Europ. J. Agronomy 84 (2017) 35–46

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Effects of walnut trees on biological nitrogen fixation and yield of intercropped alfalfa in a Mediterranean agroforestry system

Aurélie Querné[a,1], Patricia Battie-laclau[a,1], Lydie Dufour[a], Jacques Wery[b], Christian Dupraz[a,*]

[a] INRA – UMR System, Bâtiment 27, 2 Place Viala, 34060, Montpellier, Cedex 1, France
[b] Supagro – UMR System, Bâtiment 27, 2 Place Viala, 34060, Montpellier, Cedex 1, France

ARTICLE INFO

Article history:
Received 31 December 2015
Received in revised form
30 November 2016
Accepted 1 December 2016

Keywords:
Shade tolerance
Light and water competition
Lucerne
Juglans regia x nigra
Biological N fixation
Light use efficiency

ABSTRACT

While intercropping annual non nitrogen-fixing crops with deciduous hardwood species is now well documented, there is a need to investigate if nitrogen-fixing intercrops may succeed in agroforestry systems. Intercropping with trees usually leads to a decline in crop yield, and could in addition possibly reduce the biological N fixation (BNF) over time due to the competition for resources. In a Mediterranean experimental site, 17 year-old hybrid walnut trees (*Juglans nigra* x *Juglans regia* L.) planted in East-West oriented lines were intercropped with alfalfa (*Medicago sativa* L.) to assess the impact of competition for light and water on alfalfa yield and BNF. Alfalfa yield and shoot δ^{15}N values (a proxy for the proportion of N derived from the air, %Ndfa) were measured during one year at different distances from the tree row in two directions (north and south). Alfalfa yield was reduced close to the tree row (−28% and −22% on the northern and southern sides respectively), but less than the reduction of irradiation (−59% and −33% respectively). Shading improved by 35% the apparent light use efficiency (LUE: aboveground biomass produced per unit of global radiation) of alfalfa, indicating that alfalfa was shade tolerant at this Mediterranean site. Alfalfa shoot δ^{15}N values were lower close to the tree rows than at mid inter-row: BNF was stimulated close to the trees. Compensative and facilitative mechanisms between trees and alfalfa plants led to a rise in LUE and%Ndfa in shaded areas. These results contradict the frequent assumption that N fixation is reduced in the shade of trees. Appropriate tree canopy management may help maintain light competition between trees and alfalfa to a level that still enhance complementary, which would further improve the sustainability of the use of alfalfa as an intercrop in Mediterranean regions.

1. Introduction

Most crop and forest plantations are monocultures often established on soils typified by low nitrogen (N) status, which is the main limiting nutrient for growth in plants (Miller, 1990; Vitousek and Howarth, 1991; LeBauer and Treseder, 2008). The reliance on expensive and environmentally damaging N fertilizers questions the sustainability of monocultures (Bohlool et al., 1992). Innovative systems are needed to satisfy the long-term global increase in the world demand for food (FAO, 2013) and wood products while taking environmental concerns into account (FAO, 2012).

Tree-based intercropping systems display economic and environmental benefits, such as an increase in the land productivity resulting from an increase in biomass production (*i.e.* over yielding) (Dupraz and Liagre, 2008), the reduction in soil and water losses (Thevathasan and Gordon, 2004; Thevathasan et al., 2004; Eichhorn et al., 2006), biodiversity conservation (Quinkenstein et al., 2009) and carbon sequestration (Albrecht and Kandji, 2003). Moreover, the integration of N-fixing crops between hardwood trees can be a strategy to reduce the need for external inputs by enhancing the soil fertility (Herridge et al., 2008). Unfortunately, native high-value timber or fruit producing tree species capable to fix atmospheric N are not common in Europe. Therefore combining leguminous crops with non-N-fixing trees is the best option to improve N nutrition and growth of both trees and crops (Turvey and Smethurst, 1983).

Legumes contribute to supply substantial N amounts to natural and cultivated ecosystems in the long term (Ta and Faris, 1988; Mayer et al., 2003; McNeill and Fillery, 2008). The amounts of N fixed by perennial and forage legumes can be as high as the amounts of N fertilizer commonly used in conventional agriculture (Bohlool et al., 1992; Unkovich et al., 2010). Substantial N quantities become

---

[*] Corresponding author.
E-mail address: christian.dupraz@inra.fr (C. Dupraz).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.eja.2016.12.001

available to non-fixing plants by legume residues decomposition and rhizodeposition (Van Sambeek et al., 1986; McCallum et al., 2000; Khan et al., 2002). Legume cover crops increased tree growth by 28% relative to trees grown in monospecific planted forests in a temperate region, and this was attributed to increased N availability issued from BNF (van Sambeek and Garrett, 2004). Thus, the role of legumes as a N supplier and as a builder of soil organic matter in tree-crops intercropped systems will likely gain importance in the future. The success of agroforestry systems depends on maximizing complementary and minimizing competition between trees and crops (Nair, 1993; Sanchez, 1995; Dupraz and Newman, 1997; Thevathasan and Gordon, 2004). Plants mainly interact by beneficial, neutral or detrimental climatic modifications and by resource addition or depletion (Anderson and Sinclair, 1993; Ong and Huxley, 1996; Jose et al., 2004). Tree canopy and root system architecture directly modify above and below-ground resource availability and climate conditions and consequently impact crop biomass production (Bouttier et al. 2016; Reynolds et al., 2007; Dupraz and Liagre, 2008; Li et al., 2008; Muthuri et al., 2009; Rivest et al., 2009; Dufour et al., 2013; Xu et al., 2013). Over time, negative interactions can appear when trees and crops overlap in their resource use, leading to competition and hence lower productivity as compared to when the components are grown separately (Jose et al., 2004). When trees mature, intercropping usually leads to a decline in crop yields due to higher tree competition for resources (Chamshama et al., 1998; Thevathasan and Gordon, 2004; Reynolds et al., 2007; Dufour et al., 2013).

Successful tree and legume associations depend on effective BNF in the shade of trees and on subsequent N transfer to the trees. Likewise, as the potential for N fixation is directly related to *Rhizobium* survival and to plant growth factors (Wery et al., 1986; Dear et al., 1999; McCallum et al., 2000; Bowman et al., 2002), tree competition for light, water and nutrients over time is likely to limit legume growth and N fixation. Trees may modify other abiotic factors that directly affect the BNF process such as soil temperature (Ta and Faris, 1988; Bordeleau and Prevost, 1994; Aranjuelo et al., 2007) and soil N content (Peoples et al., 1995; Salvagiotti et al., 2008; Unkovich et al., 2010). So far, most studies have only considered tree effects on legume yields and the question remains of the effect of mature trees on the understory BNF. Investigating the tree competitive mechanisms on growth and BNF processes of the legume intercrop is a prerequisite for optimizing resource use complementarity and designing systems that tolerate competition in intercropping (Eichhorn et al., 2006).

Alfalfa (*Medicago sativa* L.) is a widely used temperate fodder crop noted for its superior forage quality, yield and BNF potential (Wery et al., 1986; Marten et al., 1988; Russelle et al., 1994). Depending on growing conditions, some studies showed that 70–94% of alfalfa N requirements resulted from BNF through *Rhizobium* bacteria in root nodules (Ta and Faris, 1988; Russelle et al., 1994). BNF by alfalfa provides substantial amounts of N to livestock operations, subsequent crops and soil organic matter. Literature showed that annual BNF by alfalfa ranges from 45 to 475 kg N ha^{-1} (Russelle and Birr, 2004; Russelle, 2005). Based on the amount of N in roots, aboveground biomass and regrowth, Kelner et al. (1997) estimated that 85 kg N ha^{-1} and 148 kg N ha^{-1} were added to the soil after one year and two years of alfalfa cultivation respectively. In a Mediterranean silvo-arable system, alfalfa intercrops improved the N status of young walnut trees (Dupraz et al., 1998; Simorte et al., 2001). However, as far as we are aware, the effects of mature trees on alfalfa BNF in agroforestry systems have never been documented since the only study published was performed on very small pine trees (1.5–3 m in height) resulting in a very minor impact on legume BNF (Goh et al., 1996).

Our objective was to investigate competitive effects (i.e., shading and competition for soil moisture and nitrogen) of large hybrid walnuts, commonly used in agroforestry for their high value timber on alfalfa crop yield and BNF in a Mediterranean tree-based intercrop system. The hypotheses tested in our study are that (i) alfalfa yield is reduced close to the trees and positively correlated with light availability, (ii) soil water availability is lower near the trees than in the inter-row, with a negative impact on alfalfa water status and possibly yield, and (iii) the proportion of alfalfa N derived from the air is reduced close to the trees and is positively correlated with alfalfa yield.

2. Material and methods

2.1. Site description

The research site is located 10 km north of Montpellier City Centre (southern France) at the Public farm estate of Restinclières (Prades le Lez, Hérault), a hilly area of 215 ha bordered by two rivers, the Lez and the Lirou (43°42′26″N, 3°51′34″E, elevation 61 m asl). A large experimental agroforestry site of 54 ha was established by INRA (Institut National de la Recherche Agronomique) in 1995. The climate is sub-humid Mediterranean with an average annual rainfall of 873 mm (1995–2013 average), lowest in July and highest in September to December. The mean air temperature (1995–2013) is 15.4 °C, with a maximum monthly mean in July (24.9 °C), and minimum in January (7.1 °C). The walnut-cereal agroforestry plots are located on alluvial soils of the Lez river. The soil is a silty clay (25% clay and 60% silt) deep alluvial Fluvisol (IUSS Working Group WRB 2007) and the average pH is approximately 8.0 (Dupraz et al., 1999), with a medium cation exchange capacity (<13 me/100 g) and organic matter percentage (<3.5%) and a high content in total carbonates, typical from the Montpellier region where limestones are predominant.

The field experiment was carried out in 2012 and 2013 in a 17-year-old silvoarable field. Hybrid walnuts (*Juglans nigra* × *regia cv* NG23) were planted in 1995, in East-West oriented rows at 4 m (between trees) x 13 m (between rows). A thinning was performed in 2004 and reduced the stocking density to 96 trees ha^{-1}. We applied a selective pruning scheme by removing half the trees. The average distance between trees on the line is now 8 m, but may locally be 4 or 12 m if two consecutive trees were both retained or both thinned. Leaf litter is left on the soil, and usually is not uniformly scattered in the alley. Strong winds sometimes pile the leaves close to the tree row where a soil elevation block them. But this depends on the temperature and rain pattern at the time of leaf shedding. When no early frost occurs, leaves fall progressively in two weeks. When an early frost occurs, all leaves fall in some days. Some years, late soil preparation for growing winter crops may bury the leaf litter, but this happened only few years since tree plantation. Walnut leaves may smother the winter crops germination and early growth. This was observed under some piles of leaves resulting from the wind action. It was not a major problem so far as the composed leaves of walnut usually drop separately the small leaflets and the leaves axis. So far, these hybrid walnut trees produced almost no nuts. Nuts of hybrid walnut trees are usually not edible and will be left on the ground. If grafted walnut trees were included in the system, alfalfa would be moved before nut harvest, and both hand picking and machine harvesting with skirts could be performed easily.

The site was managed by a farmer from 1995 to 2011, and a cereal-based rotation was cultivated in a 12 m wide alley between walnut tree rows including durum wheat (*Triticum turgidum* L. *subsp. durum*) and rapeseed (*Brassica napus* L.). In early 2012, average tree height and diameter at breast height were 10.6 m and 0.20 m respectively, while tree canopy diameter across the alley was 6.5 m. Soil ploughing down to 15 cm was applied only every