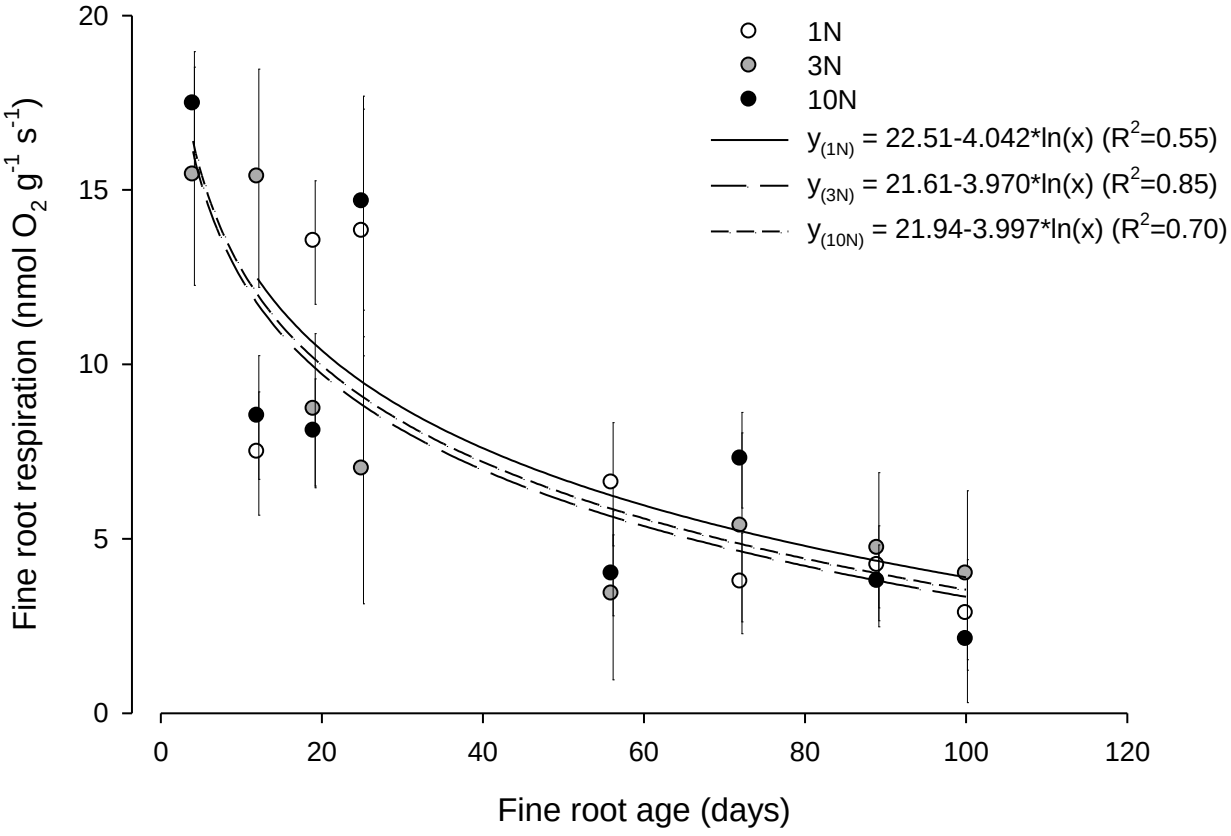


Fig. 3.5 - Mean FFR by age classes, between homogeneous N treatments (greenhouse exp.) (average ± standard error).

Pic. 3.1 - Root box as it was placed in the soil. To this window there was an equal one opposite to it.

4. Experiment three

Effects of nitrogen on total and heterotrophic soil respiration in an apple orchard assessed by continuous measurements

Introduction

Climate change, resulting from the continuous rise of CO_2 concentration in the atmosphere, has led to extend studies of carbon (C) fluxes and carbon budgets in all ecosystems. Main objectives are to determine the potential of ecosystems for C sequestration and how C fluxes are affected by plant- and environmental factors. Recently more attention was dedicated also to agricultural ecosystems, which are mainly represented by annual and perennial crops. This ecosystems are, on one hand, less studied for their C budgets in respect to forest and grassland ecosystems, and, on another hand, generally thought to be a net source of CO_2 to the atmosphere (Schulze et al. 2010). In particular this seems to be true in the case of annual crops (Ceschia et al. 2010), while perennial crops, like orchards, might have the potential to act as net sink for C due to some peculiarities that distinguish them from annual crops. Like a large partitioning of their net primary productivity (NPP) to woody organs (Panzacchi 2008), reduced C-losses from the soil due to no tillage and the possibility to have large parts of the soil surface permanently covered with herbaceous plants, that also contribute to C fixation.

At ecosystem level a major CO_2 flux is represented by soil respiration (R_s), which is the second largest C-flux after leaf photosynthesis, accounting for 60-90% of total ecosystem respiration. Therefore R_s plays an important role in ecosystems C budget (Goulden et al. 1996; Longdoz et al. 2000). R_s is the sum of all respiratory processes occurring in the soil, and it is generated by two distinct processes: microbial respiration (R_h) and root respiration (R_a). R_h is generated through the microbial decomposition of soil organic matter (SOM), while R_a derives from all root-related activities, like water and nutrient absorption, or growth and maintenance of root biomass. R_a also includes the respiration of organisms directly dependent on the presence of roots, namely microorganisms of the rhizosphere and mycorrhizal funghi. The main substrate for R_h is the SOM, whereas R_a relies on the products of the photosynthesis. Since CO_2 produced by R_a was recently fixed through photosynthesis, its release does not contribute to the increase of CO_2 concentration in the atmosphere. On the contrary CO_2 produced by R_h derives from the long term storage of C in the SOM,

therefore R_h does increase atmospheric CO_2. Since SOM represents one of the major C-pools in terrestrial ecosystems, by far exceeding the C stored in plant biomass, it is of relevant importance to understand how microbial decomposition of SOM is affected by environmental conditions. Various factors have been found to affect the components of R_s, of which soil temperature and moisture are often the most important (Tedeschi et al. 2006; Cook and Orchard 2008). Also the quality of SOM and soil N availability may affect R_s. In particular, N availability has gained interest. On one hand, it is thought that N availability is going to become larger in the future, due to a predicted increase in N depositions (Galloway et al. 2004). On another hand, N gained interest because increased N availability can have positive drawbacks in the mitigation of the climate change. The first positive effect is that increased N availability will lead to enhanced biomass production, and therefore more C will be fixed. The second effect is that N should lead to a decrease in microbial decomposition-rates of plant litter and SOM (Berg and Matzner 1997). As a consequence of these effects induced by N, there should be, in theory, an accumulation of organic C in the soil and a reduction of soil respiration, due to less CO_2 produced by the heterotrophic component of soil respiration.

To test the hypothesis of reduced soil respiration after the addition of N, and to know to which extend this may also be true in orchard ecosystems where fertilization is common, I measured R_s continuously over one growing season. The monitored parcels received either a N-treatment over a 2-year period or no N. To separate R_s into its components, R_h and R_a, I used the trenching technique (Hanson 2000). Aim of this study was to investigate how N availability influences R_s and R_h and also to identify the influence of environmental parameters (soil temperature and soil water content) on soil respiration rates in an apple orchard.

Material and methods

Site and plot description

The study was conducted in a mature apple orchard, located 2 Km south from the lake of Caldaro (Province of Bolzano, Italy; 46°21'N, 11°16'E, 224 m a.s.l.). The orchard lays within a wide valley almost totally dedicated to apple production, where commercial apple growing is practiced since many decades. The province of Bolzano is nowadays one of the most important areas for apple growing in Italy, producing 10% of European apples.

The orchard has been planted in 2001 with trees of the cultivar Fuji (grafted on M9 rootstock) on a soil previously hosting another apple planting. Trees are planted in rows oriented East-West, at 3 x 1 m spacing, corresponding to a density of 3300 trees per hectare. Trees are trained as *spindelbush* and their average height is approximately 3.5 m. The orchard is managed following the guidelines of organic farming, has a permanent grass cover in the alley (1.8 m large), while the soil area underneath the trees (1.2 m large) is kept free from weeds by periodic mechanical tillage on the top soil layer. Until 2 years before the study started, each year approximately 600 Kg of an organic fertilizer (Nutristart) were applied to the soil-stripe underneath the trees. The soil is classified as loamy (after USDA soil classification system), with an average granulometric composition (top 0-60 cm) of 12% clay, 44% silt and 44% sand. Total soil nitrogen is 0.17%, organic matter 2.46%, organic carbon 1.43% and the pH is 7.3. During 2010 rainfall was 911 mm and mean annual air and soil temperature were respectively 11.5°C (at 2 m height) and 12.3°C (at 10 cm depth) (data collected at the nearby Eddy covariance tower). The orchard is equipped with above-canopy sprinklers for irrigation. From May until August 2010 approximately 720 mm of water were distributed through irrigation.

A plot consisting of 30 trees along a row, located in the vicinity of an Eddy covariance tower used for studies of Net Ecosystem Exchange, was used for the experiment. Trees were subdivided in 6 sub-plots of 5 trees each. Out of these 6 sub-plots, 3 were randomly selected to receive a N fertilization (N+) (equivalent of 100 Kg N $ha^{-1}yr^{-1}$), while the other 3 sub-plots (N-) received no N supply.

In both the northern and southern side of each sub-plot, two plastic collars for measuring soil respiration were placed at 30 cm distance from the tree row (see Fig. 4.1). The collars had an inner diameter of 20 cm and a height of 11 cm; they were permanently inserted in the soil at a 3 cm depth and placed in a way to have its center at a distance of 30 cm from the tree row, exactly between two adjacent trees (Fig. 4.1). One of two collars was placed on soil areas previously subjected to "trenching", a technique aimed to create a portion of soil in which no living roots are present. For this purpose, in June 2009 we excavated a trench (60 cm depth) around an area of 50x50 cm. To avoid that new roots could grow into the trenched soil area, before the trench was refilled with soil, a special tissue, impenetrable to roots, but permeable to water and air, was placed all around the trenched soil area. The choice of the soil areas where locate collars was done after an initial phase of monitoring R_s (4 weeks, from March 17th to april 07th and from April 15th to April 25th 2009), to ensure initial R_s did not differ among collars.

Nitrogen supply and monitoring

Plots that had to be treated with fertilizer (N+) received an equivalent of 100 Kg N $ha^{-1}yr^{-1}$ during the year prior to the study (2009), as well as during the year of measurements (2010). The fertilizer was equally distributed in three applications each year and nitrogen was applied in the form of NH_4-NO_3 dissolved in water and homogeneously distributed over the soil (72 m^2 in total; 10 g N m^{-2} yr^{-1}). Both years the first treatment was applied on the end of March, the second in late June and the last in August. Control plots received no fertilizer treatment over the 2 year period, but the same amount of water as the fertilized plots.

On August 4th, after the last fertilization event, we sampled soil at different depths (0-20, 20-40, 40-60 cm) in all parcels and analyzed it for N content, by N-extraction in KCl (2M). Samples extracted in KCl, were analyzed for their NH_4 and NO_3 concentration by an autoanalyzer (AxFlow AA3; Bran + Luebbe, Norderstedt, Germany).

Soil respiration measurements

Soil CO_2-flux from control plots measured total soil respiration (R_s), while the heterotrophic component of soil respiration (R_h) was recorded from trenched soil portions (Fig. 4.1). R_a was calculated subtracting R_h from R_s. The CO_2-efflux was measured simultaneously and continuously (at time intervals of 30 minutes) on 8 collars, thanks to 8 automated soil respiration chambers (Li-cor LI-8100-104), managed by a multiplexer control unit (Li-cor LI-8150) and combined with an IRGA unit (Li-cor LI-8100A). To estimate CO_2 efflux from the soil, the LI-8100A uses the rate of increase of CO_2 in a measurement chamber (LI-8100-104). Each measurement lasted 3.5 minutes and to calculate the rate at which CO_2 diffuses from the soil into free air, the following equation is used:

$$F = \frac{10VP_0\left(1 - \frac{W_0}{1000}\right)}{RS(T_0 + 273.15)} * \frac{\partial C'}{\partial t}$$

where F is the soil CO_2 efflux rate (µmol m^{-2} s^{-1}), V is the volume (cm^3), P_0 is the initial pressure (kPa), W_0 is the initial water vapor mole fraction (mmol mol^{-1}), R is a gas constant (8.314 Pa m^{-3}), S is soil surface area (cm^2), T_0 is initial air temperature (°C), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO_2 mole fraction (µmol mol^{-1} s^{-1}). All these parameters are automatically measured by the instrument, with except for the volume (V), which depends from the shape of the soil surface and has to be measured separately. In addition to each CO_2-efflux measurement, also soil temperature and volumetric soil water content at 10 cm depth were recorded, respectively with a soil temperature thermistor (Li-cor 8100-203) and a soil moisture probe (Decagon EC-5). In one replicate, measurements were taken continuously over the whole period of the study, keeping 4 chambers always on the same 4 collars. The remaining 4 chambers were used to measure R_S in the other 3 replicates, with periodic cycles of 1 week for each replicate. Measurements started on March 26th and ended on November 26th 2010.

An estimate of root respiration (R_a) was obtained by subtracting heterotrophic soil respiration (R_h) from total soil respiration (R_s).

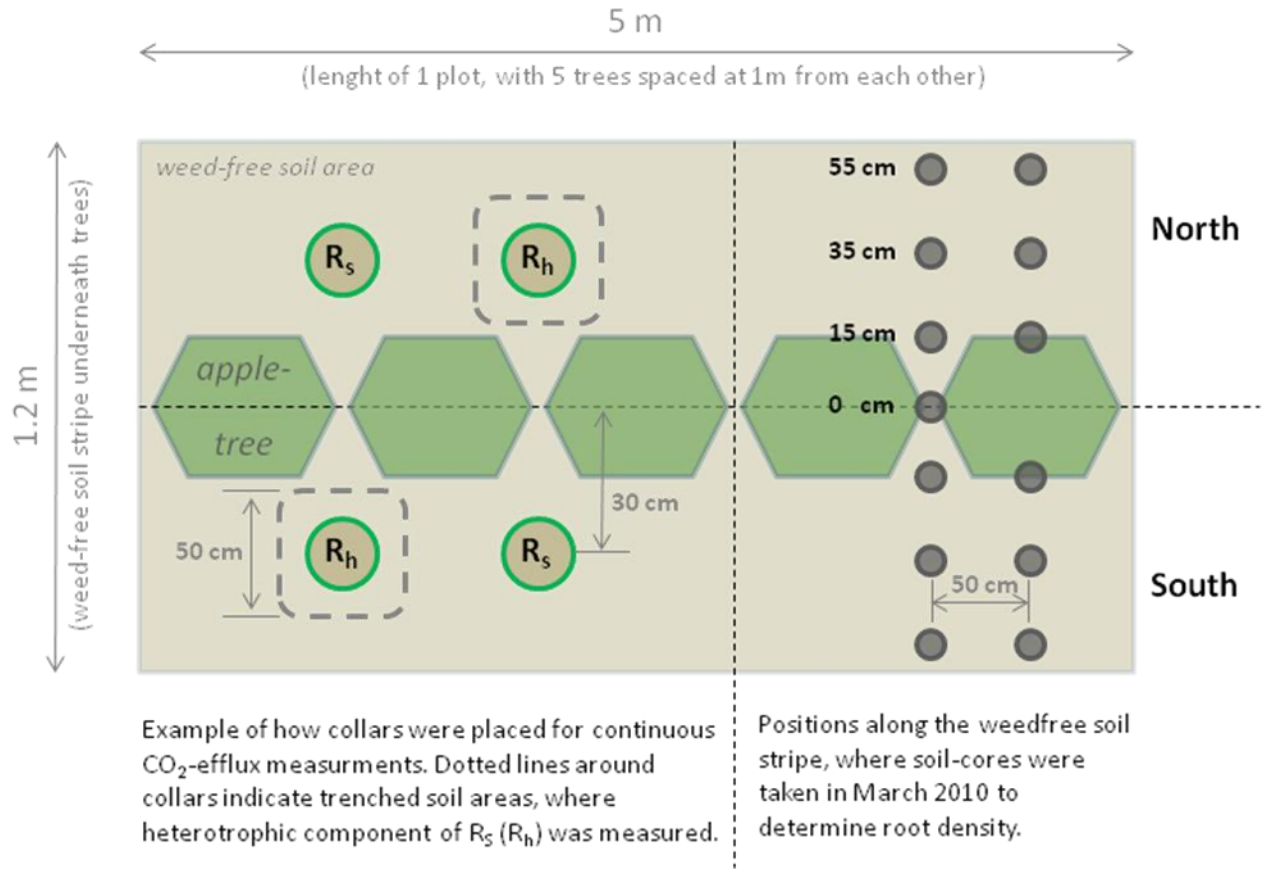


Fig. 4.1 - Representation of the location where continuous R_S-measurements were performed and where root density was determined. On the left side: position of the collars. On the right locations of soil cores collection for root density (not to scale).

Root density

Root density underneath the weed-free soil stripe of the tree row, was determined through the collection of soil-cores to 60 cm depth (with a soil-cylinder-auger) and successive separation of roots from the soil by sieving them under running water. Soil cores were collected in March 2010, prior to the beginning of vegetative growth, on 6 different locations within the orchard. On each location we collected 13 cores as shown in Fig. 4.1.

Statistical analyses

Data of soil CO_2 flux, soil temperature and moisture were analyzed in a randomized block design by one-way analysis of variance (ANOVA) with nutrient supply as factor. Data of leaves and fruits N concentration, and soil N-NO_3 concentration were subjected to one-way ANOVA with nutrient supply as the only factor. Data of fine and coarse root density was subjected to one-way ANOVA with sampling position as the only factor. The comparison between best-fit equations was performed using conditional sums of squares test. The influence of fertilization treatment on R_s, R_h and R_a was analyzed using a paired t-test. Data are reported in tables and figures as average ± standard deviation or standard error as specified in legends.

Results

Meteorological and soil conditions

During the period of measurements, from February to November 2010, we recorded 887 mm of rainfall, a mean surface soil temperature of 15.0°C (at 10 cm depth) and a mean surface soil water content of 30.7% in volume in the non trenched parcels. Precipitations were distributed homogeneously during the year, with major events in August and September (Fig. 4.2a). Surface soil temperature increased constantly from February peaking at the end of July. Soil water content in the non trenched plots showed a high seasonal variability, with highest values during late fall and lowest between June and August, reaching values as low as 19% vol. (Fig. 4.2a). Soil water content in the trenched plots (R_h) was fairly constant and mostly between 30 and 40% vol.

Tree phenological phases

Tree metabolic activity started at the beginning of March in correspondence of the phenological phase of bud break. Full bloom was observed in the second half of April and the fruit set began at the beginning of May. Fruits were harvested in late October and leaf fall spanned between November and December (Fig. 4.2b).

Soil respiration

Mean annual soil CO_2-efflux rates differed markedly between non trenched (R_s) and trenched plots (R_h), being nearly twice higher in non trenched plots (Tab. 4.1). Mean annual R_s did not differ in N-treated (N+) and in non treated (N-) parcels, while R_h resulted almost significantly different ($p=0.055$), being slower in N+ parcels (Tab. 4.1).

In non trenched plots, mean weekly soil respiration rates (R_s in Fig. 4.2b) were slowest at the beginning and at the end of the growing season, in correspondence of lowest soil temperatures (Fig. 4.2a). Between February and May, CO_2 efflux rates increased, slowly first and then sharply after the end of May, reaching the maximum in the middle of June. After this peak R_s slowly decreased despite the high temporal variability. In trenched plots, soil

respiration rates (R_h in Fig. 4.2b) were slow and almost stable until the end of May after which R_h only slightly increased, reaching its maximum at the end of July, thereafter slowly decreasing. R_s had nearly always higher rates and a broader range of soil CO_2-efflux values than R_h (Fig. 4.2b).

The connection between soil respiration and soil temperature can be particularly appreciated in figure 4.3 reporting a two-week close up of measurements taken every half hour. Soil respiration and soil temperature reached their minimum values before sunrise and their maximum in the early afternoon. In the same figure it is also possible to appreciate the influence of rainy events on soil respiration. With short intense rains (>5mm h^{-1}), R_s almost completely stopped, quickly regaining high rates after the rainy event. In association with minor persistent rainy events (<2mm h^{-1}), R_s is reduced, but not stopped, and takes longer to rise back to normal levels.

The positive relationship of R_s with soil temperature can be described by an exponential equation for trenched (Fig. 4.4a) and by a linear equation for non trenched parcels (Fig. 4.4b). On the contrary, R_s and soil moisture were negatively related by a 2^{nd} degree polynomial equation. However, this relation was evident only in the non trenched plots; due to the very narrow range of moisture in the trenched plots, we couldn't obtain a significant relationship between R_h and soil moisture.

Mineral forms of nitrogen in soil solution

The analysis of soil solution in KCl revealed that, on August 4^{th}, ammoniacal nitrogen ($N\text{-}NH_4$) was almost completely undetectable (data not shown). $N\text{-}NH_4$ was extracted in only one of the fertilized parcels in concentration ranging from 0.04 to 0.49 mg L^{-1}, decreasing with depth (data not shown). The amount of nitrate nitrogen ($N\text{-}NO_3$) recovered in the soil solution was higher in the fertilized parcels than in the non fertilized ones, especially at deeper depths (Fig. 4.6).

Total nitrogen in fruits and leaves

Total nitrogen in apple fruits was affected by the treatment, being lower in the non-treated plots than in the treated plots (Tab. 4.2) while the concentration of total carbon in fruits was not significantly different. C-to-N ratio resulted to be higher in the non-treated plots than in the treated plots, although the value of p was at the limit of statistical significance (Tab. 4.2). Total nitrogen in the leaves was not significantly affected by the treatment, being 2.5% on average. Total carbon in the leaves resulted to be lower in the non treated plots than in the treated plot. However, C-to-N ratio was not significantly different (Tab. 4.2).

Root density

Fine root density ranged from 0.3 to 1 kg m^{-3}, being highest closer to the tree and decreasing gradually edging away from it (Fig. 4.7a). Coarse root density showed high variability, ranging from 0.1 to 8 kg m^{-3}. Similarly to fine root density, the highest values were found close to the tree.

Discussion

Among the few studies on soil respiration (R_s) in apple and in general in fruit orchards, this is the first where R_s was continuously measured at half hour steps over one entire growing season. For the period from March until November 2010, I recorded a mean R_s of 0.540 g CO_2 m^{-2} h^{-1}. To be comparable to other studies, this R_s-value has to be corrected for the period between December and February where R_s was not measured. Thus the estimated mean annual R_s is 0.433 g CO_2 m^{-2} h^{-1}, calculated according to the equation reported in Fig. 4.4a, using a mean annual soil temperature of 12.3°C (this value of mean soil temperature for 2010 was measured at a nearby weather station of the research center "Laimburg"). This mean annual R_s-value is relatively high if compared to the study of Tufekcioglu et al. (2008) in Artvin (Turkey) and Ceccon et al. (2010) near Bologna (Italy), for which 0.302 and 0.380 g CO_2 m^{-2} h^{-1} are reported, respectively. This discrepancy in R_s values between the three studies might be site related, depending on different climatic conditions, soil characteristics, planting densities and ages of the trees, and also related to different methodologies used to obtain mean annual R_s. However, the interesting fact is that the highest mean annual R_s value between these three studies was found for Bolzano, where mean annual soil temperature was lowest in respect to the other sites. As soil temperature is known to be the major driving factor for R_s (Lloyd and Taylor 1994), this might suggests that factors other than soil temperature also have an important role in determining soil CO_2-efflux rates. The most evident differences between these sites, beside mean annual soil temperature, are their soil characteristics. The soil in Artvin was a loamy-clay, in Bologna a silty clay loam and in Bolzano a loamy. The first two differ particularly from the latter for their clay content, which respectively was 38, 32 and 12%, while the soil organic matter was 3.4, 2.3 and 2.5%.

During the growing season R_s followed nearly the same trend as soil temperature, with rising values from early spring until the middle of summer, after which both slowly decreased to lower values in late fall (Fig. 4.2). The strong correlation of R_s to soil temperature was true for instantaneous R_s measurements over the day (Fig. 4.3), as well as

for weekly mean values (Fig. 4.4a) and this correlation is best described by an exponential function, as proposed by other authors (Lee and Jose 2003). The overall influence that soil temperature has on R_s was also found by comparing R_s-values measured on South- and North-exposed collars. Differences for R_s were evident, and the lower values measured on the Northern side could not be explained by other factors than reduced soil temperature (data not shown). However, R_s showed a higher temporal variability than soil temperature, especially during the warmer months (Fig. 4.2b). This variability for R_s was largely due to the partial negative effect that rainy events and subsequent high values of soil water content showed on CO_2 efflux from the soil (Fig. 4.5a), especially in the case of more intense or prolonged rainfalls (Fig. 4.3). The decrease of R_s at high levels of soil water content was observed over the whole range of measured soil temperatures, indicating that low respiration rates due to high soil water content are not the consequence of the fact that high soil moisture occurs in winter when soil temperature are low (Fig. 4.8). The reduced CO_2-efflux rates observed at high soil water content can be explained, on one hand, by the physical impedance that water filled pore spaces of the soil have on the diffusion of CO_2 from the soil (Bouma and Bryla 2000; Cook and Orchard 2008), and, on other hand, by a reduced respiratory activity, especially of aerobic microbes, as a consequence of limited O_2 diffusion (Linn and Doran 1984). Root respiration can also be negatively affected by limited diffusion of O_2, but this might only be the case in situations of prolonged water logging, which never occurred during this study.

Total soil respiration (R_s) was not significantly affected by the N treatment, being 0.537 g CO_2 m^{-2} h^{-1} for the control (N-) and 0.546 CO_2 m^{-2} h^{-1} for fertilized (N+) plots (Tab. 4.1). On the contrary, in trenched plots (R_h), CO_2-efflux was higher in N- parcels compared to N+ parcels (Tab. 4.1), with a p-value of 0.055. A similar response of R_h to nitrogen availability was also observed in analogous studies performed in forest ecosystems (Janssens et al. 2010). The reduced CO_2-efflux from trenched soil portions with higher N-availability was explained as the result of a quantitative reduction of the microbial biomass in fertilized plots, as well as a qualitative shift in the microbial community. Indeed, it is assumed that a higher availability of nitrogen leads to the decrease of the microbial communities responsible for the degradation of more recalcitrant soil organic matter (Treseder 2008). The fact that I did

not found lower values for R_s in N+ parcels, as it would be expected from the results obtained in the trenched plots, might suggest a positive effect of N on autotrophic soil respiration. Autotrophic soil respiration (R_a), calculated as the difference of R_s and R_h, was 0.136 and 0.268 g CO_2 m^{-2} h^{-1} for N- and N+ parcels, respectively (Tab. 4.1), being significantly different (p=0.035). This results suggest that N have an opposite effect on root respiration in respect to microbial respiration, stimulating the former and inhibiting the latter. On one hand, N can stimulate root growth, especially of fine roots, those responsible for water and nutrient uptake (Drew and Saker 1975), and on other hand, N has been found to influence also fine root turnover, which can become faster in conditions of high N availability (Pregitzer et al. 1995, 2000). Literature reports values of contribution of R_a to R_s in ranges from 10-90% (Hanson et al. 2000), being on average 42% (Bond-Lamberty et al. 2004), which is comparable to the average value of 37.2% found for this experiment. Regardless of the broad range of values reported by Hanson et al. (2000), Bond-Lamberty et al. (2004) suggest that there is a strong correlation between R_a and R_h among a broad range of ecosystems, independently of any disturbance or factor introduced to determine these components. They suggest R_a and R_h being closely linked together, as R_h depend also on processes linked to roots, such as root turnover. Therefore, R_h measured in trenched plots in the absence of roots, might not be totally representative of microbial respiration in the presence of roots. As microbial respiration results from the degradation of soil organic matter, but also from the degradation of newly produced root-litter, it can be assumed that if the supply of fresh root-litter in trenched plots is suppressed and its degradation does not take place, then a portion of soil microbial respiration will not be measured. In addition to that, heterotrophic microbial community is also indirectly dependent on the activity of living roots, which includes the release of exudates (Nguyen 2003) and water and nutrient uptake, which all influence chemical and physical conditions in the soil. All these factors are suppressed or altered by the trenching, leading probably to different respiratory patterns of the heterotrophic component of soil respiration. Since the aim of my study was to estimate R_a and R_h and to determine their response in relation to N availability, these issues related to the trenching technique probably influenced the outcomes of my research. Soil water content for example was constantly higher in trenched plots than in non trenched plots (Fig.

4.5). This certainly resulted from the absence of water absorption by roots. Since I noticed a negative relationship between R_s and high soil moisture levels (Fig. 4.5a), the higher moisture in the trenched plot can lead to a reduced CO_2-efflux, even if all the other factors are kept constant. Another issue of this technique could have been that the applied nitrogen in non trenched plots was completely absorbed by roots. In this case, microbial community could not have been influenced by N in non trenched plots, explaining why I didn't observed the same reduction in total soil respiration in non trenched fertilized plots, as it was found in trenched fertilized plots. On the contrary, N in trenched plots might have been in excess, as it was not absorbed by roots, leading to a more emphasized response of R_h. Therefore, I think that the trenching technique may not be the most suitable methodology to estimate autotrophic respiration, especially if different treatments, like nutrient availability, are compared.

In conclusion, with this experiment I showed that R_h in trenched plots but not R_s was significantly reduced after the addition of nitrogen to the soil. I suggest that this may depend on one hand on interactions between roots and the surrounding rhizospheric and bulk microorganisms in response to N, and on other hand on the positive effect N has on root turnover and on the production of new roots, which likely enhances root respiration. If this is true, than C-balance of the soil should benefit from the addition of N, as a consequence of more biomass introduced to the soil trough root production and turnover, and reduced decomposition of more recalcitrant and long-lasting SOM. Both these responses to N would finally contribute to the mitigation of the climate change. As a final point, with this experiment I also confirmed the already accepted positive correlation between R_s and soil temperature and the inhibiting effect that high values of soil moisture have on CO_2-efflux from the soil.

Tables and figures

Tab. 4.1 - Mean annual values of soil respiration (g CO_2 m^{-2} h^{-1}) as affected by nitrogen fertilization (N+ = fertilized parcels; N- = control parcels). Total soil respiration (R_s) was measured in the non-trenched plots while heterotrophic respiration (R_h) was measured in the trenched plots; autotrophic respiration was calculated by the difference between R_s and R_h (average ± standard deviation).

	N-	N+	*p*
R_s	0.537 ± 0.12	0.546 ± 0.14	*n.s.*
R_h	0.400 ± 0.26	0.279 ± 0.32	*0.055*
R_a	0.136 ± 1.00	0.268 ± 0.10	*0.035*

Tab. 4.2 - Nitrogen (N%) and carbon (C%) concentrations in leaves sampled in July 2010 and in apple fruits harvested in October 2010 in the fertilized (N+) and non-fertilized (N-) parcels. The carbon to nitrogen ratio (C:N) is also shown (average ± standard deviation).

		N-	N+	*p*
Fruits	N%	0.30 ± 0.06	0.41 ± 0.06	*0.04*
	C%	43.06 ± 0.72	43.85 ± 0.39	*n.s.*
	C:N	150 ± 29	109 ± 16	*0.05*
Leaves	N%	2.42 ± 0.12	2.54 ± 0.05	*n.s.*
	C%	50.29 ± 0.29	51.00 ± 0.27	*0.04*
	C:N	20.84 ± 1.18	20.09 ± 0.29	*n.s.*

Fig. 4.2 - Soil moisture and temperature, rainfall and soil respiration measured from March to November 2010. (a) Weekly mean values of soil temperature (°C), weekly mean values of soil water content (%vol) in the trenched (R_h) and non-trenched (R_s) plots, and cumulative amount of rainfall (mm) per week. (b) Weekly mean values of soil respiration (g CO_2 m^{-2} h^{-1}) in the trenched (R_h) and non-trenched (R_s) plots (average ± standard deviation). The phenological phases of the trees are also shown.

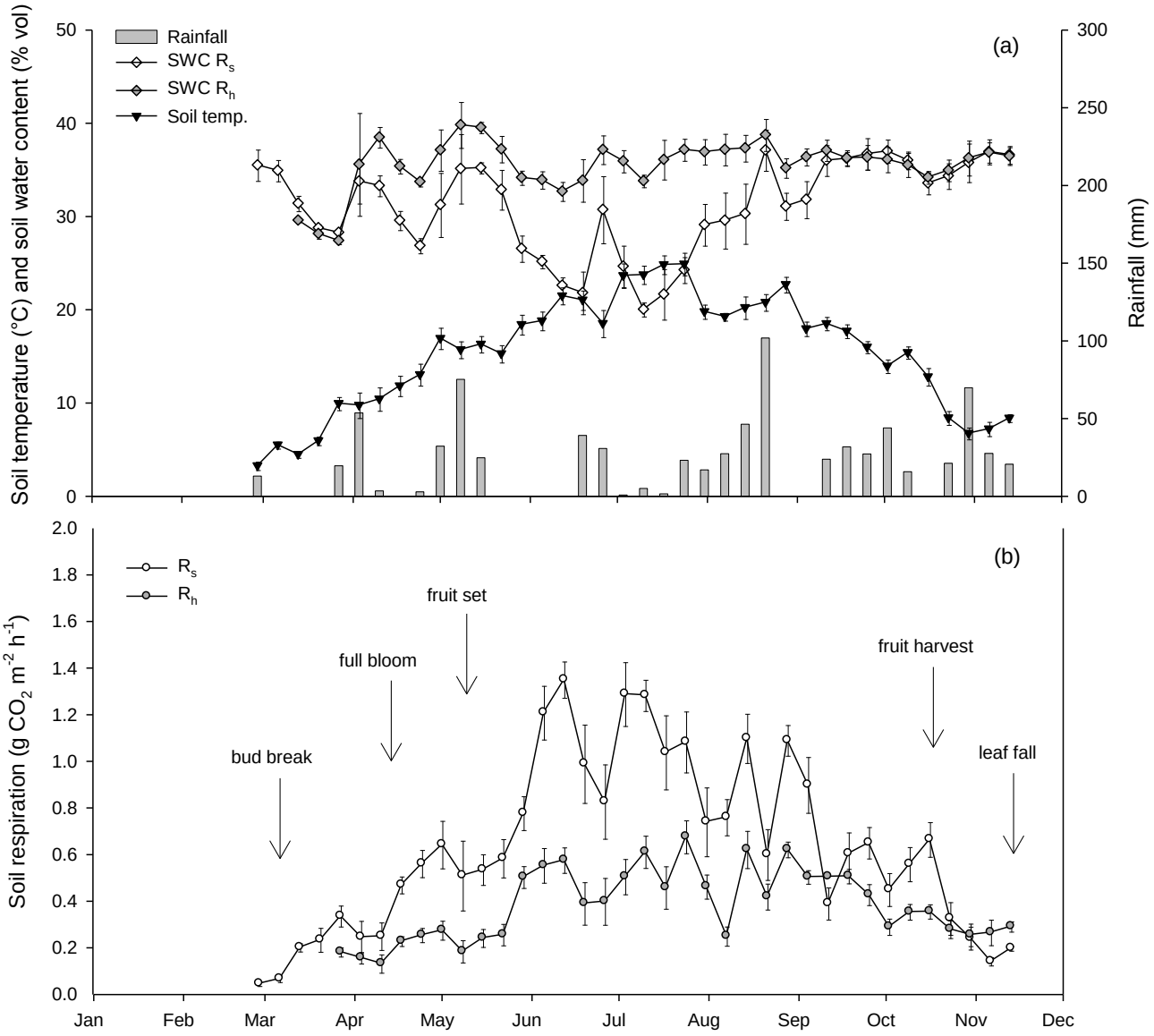


Fig. 4.3 - Close up of a three week period (from June 6 to June 27, 2010) of soil respiration, soil temperature, soil water content and rainfall measurements. (a) Rainfall (mm) (values in brackets show cumulative rainfall in mm for each rainy event). (b) Soil water content (% vol). (c) Soil temperature (°C). (d) Soil respiration (g CO_2 m^{-2} h^{-1}). All data are measured at intervals of half hour.

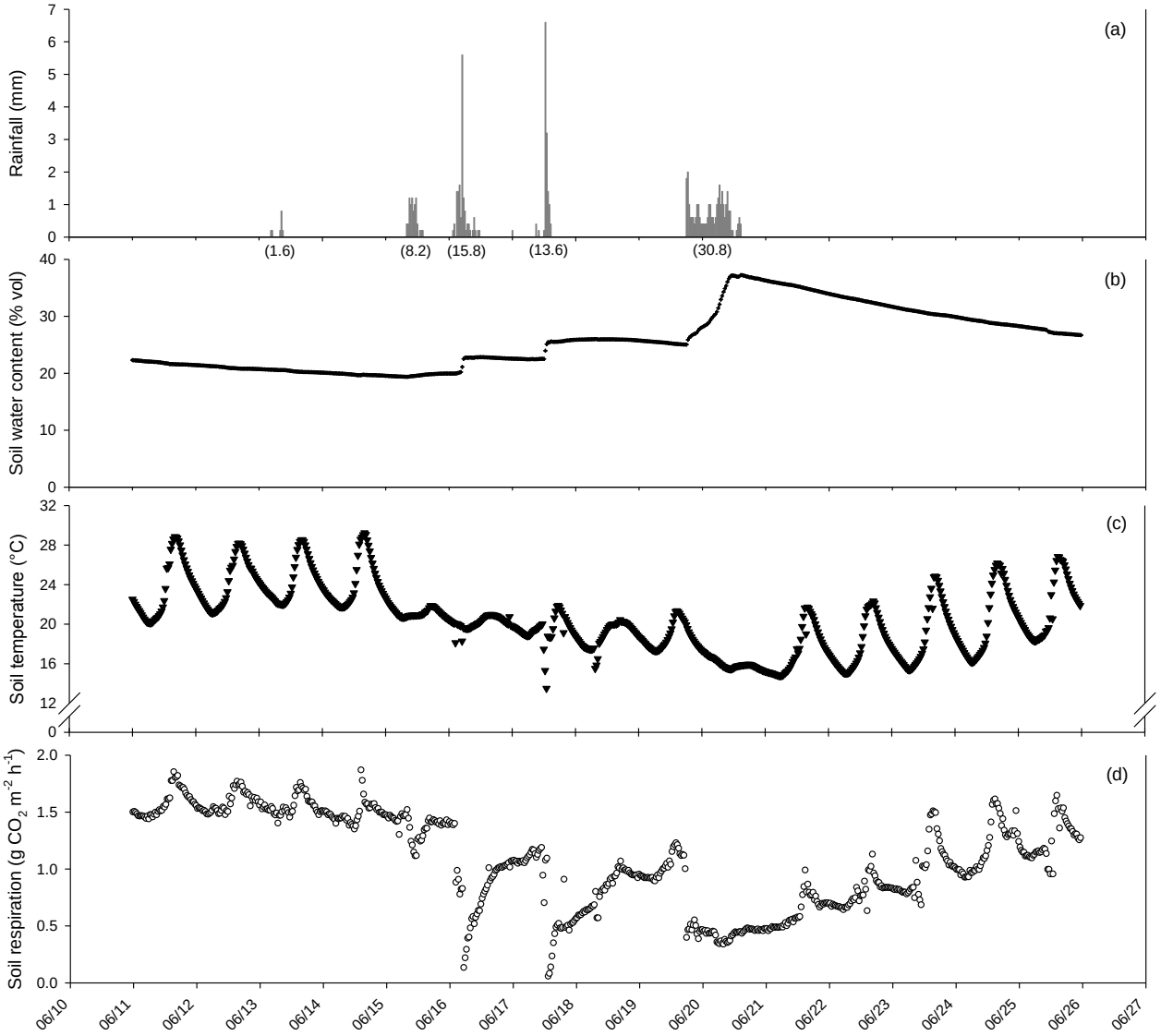
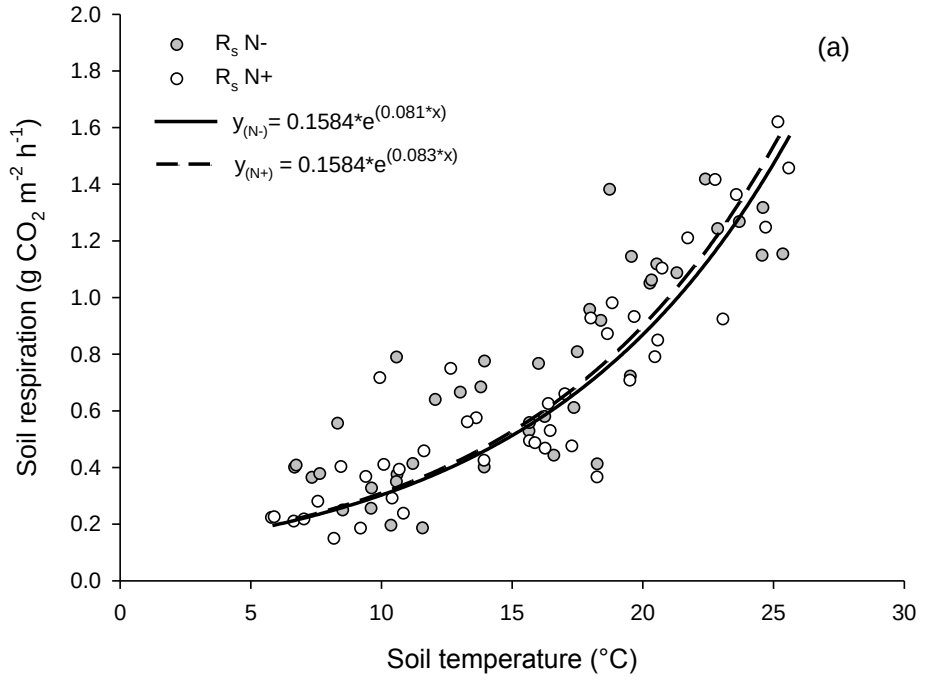


Fig. 4.4 - Relationship of mean weekly soil respiration (g CO_2 m^{-2} h^{-1}) with mean weekly soil temperature from March to November 2010 in (a) the non-trenched plots (R_s) and in (b) the trenched plots (R_h); the continuous line represents the best fit for the non-fertilized parcels while the broken line represents the best fit for the fertilized parcels (N+); (a) both lines derive from an exponential equation (b) both lines derives from a linear equation. Slopes and intercepts are significantly different ($p<0.001$).



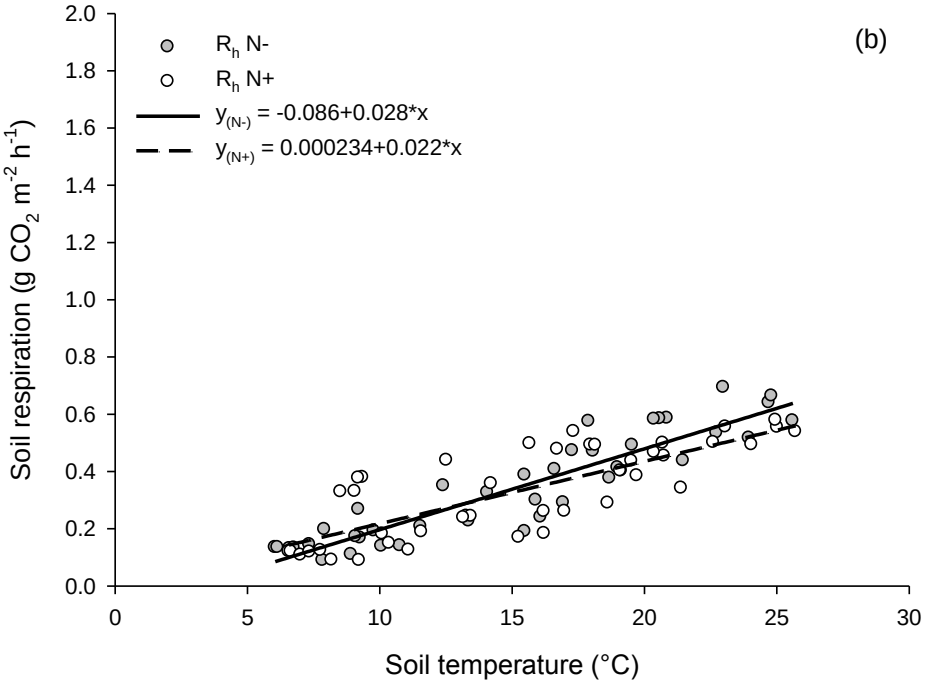
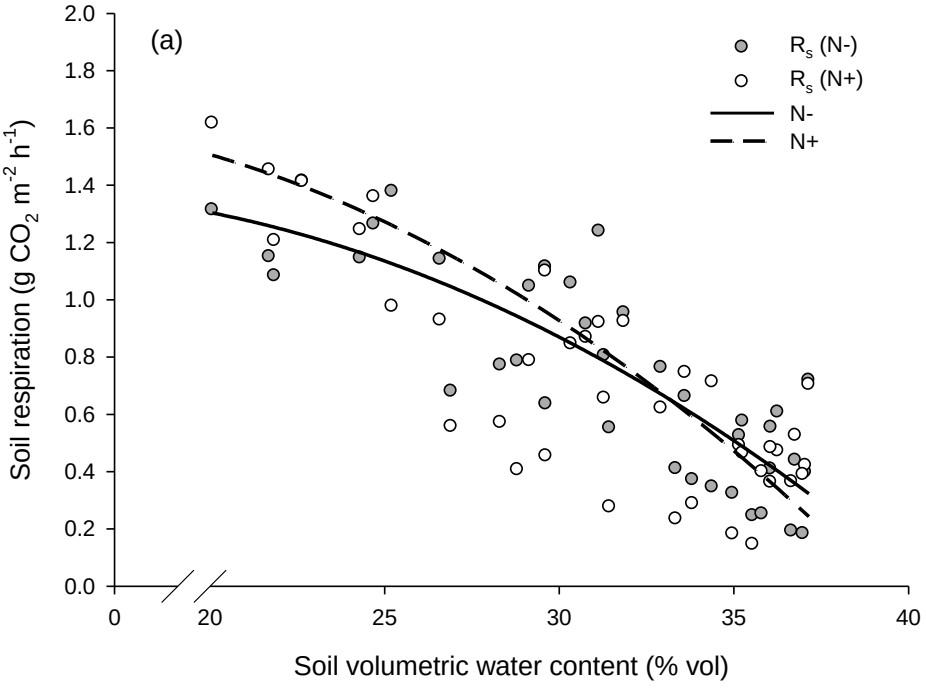


Fig. 4.5 - Relationship of mean weekly soil respiration with mean weekly soil moisture (%vol) from March to November 2010. (a) Soil respiration (g CO_2 m^{-2} h^{-1}) in the non-trenched plots (R_s); the continuous line represents the best fit for the non-fertilized parcels while the broken line represents the best fit for the fertilized parcels (N+); both lines derive from a second-degree polynomial equation. (b) Soil respiration (g CO_2 m^{-2} h^{-1}) in the trenched plots (R_h).



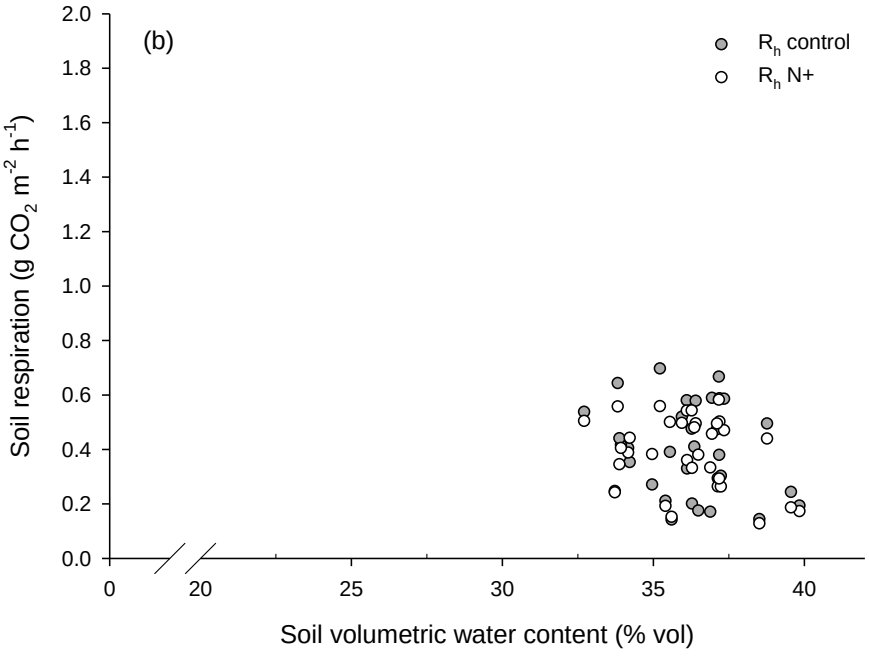


Fig. 4.6 - Nitrate nitrogen ($N\text{-}NO_3$) ($mg\ L^{-1}$) in the fertilized (N-treated) and non-fertilized (control) parcels (average ± standard deviation) extracted with 2M KCl from soil samples collected August 4, 2010.

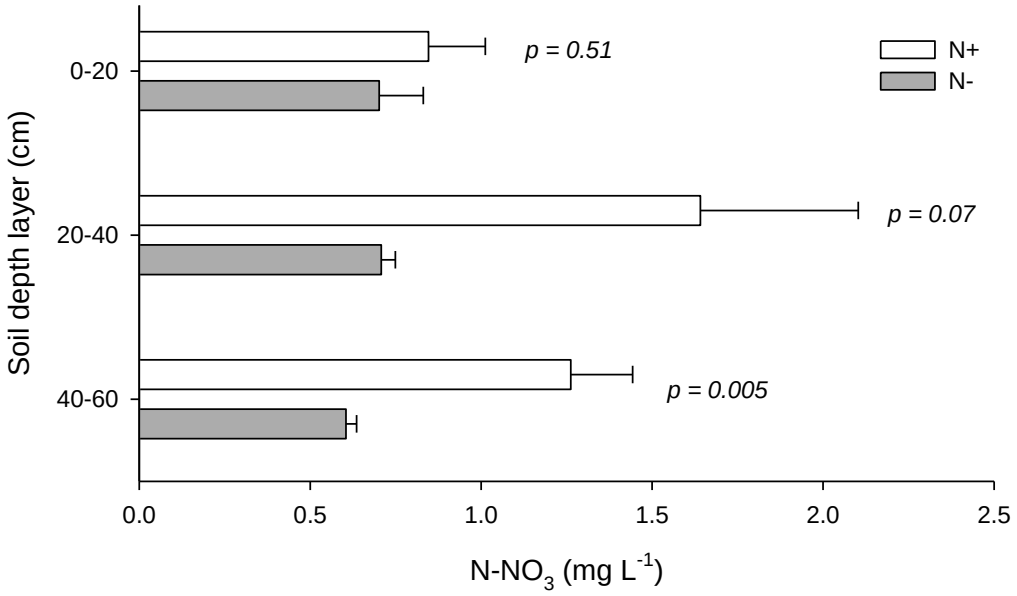
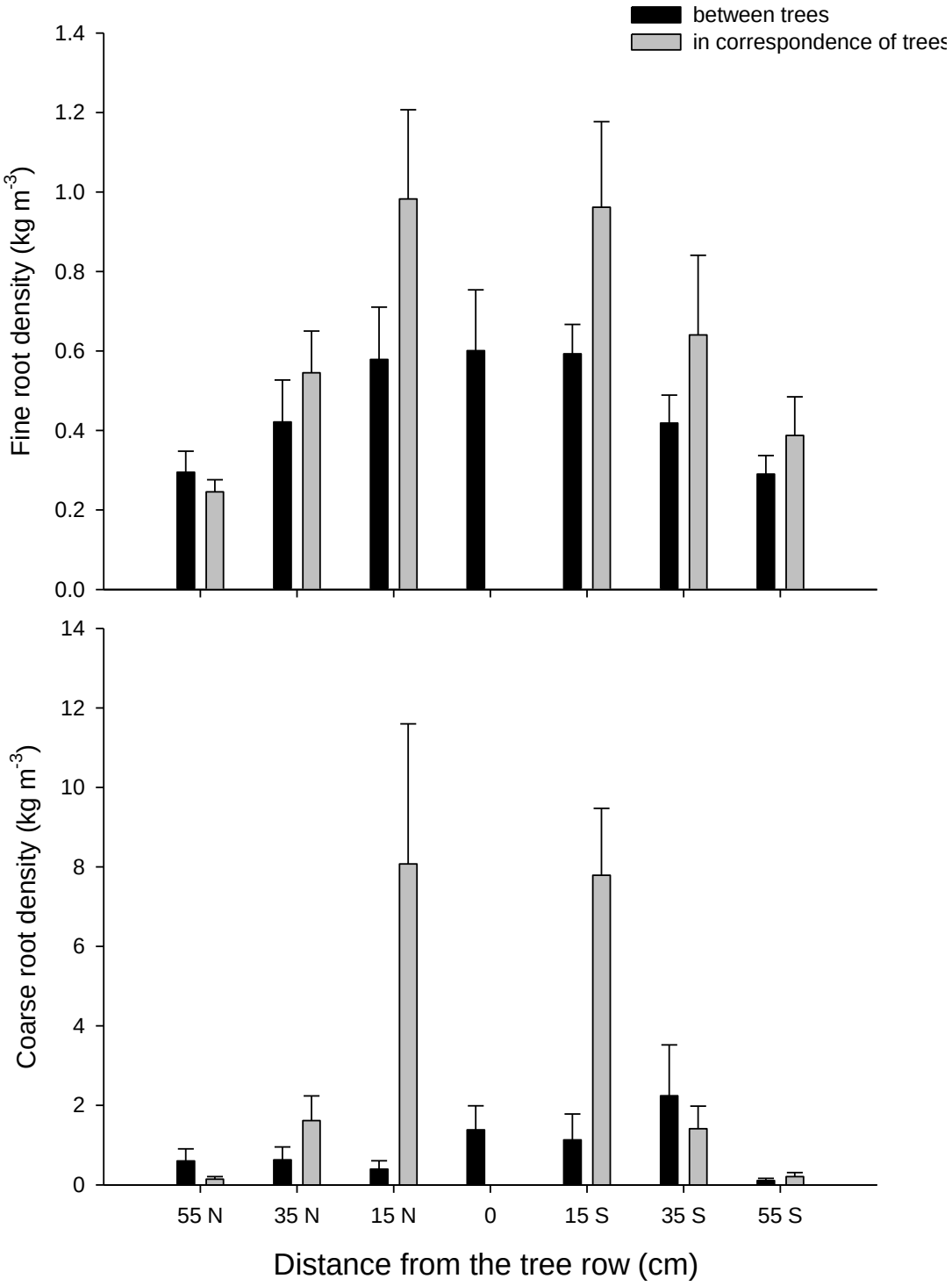


Fig. 4.7 - Root density (kg m^{-3}) in the orchard in the Northern and Southern side of the tree row. Soil cores were collected on March 2010 perpendicularly to the tree row in line with the trunk or between two trees (Fig. 4.1) (average ± standard error).



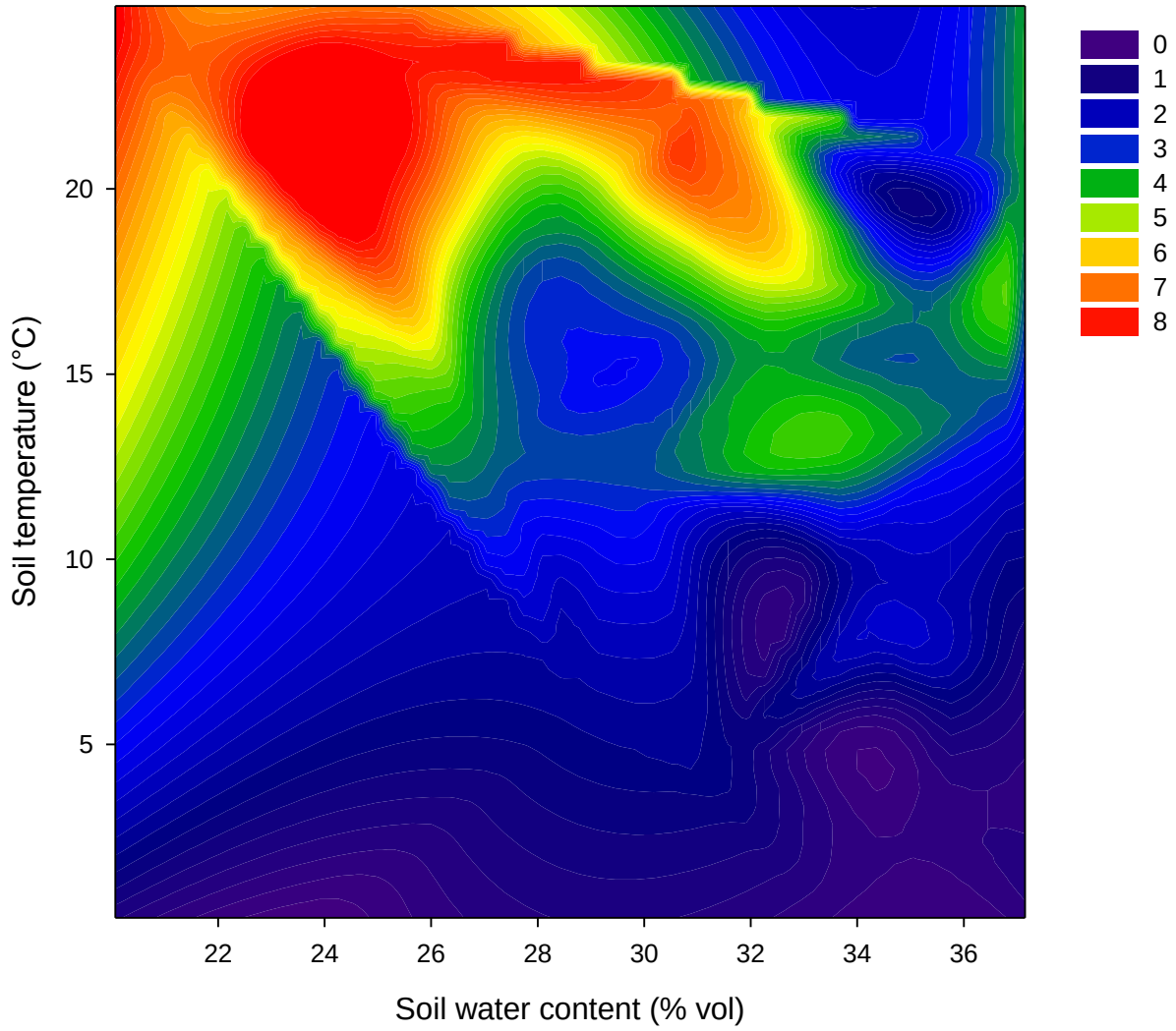


Fig. 4.8 - R_s at different levels of soil water content (% vol) and soil temperature (°C). Bluish colors represent slower soil respiration rates than reddish colors. Data include the whole period of R_s-measurements over one collar position.

5. General conclusions

General Conclusions

The major results of my investigations are that 1) temporal variability of R_s is mainly driven by soil temperature and moisture, while 2) spatial variability is more linked to fine root density. Nitrogen 3) has negligible effects on total soil CO_2 efflux. In addition with my experiments I was able to compare different techniques for the measurement of R_s and its components. The comparison of the results obtained in different environments provides some hints on the factors influencing soil respiration rates. I measured R_s in two apple orchards (Bologna, exp. 1 and Bolzano, exp. 3), differing for soil texture, total N availability, soil carbon (C) concentration, as well as for soil temperature and moisture. The comparison of R_s measurements suggests that soil temperature influences the temporal variability of R_s on a daily and on a seasonal scale. However, the overall annual range of R_s might be also related to other factors. From the comparison between the two orchards, it emerges that even if mean annual soil temperature was higher in Bologna (17.6°C), R_s was 20% lower than in Bolzano (15°C), being 2.5 and 3.1 kg CO_2 m^{-2} yr^{-1}, respectively. The main differences between the two orchards are their soil texture and soil C-concentration. The soil in the experiment 1 was silty clay loam with 1.05% organic C, while that in exp. 3 had a loamy texture with 1.43% organic C. Soil texture certainly affects soil aeration and water drainage, being both lower in soils with higher clay content. This might have adversely influenced the total R_s in Bologna, which has a higher soil clay content compared to Bolzano. On the other hand, also soil organic C concentration might have an important influence on R_s, especially regarding the heterotrophic component of soil respiration (R_h). This is because soil organic C is the main substrate for microbial decomposition processes, an important source for soil CO_2 efflux. Both these factors might have contributed to the slower R_s in the case of Bologna, even though soil temperature was higher.

The contribution of autotrophic respiration (R_a) to soil respiration, although measured with different techniques and in different period of the year, was comparable in the two experiments in the apple orchards, and equal to 35% in experiment 1 (Bologna) and

37% in experiment 3 (Bolzano), a fact indicating that heterotrophic soil respiration is mainly responsible for soil CO_2 flux from soil to the atmosphere.

In general both techniques to separate the two components of soil respiration (the root regression technique in Bologna and the trenching technique in Bolzano) have their strengths, but also some major limitations. The advantage of the root regression technique is that it allows to estimate R_h without altering soil conditions. However, this technique provided acceptable results only when soil parameters, like temperature and moisture, were homogeneous among positions where soil respiration was measured. If this was not the case, the contribution of R_a to R_s was hidden by the influence that soil temperature has on R_s, both on a spatial and temporal scale. The trenching technique allowed to estimate the two components of R_s over the whole period of the experiment. However, since R_a is calculated through the subtraction of R_h (obtained from trenched parcels) from R_s (non trenched parcels), its estimate depends on the correct measurement of R_h. If R_h is measured under conditions that are altered in terms of soil organic matter and soil moisture, the assumption for correct interpretation of results is not always matched. This could be an issue when R_h is measured, as it was in my experiment, in relation to different N availability in soil. Heterotrophic respiration depends on soil availability of the microbial substrates, that is of soil organic matter and of root litter generated from root turnover. In the trenched plots the supply of fresh organic substance is suppressed by the root exclusion, and soil moisture and the soil nutrient availability are altered because of the absence of water and nutrient uptake by roots. In these conditions heterotrophic microbial respiration is probably biased.

In all my experiments I tackled the effects of soil N on total soil, root, autotrophic (root + rhizospheric respiration) and heterotrophic respiration. In experiment 1, soil N levels were only slightly different and a small effect of the history of N supply on soil respiration was detected only in the December measurement. In experiment 2, roots with markedly differences in root N concentrations showed similar root respiration, indicating that the specific root respiration rate (per unit weight of root) is unaffected by the N supply. In experiment 3 I found a significant decrease of R_h but not of R_s, as a result of generous N supply, a fact suggesting that autotrophic soil respiration can be enhanced in N fertilized plots. In summary, root respiration was unaffected by N, while indirect evidences indicate

that autotrophic soil respiration can increase as a result of soil N supply. A possible explanation of the different results of exp. 2 and 3, in addition to the fact that they refer to different plant species, might depend on the positive effect of N supply 1) on the formation of new roots, and therefore on root density and/or 2) on root turnover. If root life span decreases and roots have a higher root turnover as a consequence of N supply, then the contribution of new roots on the whole root systems increases and this, as shown in experiment 2, has markedly enhancing effects on root respiration. It is here worth to mention that I performed an additional experiment in the same site of experiment 3 (not included in my Doctoral Thesis due to the fact that not all analysis are ready) in which I investigated by rhizotrones and root images taken by root scanner the effects of N supply on root nativity and mortality.

The outcomes of my studies also suggest that more investigation has to be done in order to assess the contribution of root and microbial respiration to total soil respiration, possibly using stable isotopes techniques.

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