

# Belowground interactions for water between trees and grasses in a temperate semiarid agroforestry system

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Received: 28 March 2007 / Accepted: 14 February 2008  
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**Abstract** A fundamental hypothesis of agroforestry is the complementary use of soil resources. However, productivity of many agroforestry systems has been lower than expected due to net competition for water, highlighting the need for a mechanistic understanding of belowground interactions. The goal of this study was to examine root–root interactions for water in a temperate semiarid agroforestry system, based on ponderosa pines and a Patagonian grass. The hypotheses were: (a) A greater proportion of water uptake by pines is from deeper soil layers when they are growing with grasses than when they are growing alone; (b) Growth of grasses is improved by the use of water hydraulically lifted by pines. We used stable isotopes of O to analyze water sources of plants, and we measured sapflow direction in pine roots and continuous soil water content with a very sensitive system. We also installed barriers to isolate the roots of a set of grasses from pine roots, in which we measured water status, relative growth and water

sources, comparing to control plants. The results indicated that pines and grasses show some complementary in the use of soil water, and that pines in agroforestry systems use less shallow water than pines in monoculture. We found evidence of hydraulic lift, but contradicting results were obtained comparing growth and isotope results of the root isolation experiment. Therefore, we could not reject nor accept that grasses use water that is hydraulically lifted by the pines, or that this results in a positive effect on grass growth. This information may contribute to understand the complex and variable belowground interactions in temperate agroforestry.

**Keywords** Complementarity · Hydraulic lift · Patagonia · Ponderosa pine · Stable isotopes

## Introduction

A fundamental hypothesis of agroforestry is that the root systems of different plant life forms, such as trees, herbaceous crops or grasses, occupy to some extent different soil strata when grown in association, leading to complementary use of soil resources (Schroth 1999). This was a general assumption in early agroforestry studies and production systems derived from them; however, productivity of many of these systems has been lower than expected (Kho 2000), particularly in semiarid conditions. In this sense, some studies (e.g. Singh et al. 1989, Rao et al.

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1998) have highlighted the limitations of different agroforestry systems in semiarid tropics, where belowground competition for water between trees and crops frequently outweighed the potential positive tree effects on soil and microclimate. Ong et al. (1991) stated that competition for water is the main problem of alley cropping in the semiarid tropics. In the same way, Jose et al. (2004) cited several studies in which competition for water resources markedly decreased crop yields in temperate regions. From these studies it is clear that research attention should focus on theories that could provide a predictive understanding of agroforestry (Kho 2000), with emphasis in belowground interactions for resources (Rao et al. 1998).

Cannell et al. (1996) proposed that biophysical yield advantages can only be expected in agroforestry systems if the trees can acquire resources that the crop would not otherwise acquire. This hypothesis accentuates the crucial role of resource capture in understanding agroforestry performance (Kho 2000). The belowground interactions between trees and understory plants can be *facilitative* through carbon enrichment, interception of leaching nutrients, soil physical improvements and/or supply of hydraulically lifted water; *complementary* if deep rooted trees may use resources which are not accessible to the crops, increasing resource use by the association as a whole; and *competitive*, if trees use limiting resources from the same pool as understory plants. Negative effects through allelopathy may also be observed. It is important to note that even when some yield depressions may exist as a result of competition, the system may still be complementary if the association as a whole captures more resources than the monoculture controls (Ong and Leakey 1999). Another possibility is that trees and understory plants use the same pool of resources, but that pool is abundant enough that neither species is negatively affected by the resource extraction of the other. As was proposed by Kho (2000), when a resource is not limiting in a particular environment, the effect of the trees on its availability (negative or even positive) has no impact on the performance of the system. Because the inherent conflict between different and simultaneous effects of root systems in agroforestry, the definition of desirable root characteristics is a complex task which requires a detailed understanding of root–soil and root–root interactions (Schroth 1999).

Some studies (e.g. Rao et al. 1993; Jonsson et al. 1988) have shown that in semiarid environments there is a considerable overlap between roots of trees and crops, especially in the top 0.5 m of soil, implying that trees will be competing with crops for both water and nutrients. However, the extraction of water and nutrients is not necessarily proportional to the abundance of roots within a soil stratum. Thus, it is important to ask, whether there can be spatial complementarity in water use when there is such a considerable overlap of the two rooting systems (Ong and Leakey 1999). Interestingly, some studies have shown that highly competitive crops (*Sorghum bicolor*, *Zea mays*) may induce the downward displacement of part of the tree root system (Lehmann et al. 1998; Huxley et al. 1994), decreasing competition for upper water resources and increasing the complementarity in the use of resources available in the whole soil profile.

Another form of facilitation can occur in some plant associations through the supply of hydraulically lifted water from deep sources by trees, that then becomes available to understory vegetation. Hydraulic lift is the process of water movement from relatively wet to dry soil layers through plant roots (Richards and Caldwell 1987). Some studies have demonstrated that in some cases the hydraulically lifted water may be used not only by the lifter tree (or other plant type), but also by associated plants (e.g. Dawson 1993; Ludwig et al. 2003). In general, this type of study has been carried out in natural plant associations and may be used to explain the coexistence of different plant life forms. In the same way, this phenomenon may occur in artificial plant associations such as agroforestry systems, reducing competitive effects of trees on crops or pastures in the upper soil layers. It has been suggested that increases in moisture in dry soil layers that result from hydraulic lift may benefit neighbouring plants in semiarid agroforestry systems during dry periods (Hirota et al. 2004). However, the occurrence of this process and its consequences is poorly documented in agroforestry systems. To our knowledge, there is no study describing the use of hydraulically lifted water by crops–pastures in agroforestry systems, except that of Hirota et al. (2004) in which the authors described a new experimental system to test the influence of hydraulic lift on crops associated with trees. Because the methodological approach of the paper, the results

of the study are limited (they had no replications) but they suggested that hydraulically lifted water by a *Markhamia lutea* (Benth.) Schumann tree was responsible of the viability of upland rice seedlings growing in association with it during a drought period.

Northwestern Patagonia has a Mediterranean climate, with cold, wet winters and hot, dry summers. The traditional economic activity is sheep or cattle raising using natural grasslands. The introduction of exotic, deep-rooting trees in these grasslands, in which deep soil water is underutilized (Gyenge et al. 2002a), could constitute a promising economic activity; the ecological interactions between native grasses and exotic trees would also differ from their interactions with native trees. The most commonly planted tree species in the region is the exotic *Pinus ponderosa* Doug. ex Laws, a North American conifer adapted to summer-dry conditions (Maherali et al. 2002). In Patagonia it is generally planted in very high densities, which is incompatible with grass growth in the understory. Recent studies have examined the conditions required for the development of agroforestry systems based on this species and native forage grasses such as *Festuca pallescens* (St. Ives) Parodi (Fernández et al. 2004, 2006a, b and 2007). These studies showed neutral to positive net tree effects on grass growth in humid as well in drought growing seasons. Laclau (2003) found that most of biomass of ponderosa pine rooting systems in Patagonia is in the upper 50 cm of soil. However, pines also develop some deep roots, as was observed in Patagonian plantations (more than 4.5 m deep, Gyenge 2005) as well in North American forests (more than 2 m in porous soils, and in soils underlying by rock with deep fissures, ponderosa pine roots have been observed at depths of 11 to 12 m, Oliver and Ryker 1990). Most grass root systems are in the upper 30 cm of soils in Patagonia (Soriano et al. 1987), as well as in other parts of the world. This information suggests an important potential overlap in rooting systems of ponderosa pine when grown together with grasses, leading to potential competitive interactions for water resources. But at the same time, the deeper roots of ponderosa pines may lead to complementarity and facilitative effects through the supply of hydraulically lifted water to the grasses. All these interactions may occur simultaneously (Holmgren et al. 1997), leading to net positive, negative or

neutral balances in different periods within the rotation time of the tree plantation, in growing seasons with different climatic conditions, and even within a growing season, in periods differing in the availability of soil water resources. At the time scale of a whole growing season, ponderosa pine/grass agroforestry systems may produce more biomass than grasslands and than pines growing in monocultures, depending on stand age (Gyenge 2005; Gyenge et al. in review), suggesting that they could be an example of net complementarity in the use of water, the most limiting resource in the region.

Based on this background, the goal of this study was to examine belowground interactions for water resources between ponderosa pines and grasses growing in agroforestry systems in semiarid Patagonia. We tested two specific hypotheses: (a) A greater proportion of water uptake by pines is from deeper soil layers when they are growing with grasses than when they are growing alone; and (b) Growth of grasses in AF is improved by the use of water that is hydraulically lifted by pines. This information will contribute to improve our understanding of belowground interactions in semiarid temperate agroforestry systems.

## Material and methods

The study was carried out in agroforestry (AF) plots installed in Estancia Lemú Cuyén (40.3° S, 71.1° W), located within Lanín National Park, Patagonia, Argentina. The climate is of Mediterranean type with wet, cold winters, and dry, hot summers. Average annual rainfall (period 1978–1999) is  $684 \pm 283$  mm (with ca. 579 mm in fall-winter and 105 mm in spring–summer). Maximum and minimum annual average temperatures are  $17.1^\circ\text{C} \pm 0.5$  and  $4^\circ\text{C} \pm 2.1$ , respectively.

The study took place in an existing, 20-year-old agroforestry trial that involved plots with two densities of ponderosa pine trees (350 and 500 trees  $\text{ha}^{-1}$ , 1,600  $\text{m}^2$  per plot) and an open grassland area. However, for the purposes of this paper, soil and plant measurements were carried out only in the plots with 350 trees  $\text{ha}^{-1}$  (hereafter referred to as AF plots). For more details about the trial see Fernández et al. (2004, 2006a). In addition, some measurements (see below) were carried out in a dense pine

plantation (pine monoculture, 1,100 pines ha<sup>-1</sup>) of the same age of the AF plots, located approximately 100 m away from them. At the time of the measurements, the trees of AF plots had 31.3 (1.4) cm of diameter at breast height (dbh) and 11.5 (0.3) m of height. The understory of the AF plots was dominated by *Festuca pallescens*. Other species presented in relatively high cover were *Stipa speciosa*, *Mulinum spinosum* and *Acaena splendens*.

#### Measurements to determine the extent of competition-complementarity in water use

In order to determine the extent of competition-complementarity in water use between trees (*P. ponderosa*) and grasses (*F. pallescens*), we studied water sources of both species in different periods within the growing season: spring (November 2004), summer (February 2005) and autumn (March 2004). Soil water content in those periods typically differs, with high amount of water in spring, very low values in summer (dry period), with a little increase in upper soil layers after the first rains fallen in autumn (Gyenge et al. 2002; Fernández et al. 2006a). Variation in the oxygen and hydrogen stable isotope composition of water sources has been extensively used to determine the zone of active water uptake by plant roots (Dawson et al. 2002). In addition, this technique has been used to look at water uptake by understory plants growing near trees that conduct hydraulic lift (e.g. Dawson 1993; Ludwig et al. 2003). Therefore, we collected soil samples at different depths (potential water sources), and plant samples. Soil samples were taken with an auger from the surface to 180 cm, at the following intervals: 10, 20, 40, 80, 140 and 180 cm, in three or four points within the AF plots and the dense pine plantation. Around each soil sampling point, twigs of the nearest three pines were collected, and around each pine, the basal non-green portion of the tillers of the nearest three grasses were also collected (only in AF plots because in dense plantations there were no plants in the understory). All precautions in sample collection and storing were taken as recommended in Brooks and Gregg (2001). The samples were kept frozen (-20°C) until water was extracted. This process was carried out using a cryogenic vacuum distillation apparatus installed and operated in the Soil Laboratory of INTA EEA Bariloche, following procedures

described in Shimabuku (2001). Water extracted from soil and plant xylem was analysed for stable isotope composition by the Center for Stable Isotope Biogeochemistry in Berkeley, CA. In some samples, ratios of both <sup>18</sup>O/<sup>16</sup>O and D/H were determined, but no additional information was provided with two elements (they were highly correlated, Spearman correlation coefficient: 0.97 and 0.98 for data of the pine monoculture and the AF plots, respectively). Therefore, we measured only the <sup>18</sup>O/<sup>16</sup>O ratio ( $\delta^{18}\text{O}$ ) in later samples. Values of  $\delta^{18}\text{O}$  are expressed in delta notation (‰) relative to the international standard, V-SMOW (Vienna standard mean ocean water):

$$\delta^{18}\text{O} = \left[ \left( \frac{{}^{18}\text{O}/{}^{16}\text{O}_{\text{sample}}}{{}^{18}\text{O}/{}^{16}\text{O}_{\text{standard}}} \right) - 1 \right] \times 1000.$$

To quantitatively determine the amount of water used by plants from the different sources, water source mixing models can be applied (e.g. Dawson 1993). Because only two different sources could be separated based on isotope results in our study (see below), we used a simple two end-member model, considering the water in the upper soil layer (0–20 cm) and the average of deeper soil layers (40, 80, 140 and 180 cm). We applied the same analysis considering as the upper soil layers the average of 0–20 and 20–40 cm of soil depth. Briefly, the  $\delta^{18}\text{O}$  value of a mixture of different sources (water in plant xylem) is:

$$\delta_m = \delta_A p_A + \delta_B p_B$$

$$p_A + p_B = 1$$

where  $\delta_m$ ,  $\delta_A$ , and  $\delta_B$  are delta values in the mixture, in water source A and water source B, respectively; and  $p_A$  and  $p_B$  are the proportions of sources A or B in the mixture. With the above two equations it can be easily derived any of both proportions. Therefore,

$$p_A = (\delta_m - \delta_B) / (\delta_A - \delta_B).$$

#### Measurements to detect hydraulic lift and use of hydraulically lifted water by grasses

To determine whether hydraulic lift occurs in agroforestry system plots, two methodological approaches were applied. Firstly, four multiple-sensor, frequency domain capacitance probes (Sentek EnviroSMART, Sentek Pty Ltd., Adelaide, Australia) were used to

continuously monitor volumetric soil water content; data were recorded every 15 s and 30 min averages were stored in a CR10X datalogger (Campbell Scientific, Logan, UT, USA). The Sentek measurement system employs long probes which are inserted into 5.5 cm diameter PVC casings; the casings, in turn, are installed in holes augured into the soil. The probes were 2 m long, with six sensors per probe at 10, 20, 40, 80, 140, and 180 cm depths. This system is able to detect very small changes in soil water content, and thus if hydraulic lift occurs, the daily patterns of soil water variation will show an increase in soil water content at night. Additionally, we installed in some pine roots a modified version of sapflow sensors based on Granier's heat dissipation method (Granier 1985). This technique consists of one central heat source (electrical resistance) and two pairs of thermocouples, one located at each side of the resistance. Each of these pairs has a reference sensor located distal to the heat source (for more detail of the system, see Fernández (2003) and Gyenge et al. (2002b)). This system is able to detect the direction of water flux inside the roots, and thus it is possible to determine if there are reverse fluxes (from the base to the tip of the root). Direction of sapflow in roots was measured in three pines of the AF plots. Within each of these trees, two different types of roots were measured: one relatively fine and horizontal root (<3 cm of diameter, approximately 15 cm of soil depth), and one very thick and vertical root (more than 10 cm of diameter). Data were recorded during February 2005 (very low values of soil water), and November and December 2005 (high soil water content).

To determine whether grasses growing near the pines in agroforestry systems are able to use hydraulically lifted water and the effects of this on their water status and growth, a manipulative approach was used. The roots of five grass tussocks growing near (less than 1.5 m away) different pines were isolated from the roots of the trees with barriers (stainless sheets of metal) installed inside the soil around each grass tussock. The diameter of the barriers was 50 cm and the depth was 60 cm. Another five grass tussocks growing in similar positions respect to other pines were selected as control plants, in which no barriers were installed to separate plant roots. Pre-dawn leaf water potential and soil water content in the upper 20 cm were

measured monthly in the ten grasses and their surrounding soil, from September 2004 to May 2005 (a dry growing season), and from October 2005 to April 2006 (a wet growing season). Leaf water potential was measured with a pressure chamber (PMS Instruments Co., Corvallis, USA). Soil water content was determined with a TDR equipment (T3 probe, Imko GmbH, Germany), as the average of measures in two points as near as possible to the base of each tussock.

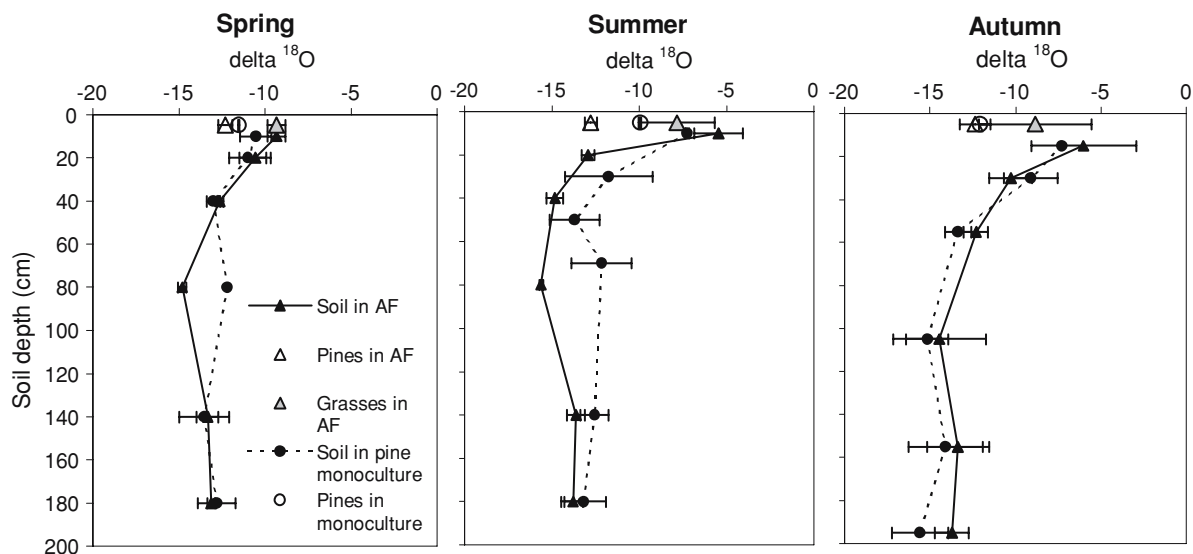
Relative growth of each plant was measured as described in Fernández et al. (2006a), measuring the difference between two dates (beginning and end of the growth period) in the number of tillers, number of leaves per tiller and length of the leaves, in a subset of marked tillers per plant. A relative growth index was estimated in a multiplicative form considering the mentioned variables.

We expected hydraulic lift to be most likely in January of 2005 due to very low water content in the upper soil layers. Thus, at that time we collected soil (0–20 and 20–40 cm depth) and plant samples (base of the tillers of the ten grasses, and twigs of the three pines closest to each grass) and water was cryogenically extracted (as above) to determine the ratio of  $^{18}\text{O}/^{16}\text{O}$ . If grasses are able to use hydraulically lifted water, we expected similar  $\delta^{18}\text{O}$  values in grass and pine samples in the control situations (without barriers), and different values in those cases in which roots were isolated.

## Results

### Extent of competition-complementarity in water use

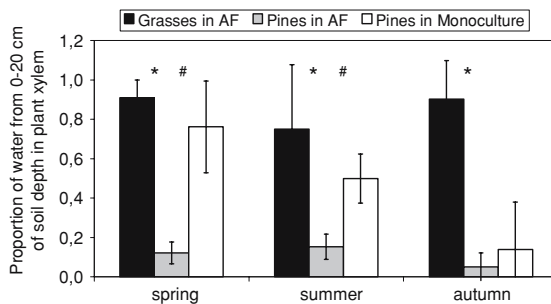
A significant difference in isotope signal (more enriched in heavy isotopes of oxygen and hydrogen) was observed between upper soil layers (from 5 to approximately 30 cm), and those below that depth (until approximately 2 m) (Fig. 1 shows average  $\delta^{18}\text{O}$  values). The same pattern of isotopes in the soil profile was observed in all studied periods within the growing season (spring, summer and autumn). Significant differences were observed between  $\delta^{18}\text{O}$  of grasses and AF pines in spring and summer (*t*-tests,  $P < 0.05$ ). Although this trend was also evident in autumn, the differences were not statistically



**Fig. 1** Ratio of  $^{18}\text{O}/^{16}\text{O}$  ( $\delta^{18}\text{O}$ , average and standard deviation) observed in xylem water of pines and grasses, and in soil water of AF plots and pine monoculture ( $n = 3\text{--}4$ )

significant due to a higher variation in samples from grasses (Fig. 1). Values of  $\delta^{18}\text{O}$  in xylem water of pines growing in monoculture and their potential water sources are also shown in Fig. 1. In this system the soil profile had a similar pattern than in the AF plots, characterized by a high uniformity of soil water  $\delta^{18}\text{O}$  below 40 cm of soil depth.

In order to quantify the use of different water sources, a simple two end-member mixing model was applied to the upper soil layer (0–20 cm) and deeper soil layers (>20 cm). The same analysis was also applied using discreet values from 20 to 40 cm of soil depth. Very similar results were obtained with both models. For this reason, we present only results of the first model in Fig. 2. The degree of potential competition between pines and grasses growing together in the AF plots can be estimated as the proportion of water used by both species from the same water source. In spring and autumn, grasses extracted approximately 90% of the water from the upper soil layer, whereas in summer, when soil water content is very low, this proportion decreased to 75% (Fig. 2). In contrast, less than 20% of water used by pines of AF systems came from the upper soil layer. However, pines growing in monoculture used proportionally more water from the upper layer (Fig. 2). In the pine monoculture, about 75% of the water used in spring came from the shallow source, falling to about 18% in autumn, with an intermediate value in summer.

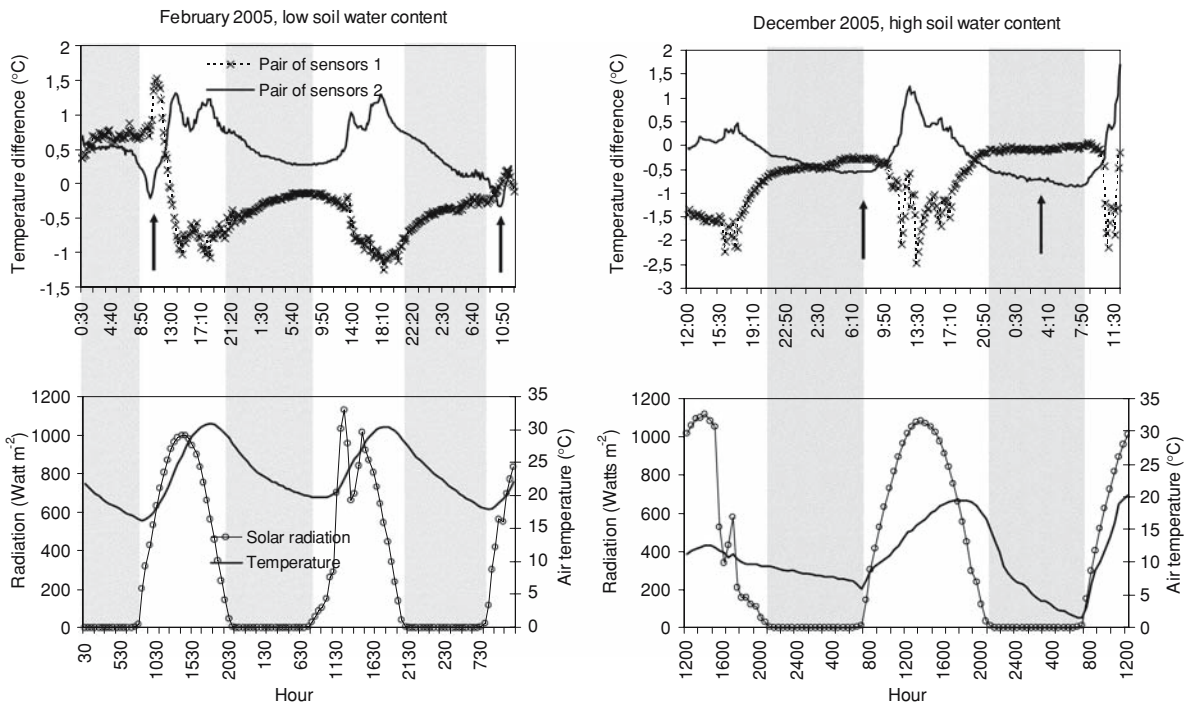


**Fig. 2** Proportion of water extracted from the upper 20 cm of soil by grasses and pines growing in the AF plots, and by pines growing in monoculture ( $n = 3\text{--}4$ ) estimated with a simple two end-member mixing model. Asterisks indicate significant differences between grasses and pines in AF within each period ( $t$ -test,  $P < 0.05$ ). Numerals indicate significant differences between pines of the AF and pines in monoculture within each period ( $t$ -test,  $P < 0.05$ )

Significant differences in the proportions of use of shallow water were observed between the pines growing in both systems in spring and summer, but not in autumn when trees of both systems use a high amount of water from deeper layers.

Determination of hydraulic lift and use of hydraulically lifted water by grasses

Two different sources of evidence suggest that hydraulic lift occurs in pines growing in AF plots.



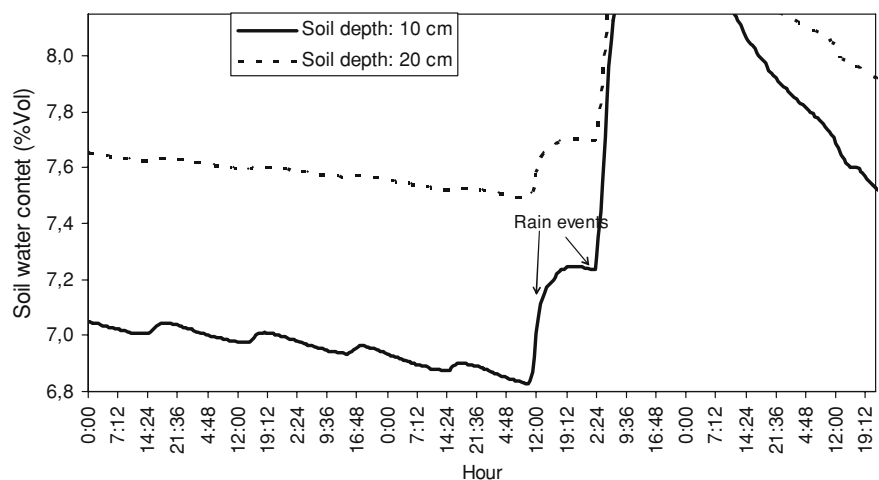
**Fig. 3** Upper panel: Temperature difference ( $\Delta T$ ) between pairs of sensors installed in one ponderosa pine root in February and December 2005. Lower panel: solar radiation and air temperature in the same dates as in upper panel. Reverse

fluxes (indicated with arrows) occurred when  $\Delta T$  in the pair of sensors 1 increases towards a positive difference and at the same time,  $\Delta T$  in the pair of sensors 2 increases towards a negative difference

Some roots showed reverse fluxes during some nights (Fig. 3). These reverse fluxes were detected not only during a dry period with very low soil water content (February 2005, soil water content: 6–8 Vol% in the top 40 cm of soil), but also during periods with a higher amount of soil water (November and December 2005, soil water: 16–30 Vol% in the top 40 cm of

soil). Other roots did not show reverse fluxes at all, suggesting that the process is not uniform through the whole rooting system, perhaps because of heterogeneity in soil water content with soil strata. Similarly, soil moisture measurements from the Sentek system shown daily patterns of increases and decreases in soil water content (Fig. 4 with an example of some

**Fig. 4** Average of soil water content ( $n = 4$ ) measured with the Sentek system installed in the AF plots, showing daily little increases (Hydraulic lift?) and a net decrease until a rain event. Period of measurement: February 3–11, 2005



days in February 2005), suggesting that hydraulic lift is occurring in some situations. These daily variations in soil water were observed at the different soil depths, but the magnitude of the increases in soil water was higher in the top soil layer (10 cm), and when soil water content was below 7.5 Vol%. Figure 4 shows the average of the four measurement points. Considering each sensor separately, the maximum daily variation observed in soil water content was 0.11 Vol%, but in general the variation was around 0.07 Vol% (data not shown). However, results of the upper soil layer have to be interpreted with caution as an evidence of hydraulic lift because soil water increased during daylight hours and decreased at night (Fig. 4), the opposite of what is expected from hydraulic lift.

Soil water content and plant water status of the grasses growing with and without root-isolating barriers are shown in Figs. 5 (season 2004–2005) and 6 (season 2005–2006). In both seasons, soil water content was slightly higher around the control plants (significant differences were detected only in the spring 2004, and in one date in December 2005, *t*-test,  $P < 0.05$ ). However, plant water status (estimated with pre-dawn leaf water potential) was similar in both treatments during both growing seasons. The observed higher soil water content in some days in the control situation had no effect on pre-dawn water potential of *F. pallescens* because this variable markedly decreased only when soil water content was below 7–10 Vol% (data not shown).

Water extracted from grasses, both with and without barriers, was more enriched in  $\delta^{18}\text{O}$  than water from pines (ANOVA, LSD tests,  $P < 0.05$ ).

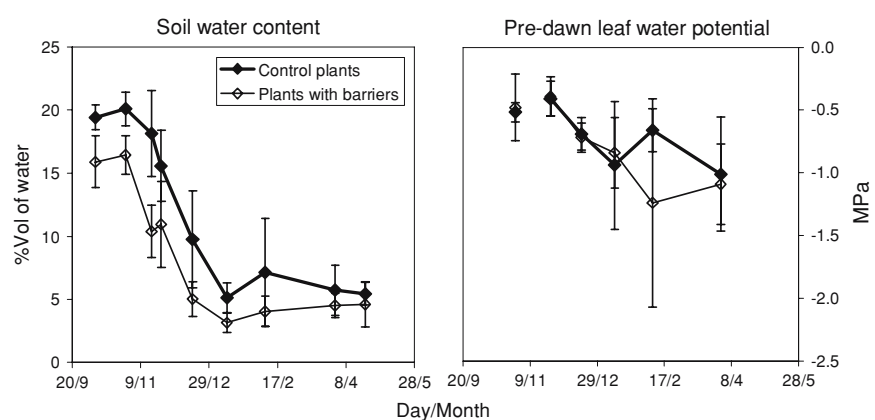
Delta  $^{18}\text{O}$  of xylem water of the grasses was intermediate to that of both 0–20 and 20–40 cm soil layers. The two-end member mixing model indicated that grasses with isolated roots used 60% (SD: 0.34) of water from 0–20 cm, and control grasses used 48% (SD: 0.33) of the same soil depth. Delta  $^{18}\text{O}$  of pine xylem water did not differ from that of 20–40 cm of soil depth. In addition, at this soil depth,  $\delta^{18}\text{O}$  of soil water was similar between the control and the barriers treatment (Fig. 7). In contrast, soil water at 0–20 cm in the control situation was more enriched in the heavy isotope than in the treatment with barriers.

In the first very dry growing season, the relative growth of grasses growing inside the barriers was significantly less than in the control situation (Fig. 8). Actually, those plants presented a net decrease in biomass (mainly due to tiller mortality, data not shown) when the beginning and the end of the growing season are compared (relative growth index  $< 1$ ), whereas control plants had positive but very low growth values. In contrast, in the second growing season, which was characterized by a high soil water content during both spring and summer (Fig. 6), relative growth did not differ between treatments, with a trend of a higher growth in the barrier treatment.

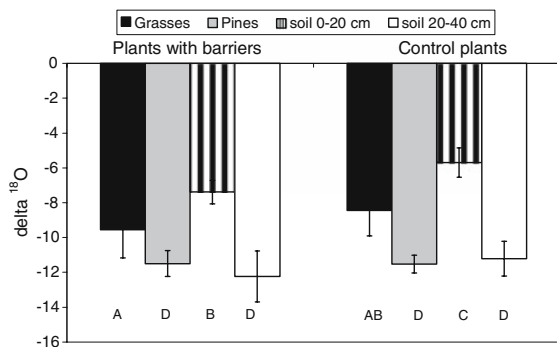
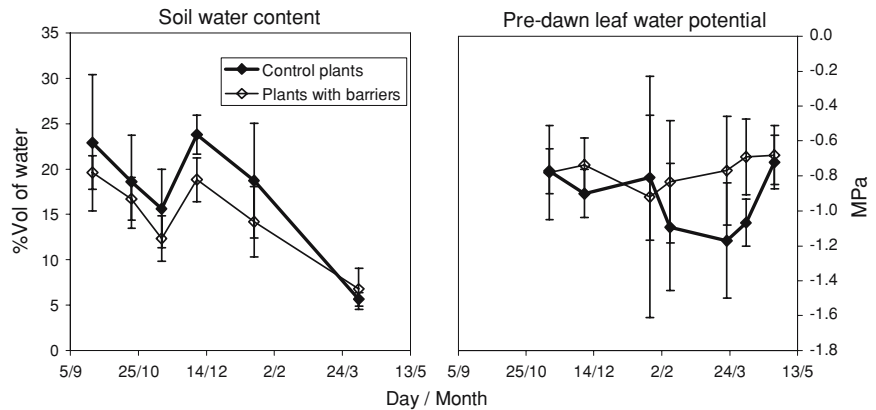
## Discussion

To examine belowground interactions between trees and grasses in agroforestry systems, we tested two hypotheses: (a) A greater proportion of water uptake by pines is from deeper soil layers when they are

**Fig. 5** Average and standard deviation of soil water content (left panel) and pre-dawn leaf water potential (right panel) measured in *Festuca pallescens* plants growing with and without (control) barriers around their roots. Growing season 2004–2005 (dry summer and autumn)  $n = 5$



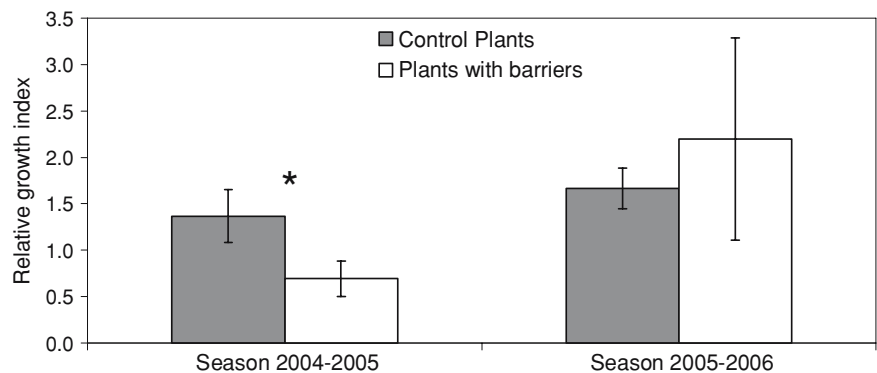
**Fig. 6** Average and standard deviation of soil water content (left panel) and pre-dawn leaf water potential (right panel) measured in *Festuca pallescens* plants growing with and without (control) barriers around their roots. Growing season 2005–2006 (wet summer and dry autumn)  $n = 5$



**Fig. 7** Ratio of  $^{18}\text{O}/^{16}\text{O}$  (delta  $^{18}\text{O}$ , mean and standard deviation) presented in grass and pine xylem water, and in upper soil water (two depths: 0–20 and 20–40 cm) in the situations with and without (control) barriers around grass roots.  $n = 5$ . Sampling carried out in January 2005. Different letters indicate significant differences (ANOVA, LSD tests for post-hoc comparisons,  $P < 0.05$ )

growing with grasses than when they are growing alone; and (b) Growth of grasses in AF is improved by the use of water that is hydraulically lifted by pines.

**Fig. 8** Relative growth of grasses growing in the situations with and without (control) barriers around their roots  $n = 5$ . Significant differences were only detected in the first growing season (non-parametric comparison between two samples applied to data of each growing season, Kolmogorov-Smirnov test,  $P < 0.05$ )



The first hypothesis is based on results of other authors, who found situations in which very competitive crops induce changes in tree root development. In our study, results from measurements of isotopes suggest that ponderosa pines in AF systems use less shallow water resources than pines grown in monoculture. In AF, 80–100% of water used by the grasses came from the upper 20 cm of soil, whereas the pines growing in those systems extracted more than 80% of their water from deeper soil layers during the whole growing season (Fig. 2). In contrast, trees in monoculture extracted approximately 70% of their water from the upper 20 cm of soil during spring. In summer, the proportion of water derived from shallow sources decreased to about 50%, and in autumn, pines in monoculture decreased the use of shallow water to 15% mainly due to the very low soil water content before fall rains. Our results are similar to those found by Lehmann et al. (1998), who studied an agroforestry system with *Acacia saligna* and *Sorghum bicolor* in Kenya. They found that when intercropping trees and crops, the tree root system

expanded more into the subsoil and contracted below the tree canopy than in sole tree systems. Huxley et al. (1994) also found a deeper root penetration of trees influenced by an annual intercrop. Both of these studies were based on direct measurements of root systems. Those authors suggested that when grown in association, trees may invest more into the subsoil root zone to avoid competition for water and nutrients with the aggressive root system of the crop.

Our results suggest that there exists an important degree of complementarity in the use of soil water resources in the AF, and that ponderosa pines acclimate somewhat flexibly to the presence of herbaceous root systems. At the same time, approximately 20% of the water used by pines in AF systems came from the upper 20 cm of soil, indicating a certain degree of sharing of resources with grasses, possibly leading to some level of competitive effects of the trees on the grasses. However, if soil water resources are abundant, no negative effects of the trees might be expected. In this sense, Kho (2000) stated that the positive or negative effects of trees on resource availability will have any effect on crops depending on the balance of resources in the particular environment, i.e. in the relative limitation of each resource. Fernández et al. (2007) also highlighted the importance of the physiology of the crop-pasture species, because within any particular environment a certain amount of resource may be more or less limiting to its development. In the case of the AF trial examined in the current study, previous results shown that pre-dawn water potential of *F. palleescens* was more negative under pines than in the open grassland only during a very dry summer (Fernández et al. 2007), which was similar in climatic conditions to the present study. These results indicate that the estimated degree of overlapping between tree-grass water sources are likely to be negative to the grasses only during periods of very low soil water content combined with high atmospheric demand.

A complementary study measuring soil water depletion and sapflow of pines in AF and in monoculture also indicates that a high proportion of water is extracted from upper soil layers in the monoculture during periods with high soil water content, and that this proportion decreases when soil water decreases (Licata et al. 2007). However, soil water depletion suggests that in spring the

proportion of shallow soil water used by pines in monoculture is lower than that estimated with the isotope mixing model, but estimations from both studies are very similar in summer and autumn. Conclusions based on the application of mixing models must be taken with caution because we did not find a marked differentiation in the isotope signal along the soil profile. A similar lack of differentiation in the soil profile, particularly below a relatively shallow soil depth, has been reported by several authors in other systems (e.g. Le Roux et al. 1995; Jackson et al. 1995), including an agroforestry system with *Eucalyptus* spp. (Burgess et al. 2000). In that case, authors suggested that the uniformity in the soil profile could be the result of the contribution of large winter rain events, but also of mixing of water from different sources by tree roots redistribution. In our system both possibilities could also apply.

The second hypothesis tested in this study was related to the potential facilitative effects of trees on grasses through the supply of hydraulically lifted water. Some studies have demonstrated or at least suggested that hydraulically lifted water by trees may be used by other species growing near the trees. One pioneer study was that of Caldwell and Richards (1989), who demonstrated that the water enriched in heavy isotope put in the tips of *Artemisia tridentata* shrubs appears in the xylem of herbaceous plants growing with them. Dawson (1993) demonstrated that some species (but not others) growing in the understory of open grown sugar maple (*Acer saccharum*) use water that is hydraulically lifted by the tree, and this use was correlated with a better water status and higher stomatal conductance and relative growth of those plants. Ludwig et al. (2003) detected hydraulic lift in *Acacia tortillis* growing on an East African savanna. They concluded that grasses growing near the trees use hydraulically lifted water (the grasses had  $\delta^{18}\text{O}$  values similar to those of groundwater and trees). However, in contrast to Dawson's results, lower soil water content under the canopies resulted in more negative pre-dawn water potential of those plants without impact on grass production. Their results suggest that in savannas both facilitation via hydraulic lift and competition are concurrent processes, and the importance of each process may depend upon how wet or dry that particular site or year is. In our study, to test whether hydraulically

lifted water is used by grasses, we first wanted to know whether hydraulic lift was actually occurring. Data from the sap flux sensors installed in the roots were variable, but it appears that some roots have reverse fluxes during some days. Surprisingly, when we found reverse fluxes, these occurred both in periods with very low soil water content (February 2005) and also with high soil water availability (December 2005). A similar pattern of hydraulic lift during periods with relatively high soil water content was observed by Ludwig et al. (2003), who did not detect hydraulic lift when the soil was extremely dry. In our study, some roots showed reverse fluxes during both measurement periods, but others did not on either of the measurement dates. These results suggest that hydraulic lift is a complex phenomenon that may occur in one part of the root system but not in other at the same soil depth, in some trees but not in others. This complexity makes hydraulic lift detection and quantification difficult. Considering the results of the Sentek system, they also suggested that hydraulic lift is occurring. However, some odd patterns were found in the upper soil layer. This offset pattern of hydraulic lift in soil sensors was also found by other authors (Millikin et al. 2000; Espeleta et al. 2004). Millikin et al. (2000) suggested that it could have been due to an artifact of overcorrection for changes in soil temperature. In our case, soil sensors were not compensated by soil temperature, and the temperature diel pattern was different than the soil water content pattern observed during hydraulic lift (data not shown). Espeleta et al. (2004) concluded in their study that their offset pattern of hydraulic lift was likely due to early afternoon stomatal closure, which would cause an increase in water potential in the plant. In our study, soil water content in the upper soil layer began to increase while sapflow was still at maximal daily values (data not shown). We speculate that the offset wave of hydraulic lift we observed in the uppermost soil layer was due to evaporation of water from the soil, which decreased soil water potential below that of the water potential of roots near the soil surface, causing a release of water from roots. In this case, we can not know if the water was taken from a deeper soil layer, resulting in a case of hydraulic lift, or if the water was taken from the same soil layer in which it is later released.

Similarly, results of our root-barrier experiment were variable. Significantly higher soil water content occurred in the control situation only during some periods with overall high soil moisture, and not during periods with extreme drought in which hydraulic lift is more expected. As was pointed out above, Ludwig et al. (2003) found that hydraulic lift was present in a relatively wet period and not during a very dry season in an East African savanna. Those authors suggested that this might be the result of no fine root production in very dry soils, a poor soil-root contact reducing the efficacy of root water exudation, root death, and/or the inhibition of hydraulic lift due to root cavitation. In this sense, Jackson et al. (2000) proposed that, under drought conditions, if vulnerability to cavitation of shallow roots is higher than that of other parts of the plant, the smaller roots may operate as a “hydraulic fuse”, localizing the conductance failure to relatively cheap units compared with tap roots, stems and branches. This strategy will result in hydraulic lift inhibition in very dry soils. In the present study, results of sapflow direction measured in roots (Fig. 3) also suggested reverse fluxes during periods with high soil water content, but also in dry periods. However, in general the magnitude of hydraulically lifted water is not big enough to be detected with the TDR method with which we measured soil water content around the grasses. For this reason, it is more probable that the lower amount of water inside the barriers was not due to the inhibition of hydraulic lift, but due to the depletion of water very close to the grasses disabled to explore higher distances horizontally due to the barriers presence. In addition, significant differences between  $\delta^{18}\text{O}$  values of xylem water of grasses and pines in the treatment with barriers as well in the control situation would suggest that grasses are not using hydraulically lifted water by pines (at least in the case of the particular date and individuals we measured).

On the other hand, during the studied dry season (2004–2005), grasses growing with barriers decreased their biomass, whereas those growing in contact with tree roots, had positive growth values. These results would indicate a significant positive effect of tree roots on grasses, particularly during dry seasons, contradicting isotope results. An alternative explanation for the roots barriers decreasing the biomass of grasses within

them under very dry conditions might be that their roots were prevented from exploring a larger volume of soil, rather than the association with tree roots giving a positive effect. In this sense, our results are not conclusive, and we can not accept nor reject with some confidence that hydraulically lifted water by pines is actually used by the grasses and that it has a quantifiable effect on grass performance.

## Conclusion

In conclusion, our results indicate that pines and grasses growing in the studied agroforestry systems in Patagonia show some complementarity in the use of soil water resources. In this regard, a degree of sharing of water in shallow soil strata was also observed; however negative effects of this sharing could only be expected during periods with very low soil water contents. We found evidence that hydraulic lift by the pines occurs in these systems. However, we could not prove that hydraulically lifted water is actually used by understory grasses. From a production point of view, complementarity in the use of water resources by both species is another reason to recommend this particular association of plants, which may lead to a sustainable production system in NW Patagonia. On the other hand, our results highlight the complexity in below-ground interactions in water-limited environments, which may be positive, neutral or negative depending on the availability of resources and the abilities of the interacting species. This complexity demands the use of complex methodological approaches, which not always lead to conclusive interpretations. In this regard, in spite of the fact that the use of stable isotopes may be a powerful tool in water sources and ecological interaction studies, it has limitations in some particular systems, particularly where there is not a clear differentiation in isotope signals along the soil profile.

**Acknowledgements** We gratefully acknowledge Mr R. Von Haniel, the owner of the ranch in which we carried out all the field measurements. This research was funded by the Grant DEB 0316392 from NSF, and the Grant PICT N° 08-12741 from FONCyT-SECyT (Argentina). We also acknowledge personnel of the Soil Laboratory of INTA EEA Bariloche, who allowed us to install the cryogenic vacuum line to extract water from soil and plant samples. Thanks also to M. Weigandt and S. Varela, who did the water extraction of all the samples.

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