Agroforest Syst (2014) 88:671–678
DOI 10.1007/s10457-014-9680-y

Intercropping trees' effect on soil oribatid diversity in agro-ecosystems

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Received: 26 September 2013 / Accepted: 4 March 2014 / Published online: 13 March 2014

Abstract The benefits of tree-based intercropping (TBI) compared to conventional agro-ecosystems in North America could include climate change mitigation and adaptation, although enhancing resilience to climate change through increasing soil diversity remains poorly explored. Diversity of soil microarthropods supports a series of ecological services that may be altered by soil desiccation due to climate change. Here we study the effect of red oak and hybrid poplar TBI on soil oribatid mite species assemblages associated to forage crops (mix of Timothy-grass and red clover). Abundance and species density of oribatids were affected by treatment, depth and the interaction of both variables. Abundance of oribatid mites was significantly lower in the oak TBI, showing a homogeneous vertical distribution in opposition to a decreasing with depth distribution under poplar TBI and conventional crops. Species density was significantly higher in the conventional crop, showing again significant differences in depth that were not present in both TBI treatments. Distance to tree did not affect mite abundance nor species density. TBI increased oribatid richness (obtained by sample-based rarefaction and extrapolation) only in the presence of oaks. The distribution of oribatids was strongly associated to tree fine root biomass and stress the importance of underground organic resources for the oribatid fauna and their ecological functions. If increasing drought associated with climate change desiccates superficial levels of agro-ecosystem soils, deeper sources of organic resources, such as tree roots, should become crucial in the maintenance of diverse microarthropod communities.

Keywords Ecological services · Forage crops · Hybrid poplar · Red oak · Vertical distribution · Tree-based intercropping

Introduction

The benefits of tree-based intercropping (TBI) in north-American agro-ecosystems include carbon sequestration (Bambrick et al. 2010), reduced soil nutrient leaching (Bergeron et al. 2011), increasing microbial biomass (Rivest et al. 2010) and financial returns of wood that compensate variations in annual crop profits (Cardinael et al. 2012; Toor et al. 2012). However, effects of TBI on soil microbial biodiversity are controversial, TBI does not increase bacterial or fungal diversity in all cases and these increases are not always beneficial for the crop (e.g. Lacombe et al. 2009; Bainard et al. 2013; see also Bainard et al. 2011 for a complete review in tropical agro-ecosystems).

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TBI effects on soil microarthropods have not been analyzed to date.

Both abundance and diversity of soil microarthropods are considered important ecological components of the organic and lower soil strata and play a significant role in the decomposition process. Abundance of soil arthropods affects porosity, aeration, infiltration, and the distribution of organic matter within the soil (Seastedt 1984; Moore et al. 1988). There is also evidence that soil microarthropod diversity affects microbes, nematodes, fungi and other components of the soil food web, altering ecosystem processes such as decomposition rates (Crossley et al. 1992; Heneghan and Bolger 1998; Bradford et al. 2002). This has led to increased interest in the effects of manipulation of soil microarthropod communities in agro-ecosystem in order to maintain ecosystem services (Osler et al. 2008), especially in the face of climate change (Schoeneberger et al. 2012). Several agricultural practices such as tillage, pesticide use and fertilization affect soil microarthropods (Behan-Pelletier 1999, 2003), although the effects of TBI are still poorly documented.

Soil microarthropod assemblages respond to differences in overlying tree diversity (Badejo and Tian 1999; Hansen 2000; Eissfeller et al. 2013). Most studies consider the effects of trees in relation to leaf litter addition in the soil surface, but have paid less attention to biomass of coarse and fine roots below the soil surface (Eisenhauer and Reich 2012). However, the influence of roots on soil communities may be especially important in agro-ecosystems (Crossley et al. 1992). These systems usually lack surface organic litter and therefore microarthropods depend on roots and associated fungi as principal resource (Garrett et al. 2001). The associated benefits of increasing microarthropod diversity in deeper soil levels could be a considerable advantage in view of climate change. High temperatures and drought associated to climate change could desiccate superficial soil levels and therefore negatively affect the soil fauna (Lindberg and Bengtsson 2005; Blankinship et al. 2011), which would migrate to deeper levels as has been demonstrated previously (Whitford et al. 1981). The capacity of soil arthropods to adapt to proposed climate changes, both in terms of abundance and species composition, is of course dependant on the presence of palatable food resources at those deeper soil layers.

Here we study the effect of intercropping tree lines of red oaks and poplars compared to conventional forage crops (mix of Timothy-grass and red clover) on soil oribatid mites, considering differences among tree species and depth and distance to tree levels. Mites are the most diverse and abundant arthropods in Canadian agricultural soils (Behan-Pelletier 2003) while oribatids are the most numerous and species-rich mite group and are specially recommended as bioindicators in agro-ecosystems (Behan-Pelletier 1999; Gulvik 2007). We hypothesize that TBI will show higher diversity of oribatids than conventional agro-ecosystems, especially at deeper soil levels and close to the tree, and that these effects may depend on tree species.

Materials and methods

Study site

The study was conducted in St-Paulin (46°27′6″ N, −72°59′26″E), in southwestern Québec. Average annual temperatures average 6 °C while annual precipitations approximate 1,027 mm (Environment Canada; http://climate.weather.gc.ca/). The soil is classified as an orthic melanic brunisol (Agriculture Canada Expert Committee on Soil Survey 1987) with a loam soil texture (19 % clay, 34.5 % silt and 46.5 % sand). The experimental area was set up in 2004 with a plantation composed of two hardwood species; red oak (*Quercus rubra* L.) and black cherry (*Prunus serotina* Ehrh), and two *Populus deltoides* x *Populus nigra* hybrid poplar clones; DN3333 (cv. Stormont) and DN3570 (Belgium). From 2009 to 2012 intercropping was composed of a mix of Timothy-grass (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) under organic cultivation (without herbicides).

Experimental design and methodology

Trees were planted in 9 lines (alternating hardwoods and poplars) separated by a 12 m band left for intercropping. Within each tree line, trees are separated 3 or 4 m from one another for hardwoods, and 2 m for poplars. Four random areas (minimal size of 48 × 24 m^2) were left without trees in order to provide conventional agro-ecosystems for comparison (controls), one in each of four complete replication blocks (TBI + control), from which samples were thus extracted (Fig. 1). Only red oaks and DN3570 clones were used in the present experiment. We sampled soil cores (a square coring device consisting of two tightly fitting pieces of sharpened aluminum; 25 cm^2) from July 3rd to August 4th 2011 (temperatures ranged between 8.0 and 33.0 °C during the sampling period) at three distances from trees (1.5, 3.5 and 5.75 m) and at 7 soil depths (0, 5, 10, 15, 25, 35 and 45 cm). We collected a total of 18 cores from the control plots and a total of 199 cores from the TBI plots (108 cores from the red oak and 91 plots from poplar). These samples were collected from inside of larger trenches that were dug with an excavator. Samples were weighed and stored in a freezer until microarthropod extraction. We extracted soil microarthropods using an oil-based flotation method which relies on the affinity of the arthropod cuticle for olive oil (Kuenen et al. 2009). Each soil sample was added to an Erlenmeyer flask filled up to 500 ml with 35 % ethanol and 20 ml of olive oil. The Erlenmeyer was agitated three times, each time for 3 min, with 15 min between agitations to permit soil and other debris to settle. After material had settled following the final agitation, oil on the top of the flask was removed and passed through a 44 µm filter, using a vacuum to accelerate the process. We then washed the filter with ethanol and oribatids were sorted from the remaining debris. Specimens were placed in lactic acid for 2 days and finally in mounting medium on a slide for identification to the species level (Balogh 1972; Moldenke and Fichter 1988; Krantz and Walter 2009, among others).

Samples were subsequently dried and weighted again to calculate soil moisture. Fine root biomass of trees and forage was also assessed by in situ counts (Böhm 1976) and mean values were assigned for each microarthropod sample.

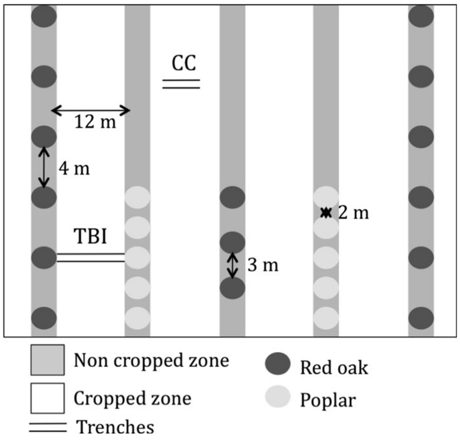

Fig. 1 Visual scheme of the experimental area. *TBI* tree-based intercropping, *CC* conventional crop

Statistical analyses

ANOVA was used to compare treatment (poplar TBI, oak TBI and conventional crop), depth (0, 5, 10, 15, 25, 35 and 45 cm) and their interaction on oribatid abundance (individuals/gram of dry soil) and species density (number of species/gram of dry soil), using block as a random factor and pooling tree distances for that model. Another ANOVA was used to analyse the effect of tree species, depth and distance from tree (1.5, 3.5 and 5.75 m) on oribatid variables only in TBI. Post hoc analyses were performed by the Tukey's 'Honest Significant Difference' method to compare means between treatments and between depth levels for each treatment. The influence of soil moisture and fine root biomass of trees and forage on belowground (from 5 to 45 cm depth) oribatid abundance and species density was assessed using linear models. Similarly, the effect of soil moisture and aerial biomass of forage on surface oribatids (0 cm depth) was also analysed (R v2.8.1; R Development Core Team 2010).

In addition, we compared estimated species richness of oribatids using individual-based rarefaction and sample-based rarefaction and extrapolation (Colwell et al. 2012). Individual based rarefactions solve the problem of comparing different abundances, while sample based rarefactions and extrapolations permitted comparison between control and TBI plots under similar sampling conditions in order to add reliable confidence intervals. Rarefaction estimates were produced from pooled abundances of individuals and samples taken within treatments and tree species using EstimateS (Version 9, R. K. Colwell, http://purl.oclc.org/estimates). Depth levels were not considered in rarefaction analyses because the low number of individuals was insufficient to perform meaningful species accumulation curves.

Results

We found a total of 400 mite individuals belonging to 8 different oribatid species (Table 1).

Table 1 Total number of mite individuals found for each oribatid species, at each depth level considered in the present study

	0 cm	5 cm	10 cm	15 cm	25 cm	35 cm	45 cm
Micropia minus	58	77	58	33	16	2	1
Suctobelbella frothinghami	0	2	0	2	1	0	0
Eniochthonius minutissimus	3	4	23	15	15	4	0
Opiella nova	5	24	1	3	2	0	0
Tectocepheus velatus	42	3	0	0	0	0	1
Scheloribates pallidulus	1	0	0	0	0	0	0
Scheloribates laevigatus	0	0	0	0	0	1	0
Brachioppiella periculosa	0	0	2	1	0	0	0

Table 2 Summary of the factorial Anova used to test the effects of treatment, depth and their interaction on oribatid species density and abundance

Response variable	Factors	Df	F value
Species density	Treatment	2	11.80***
	Depth	6	9.06***
	Treatment:Depth	12	4.57***
	Residuals	119	
Abundance	Treatment	2	5.98**
	Depth	6	6.12***
	Treatment:Depth	12	3.04***
	Residuals	119	

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

Models

Species density and abundance of oribatids were affected by treatment, depth levels and the interaction of treatment and depth (Table 2). Although greater species density of oribatids was found in conventional crops, the vertical distribution of species density is more homogeneous in TBI treatments (Fig. 2). Abundance of oribatids was significantly lower only in the case of oak TBI, which also showed a homogeneous vertical distribution of oribatids in opposition to conventional crops and poplar TBI (Fig. 2).

When only the intercropping treatment is considered, tree species and distance to tree did not affect oribatid species density, although tree species and its interaction with depth significantly affected oribatid abundance (Table 3).

Abundance and species density of oribatids depended significantly on fine root biomass of the targeted tree, while soil moisture only affected species density (Table 4). Forage aerial biomass and moisture did not affect surface oribatids ($p > 0.05$ in all cases).

Rarefactions

Individual-based rarefactions indicate that species richness of oribatids was similar in both TBI and conventional crop treatments (Table 5). However, when we compared tree species separately, we observed differences in species richness. Species richness in poplar TBI was lower than in conventional crops while species richness in oak TBI was greater than in conventional crops (Fig. 3). Only nine different species were found (Table 5). The abundant *Micropia minus* was similarly present in both treatments. *Tectocepheus velatus* and *Oppiella nova* were more abundant in the TBI treatment while *Eniochthonius minutissimus* was especially abundant in conventional cropping.

Discussion

Initially, we predicted that diversity of oribatid mites would be higher in the TBI treatments particularly deeper soils. While oribatid species density was more similar throughout lower soil depths in TBI treatments, we still observed a decrease with increasing soil depth albeit less apparent than with depth in conventional crops. Moreover, the vertical distribution of mite abundance was homogeneous only in the case of oak TBI. This also supports previous suggestions that environmental conditions along soil depth gradients may play a stronger role than agricultural operations on soil microarthropods (Osler et al. 2008). Lower abundance and especially species

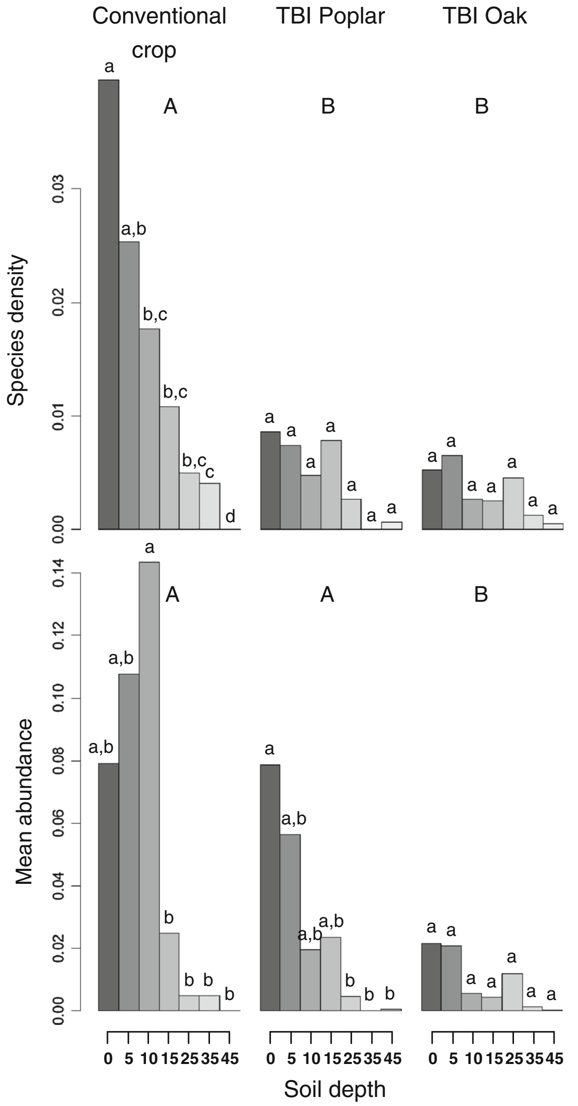

Fig. 2 Species density of oribatids in number of species/gram of dry soil (*top*) and mean abundance in number of individuals/gram of dry soil (*down*) showed under different soil depths (from *dark* to *light grey*; 0, 5, 10, 15, 25, 35, 45 cm depth) for control (conventional crop) and experimental plots of both tree species (TBI Poplar and TBI Oak). *Capital letters* indicate significant differences among treatments while *lower case letters* indicate significant differences among depth levels for each treatment by Tukey's 'Honest Significant Difference'

Table 3 Summary of the factorial Anova used to test the effects of tree species, distance to tree, depth and their interaction on oribatid species density and abundance

Response variable	Factors	Df	F value
Species density	Tree species	1	2.29
	Distance to tree	2	1.47
	Depth	6	4.12**
	Tree:Distance	2	0.44
	Tree:Depth	6	1.18
	Distance:Depth	12	0.81
	Tree:Distance:Depth	9	0.88
	Residuals	83	
Abundance	Tree species	1	9.32**
	Distance to tree	2	1.24
	Depth	6	4.95***
	Tree:Distance	2	0.96
	Tree:Depth	6	2.25*
	Distance:Depth	12	0.64
	Tree:Distance:Depth	9	1.95
	Residuals	83	

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

Table 4 Coefficients of the linear model used to test the effects of soil moisture and root biomass of trees and forage on oribatid species density and abundance

Coefficients		t value
Species density	(Intercept)	−0.58
	Moisture	2.55*
	Tree roots	3.11**
	Forage roots	1.07
Abundance	(Intercept)	−0.53
	Moisture	0.50
	Tree roots	5.77***
	Forage roots	0.77

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

density of oribatids at the surface level in the case of TBI could be the result of a surface desiccation due to tree root water absorption. However, moisture only affected oribatid species density at belowground depth levels. In any case, the relative homogeneous vertical distribution of mite abundance in oak TBI and species richness in both TBI treatments suggest that TBI is at least partially successful in extending habitat deeper into the soil than conventional crops. This fact may be related to the trees fine root material, which showed to be the most important factor affecting abundance and species density of soil fauna in the present study. TBI thus likely results in richer soils for oribatid mites at deeper levels, providing the habitat for a diverse soil mesofaunal community (Pollierer et al. 2007). It also suggests that TBI may provide some mitigative benefits for reducing negative impacts of climate change. Models of future climatic conditions predict a clear increase of temperature and a less likely decrease of moisture (IPCC 2007). In addition to predicted increases in severity, more frequent drought events are also predicted (Bonsal et al. 2011). The effects of drought in the more superficial soil levels may force the soil fauna to migrate to deeper levels of the soil profile (Whitford et al. 1981; Doblas-Miranda et al. 2009).

Nevertheless oribatids showed higher species density in conventional crops than TBI plots as observed in Fig. 2. The number of oribatid species is highly dependent on oribatid abundance (Osler and Beattie 1999), because a few species are greatly abundant within oribatid communities, especially in arable fields (Paoletti 1988). In view of individual based rarefactions (Fig. 3), oak TBI plots showed more richness than conventional crops, but not in the case of poplar intercropping. These species dependent effects could be related to a higher fine root quality in red oak than poplar, showing higher palatability for soil oribatid mites. This suggests that only particular tree species, in this case showing high root quality, may be preferable as mitigative strategies rather than any tree species (Bainard et al. 2012). Lower abundance of soil oribatids in oak TBI could be related to several variables not accounted in this study as tree and crop above-ground litter properties, allelopathy, soil physical and biochemical properties could be affected by oak. Among them, soil food web structure may be one of the key factors, as high root quality of oaks may affect not only oribatids but many other potential competitors and predators (Witt and Setala 2010; Scharroba et al. 2012).

We observed interesting differences in oribatid species responses. *T. velatus* and *O. nova*, which were more abundant in the TBI treatments than conventional cropping, may be characterized as species with a rapid development time, high reproduction rates and a great plasticity to switch between resources (Siepel 1995; Behan-Pelletier 1999; Hansen 2000). These are characteristics that are often associated with species that are good colonizers. Other species such as *E. minutissimus* are more typically associated with forest litter, fungi and wood materials (Norton and Behan-Pelletier 2007). Interestingly, this species was more abundant in conventional crops.

Apart from the specificities of the oribatid species assemblage, our results suggest the importance of tree species, particularly high root quality species, in TBI increasing soil fauna diversity, providing crucial resources not only at superficial but also at deeper soil levels. Soils are a critical medium for the interchange of water, organic matter and gasses and harbor significant biodiversity (Bardgett et al. 2001). Maintaining soil quality in agroecosystems is thus important as rapid changes could alter the soil fauna and its associated ecological services (Behan-Pelletier 2003), and soil maintenance could help buffer against

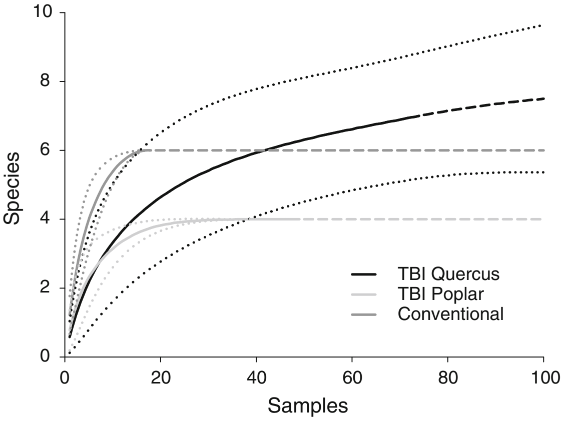

Fig. 3 Sample based rarefaction (*soild lines*) and extrapolation (*dashed lines*) for oribatids from three different treatments (*black* and *grey colors* in legend) under the Bernoulli product model, with 95 % unconditional confidence intervals

Table 5 Individual-based rarefactions up to 100 individuals for each species, and total number of species within treatments

	Conventional	TBI	TBI poplar	TBI oak
Microppia minus	54	56	76	45
Suctobelbella frothinghami	2	0	0	3
Eniochthonius minutissimus	32	12	6	18
Opiella nova	3	15	4	17
Tectocepheus velatus	6	15	14	15
Scheloribates pallidulus	0	1	0	1
Scheloribates laevigatus	0	1	0	1
Brachioppiella periculosa	3	0	0	0
Number of species	6	6	4	7

drought and disease disturbances (Johnston and Crossley 2002). TBI has the potential to address climate change mitigation and adaptation by carbon sequestration, diversifying production opportunities and providing greater biodiversity (Schoeneberger et al. 2012). The advantages of increasing soil diversity would be however conditioned by the chosen tree species, with high root quality species probably providing best effects.

Acknowledgments This work benefitted from funds from the *Fonds vert*, part of the *Plan d'action* 2006–2012 *sur les changements climatiques* of the government of Québec. Lea Bouttier kindly provided her data on root biomass. We would like to thank Luana Graham-Sauvé for her help during the fieldwork and Émilie Rivest and Evick Mestre for their work during the extraction and identification of oribatids. EDM was partially supported by the BE-DGR 2011 program of the Catalonian Agency for Management of University and Research Grants, the MONTES-Consolider project (CSD2008-00040), funded by the Spanish Ministry of Economy and Competitiveness, and the NEWFORESTS project (612645), from the European 7FP.

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