

1 For the Special Issue of **Agroforestry Systems**

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

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Post-print of: Doblas-Miranda, E. ; Paquette, A. and Work, T.T. "Intercropping trees'effect on soil oribatid diversity in agro-ecosystems" in Agroforestry systems, vol. 88, issue 4 (August 2014), p. 671-678. Published by Springer. The final versión is available at DOI 10.1007/s10457-014-9680-y

1 **Abstract**

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

21

22 **Key words:** ecological services, hybrid poplar, red clover, red oak, Timothy-grass, vertical
23 distribution, tree-based intercropping

24

1 **Introduction**

2 The benefits of tree-based intercropping (TBI) in north-American agro-ecosystems include
3 carbon sequestration (Bambrick et al. 2010), reduced soil nutrient leaching (Bergeron et al.
4 2011), increasing microbial biomass (Rivest et al. 2010) and financial returns of wood that
5 compensate variations in annual crop profits (Cardinael et al. 2012; Toor et al. 2012).
6 However, effects of TBI on soil microbial biodiversity are controversial, TBI does not
7 increase bacterial or fungal diversity in all cases and these increases are not always beneficial
8 for the crop (e.g. Lacombe et al. 2009; Bainard et al. 2013; see also Bainard et al. 2011 for a
9 complete review in tropical agro-ecosystems). TBI effects on soil microarthropods have not
10 been analyzed to date.

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

23 Soil microarthropod assemblages respond to differences in overlying tree diversity (Badejo
24 and Tian 1999; Hansen 2000; Eissfeller et al. 2013). Most studies consider the effects of trees

1 in relation to leaf litter addition in the soil surface, but have paid less attention to biomass of
2 coarse and fine roots below the soil surface (Eisenhauer & Reich 2012). However, the
3 influence of roots on soil communities may be especially important in agro-ecosystems
4 (Crossley et al. 1992). These systems usually lack surface organic litter and therefore
5 microarthropods depend on roots and associated fungi as principal resource (Garrett et al.
6 2001). The associated benefits of increasing microarthropod diversity in deeper soil levels
7 could be a considerable advantage in view of climate change. High temperatures and drought
8 associated to climate change could desiccate superficial soil levels and therefore negatively
9 affect the soil fauna (Lindberg and Bengtsson 2005; Blankinship et al. 2011), which would
10 migrate to deeper levels as has been demonstrated previously (Whitford et al. 1981). The
11 capacity of soil arthropods to adapt to proposed climate changes, both in terms of abundance
12 and species composition, is of course dependant on the presence of palatable food resources at
13 those deeper soil layers.

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

23

24 **Material and methods**

1 *Study site*

2 The study was conducted in St-Paulin (46° 27' 6" N; -72° 59' 26" E), in southwestern
3 Québec. Average annual temperatures average 6 °C while annual precipitations approximate
4 1027 mm (Environment Canada 2008). The soil is classified as an orthic melanic brunisol
5 (Agriculture Canada Expert Committee on Soil Survey 1987) with a loam soil texture (19%
6 clay, 34.5% silt and 46.5% sand). The experimental area was set up in 2004 with a plantation
7 composed of two hardwood species; red oak (*Quercus rubra* L.) and black cherry (*Prunus*
8 *serotina* Ehrh), and two *Populus deltoides* x *Populus nigra* hybrid poplar clones; DN3333
9 (cv. Stormont) and DN3570 (Belgium). From 2009 to 2012 intercropping was composed of a
10 mix of Timothy-grass (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) under
11 organic cultivation (without herbicides).

12 *Experimental design and methodology*

13 Trees were planted in 9 lines (alternating hardwoods and poplars) separated by a 12m band
14 left for intercropping. Within each tree line, trees are separated 3 or 4 m from one another for
15 hardwoods, and 2 m for poplars. Four random areas (minimal size of 48 m x 24 m) were left
16 without trees in order to provide conventional agro-ecosystems for comparison (controls), one
17 in each of four complete replication blocks (TBI + control), from which samples were thus
18 extracted (Figure 1). Only red oaks and DN3570 clones were used in the present experiment.
19 We sampled soil cores (a square coring device consisting of two tightly fitting pieces of
20 sharpened aluminum; 25 cm²) from July 3rd to August 4th 2011 (temperatures ranged between
21 8.0 and 33.0 °C during the sampling period) at three distances from trees (1.5, 3.5 and 5.75 m)
22 and at 7 soil depths (0, 5, 10, 15, 25, 35 and 45 cm). We collected a total of 18 cores from the
23 control plots and a total of 199 cores from the TBI plots (108 cores from the red oak and 91
24 plots from poplar). These samples were collected from inside of larger trenches that were dug

1 with an excavator. Samples were weighed and stored in a freezer until microarthropod
2 extraction. We extracted soil microarthropods using an oil-based flotation method which
3 relies on the affinity of the arthropod cuticle for olive oil (Kuenen et al. 2009). Each soil
4 sample was added to an Erlenmeyer flask filled up to 500 ml with 35% ethanol and 20 ml of
5 olive oil. The Erlenmeyer was agitated 3 times, each time for 3 minutes, with 15 minutes
6 between agitations to permit soil and other debris to settle. After material had settled
7 following the final agitation, oil on the top of the flask was removed and passed through a 44
8 μm filter, using a vacuum to accelerate the process. We then washed the filter with ethanol
9 and oribatids were sorted from the remaining debris. Specimens were placed in lactic acid for
10 2 days and finally in mounting medium on a slide for identification to the species level
11 (Balogh 1972; Moldenke and Fichter 1988; Krantz and Walter 2009, among others).

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

15 *Statistical analyses*

16 ANOVA was used to compare treatment (poplar TBI, oak TBI and conventional crop), depth
17 (0, 5, 10, 15, 25, 35 and 45 cm) and their interaction on oribatid abundance (individuals/gram
18 of dry soil) and species density (number of species/gram of dry soil), using block as a random
19 factor and pooling tree distances for that model. Another ANOVA was used to analyse the
20 effect of tree species, depth and distance from tree (1.5, 3.5 and 5.75 m) on oribatid variables
21 only in TBI. Post hoc analyses were performed by the Tukey's 'Honest Significant
22 Difference' method to compare means between treatments and between depth levels for each
23 treatment. The influence of soil moisture and fine root biomass of trees and forage on
24 belowground (from 5 to 45 cm depth) oribatid abundance and species density was assessed

1 using linear models. Similarly, the effect of soil moisture and aerial biomass of forage on
2 surface oribatids (0 cm depth) was also analysed (R v2.8.1; R Development Core Team
3 2010).

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

14

15 **Results**

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

17 *Models*

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

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

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

7 *Rarefactions*

8 Individual-based rarefactions indicate that species richness of oribatids was similar in both
9 TBI and conventional crop treatments (Table 5). However, when we compared tree species
10 separately, we observed differences in species richness. Species richness in poplar TBI was
11 lower than in conventional crops while species richness in oak TBI was greater than in
12 conventional crops (Figure 3). Only nine different species were found (Table 5). The
13 abundant *Micropoppia minus* (Paoli, 1908) was similarly present in both treatments.
14 *Tectocephus velatus* (Michael, 1880) and *Oppiella nova* (Oudemans, 1902) were more
15 abundant in the TBI treatment while *Eniochthonius minutissimus* (Berlese, 1904) was
16 especially abundant in conventional cropping.

17

18 **Discussion**

19 Initially, we predicted that diversity of oribatid mites would be higher in the TBI treatments
20 particularly deeper soils. While oribatid species density was more similar throughout lower
21 soil depths in TBI treatments, we still observed a decrease with increasing soil depth albeit
22 less apparent than with depth in conventional crops. Moreover, the vertical distribution of
23 mite abundance was homogeneous only in the case of oak TBI. This also supports previous

1 suggestions that environmental conditions along soil depth gradients may play a stronger role
2 than agricultural operations on soil microarthropods (Osler et al. 2008). Lower abundance and
3 especially species density of oribatids at the surface level in the case of TBI could be the
4 result of a surface desiccation due to tree root water absorption. However, moisture only
5 affected oribatid species density at belowground depth levels. In any case, the relative
6 homogeneous vertical distribution of mite abundance in oak TBI and species richness in both
7 TBI treatments suggest that TBI is at least partially successful in extending habitat deeper into
8 the soil than conventional crops. This fact may be related to the trees fine root material, which
9 showed to be the most important factor affecting abundance and species density of soil fauna
10 in the present study. TBI thus likely results in richer soils for oribatid mites at deeper levels,
11 providing the habitat for a diverse soil mesofaunal community (Pollierer et al. 2007). It also
12 suggests that TBI may provide some mitigative benefits for reducing negative impacts of
13 climate change. Models of future climatic conditions predict a clear increase of temperature
14 and a less likely decrease of moisture (IPCC 2007). In addition to predicted increases in
15 severity, more frequent drought events are also predicted (Bonsal et al. 2011). The effects of
16 drought in the more superficial soil levels may force the soil fauna to migrate to deeper levels
17 of the soil profile (Whitford et al. 1981; Doblus-Miranda et al. 2009).

18 Nevertheless oribatids showed higher species density in conventional crops than TBI plots as
19 observed in Figure 2. The number of oribatid species is highly dependent on oribatid
20 abundance (Osler and Beattie 1999), because a few species are greatly abundant within
21 oribatid communities, especially in arable fields (Paoletti 1988). In view of individual based
22 rarefactions (Figure 3), oak TBI plots showed more richness than conventional crops, but not
23 in the case of poplar intercropping. These species dependent effects could be related to a
24 higher fine root quality in red oak than poplar, showing higher palatability for soil oribatid
25 mites. This suggests that only particular tree species, in this case showing high root quality,

1 may be preferable as mitigative strategies rather than any tree species (Bainard et al. 2012).

2 Lower abundance of soil oribatids in oak TBI could be related to several variables not
3 accounted in this study as tree and crop above-ground litter properties, allelopathy, soil
4 physical and biochemical properties could be affected by oak. Among them, soil food web
5 structure may be one of the key factors, as high root quality of oaks may affect not only
6 oribatids but many other potential competitors and predators (Witt and Setälä 2010; Scharroba
7 et al. 2012).

8 We observed interesting differences in oribatid species responses. *Tectocephus velatus* and
9 *Oppiella nova*, which were more abundant in the TBI treatments than conventional cropping,
10 may be characterized as species with a rapid development time, high reproduction rates and a
11 great plasticity to switch between resources (Siepel 1995; Behan-Pelletier 1999; Hansen
12 2000). These are characteristics that are often associated with species that are good
13 colonizers. Other species such as *Eniochthonius minutissimus* are more typically associated
14 with forest litter, fungi and wood materials (Norton and Behan-Pelletier 2007). Interestingly,
15 this species was more abundant in conventional crops.

16 Apart from the specificities of the oribatid species assemblage, our results suggest the
17 importance of tree species, particularly high root quality species, in TBI increasing soil fauna
18 diversity, providing crucial resources not only at superficial but also at deeper soil levels.

19 Soils are a critical medium for the interchange of water, organic matter and gasses and harbor
20 significant biodiversity (Bardgett et al. 2001). Maintaining soil quality in agroecosystems is
21 thus important as rapid changes could alter the soil fauna and its associated ecological
22 services (Behan-Pelletier 2003), and soil maintenance could help buffer against drought and
23 disease disturbances (Johnston and Crossley 2002). TBI has the potential to address climate
24 change mitigation and adaptation by carbon sequestration, diversifying production
25 opportunities and providing greater biodiversity (Schoeneberger et al. 2012). The advantages

1 of increasing soil diversity would be however conditioned by the chosen tree species, with
2 high root quality species probably providing best effects.

3

4 **Acknowledgements**

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

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- 10

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

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

3

1 Table 2. Summary of the factorial Anova used to test the effects of treatment, depth and their
 2 interaction on oribatid species density and abundance (*p < 0.05, **p < 0.01, and ***p <
 3 0.001).

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	

4

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1 Table 3. Summary of the factorial Anova used to test the effects of tree species, distance to
 2 tree, depth and their interaction on oribatid species density and abundance (*p < 0.05, **p <
 3 0.01, and ***p < 0.001).

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	

4

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1 Table 4. Coefficients of the linear model used to test the effects of soil moisture and root
 2 biomass of trees and forage on oribatid species density and abundance (*p < 0.05, **p < 0.01,
 3 and ***p < 0.001).

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

4

5

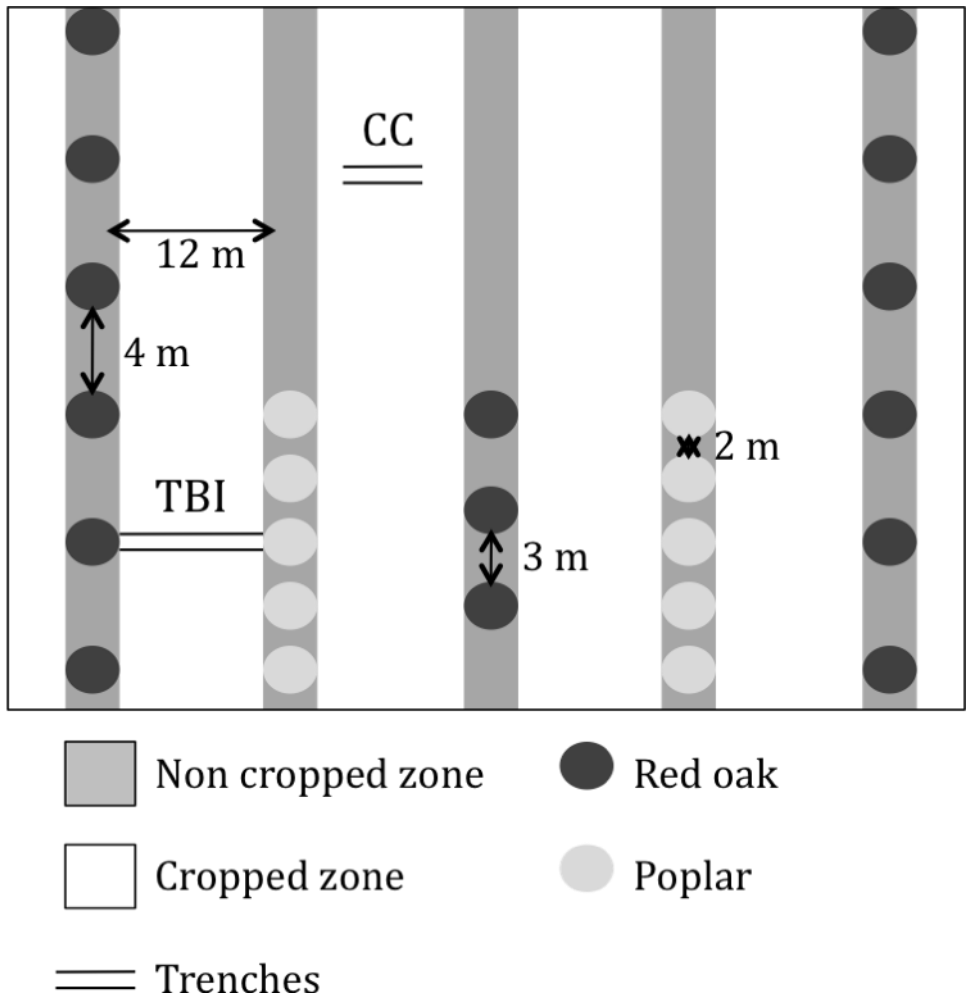
- 1 Table 5. Individual-based rarefactions up to 100 individuals for each species, and total
- 2 number of species within treatments.

	Conventional	TBI	TBI Poplar	TBI Oak
<i>Microppia minus</i>	54	56	76	45
<i>Suctobelbella frothingami</i>	2	0	0	3
<i>Eniochthonius minutissimus</i>	32	12	6	18
<i>Opiella nova</i>	3	15	4	17
<i>Tectocephus velatus</i>	6	15	14	15
<i>Scheloribates pallidulus</i>	0	1	0	1
<i>Scheloribates laevigatus</i>	0	1	0	1
<i>Brachioppiella periculosa</i>	3	0	0	0
Number of species	6	6	4	7

3

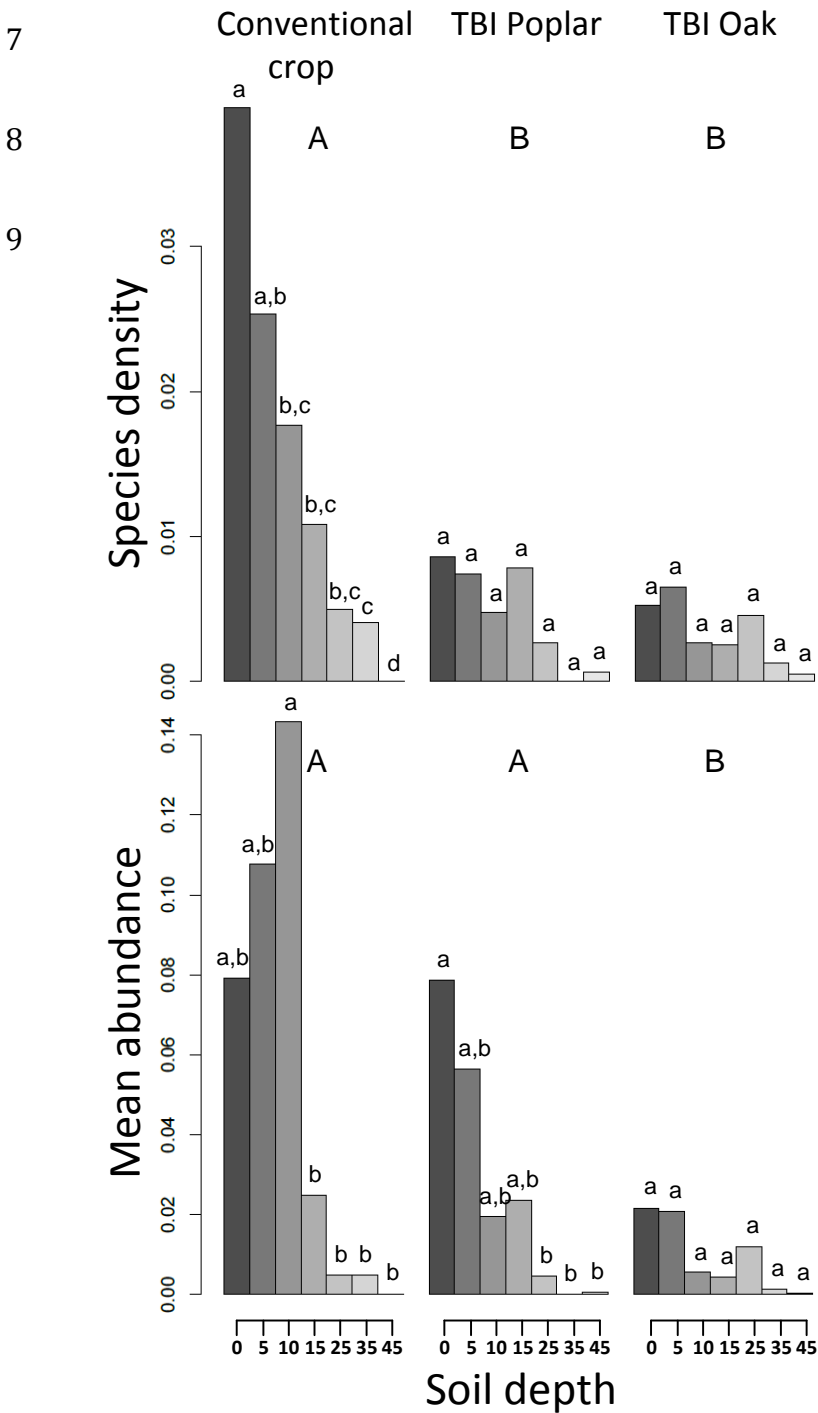
4

- 1 Figure 1. Visual scheme of the experimental area. TBI = tree-based intercropping, CC =
- 2 conventional crop

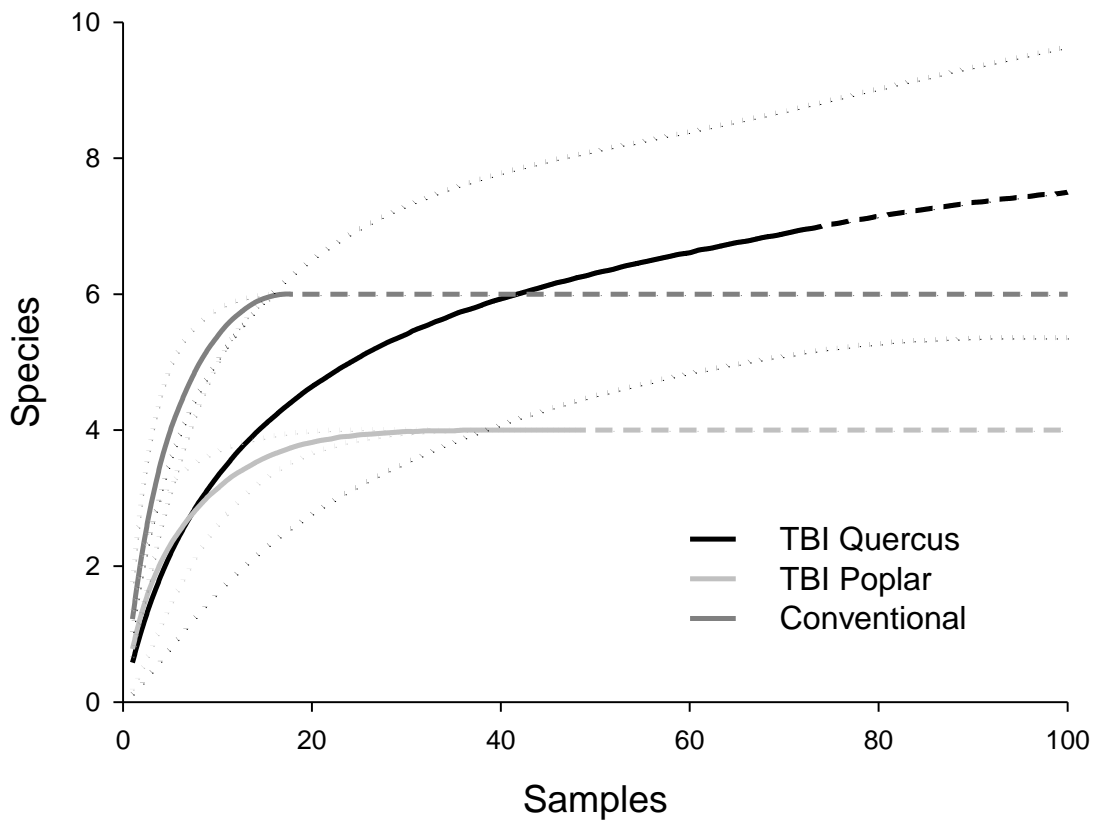


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1 Figure 2. Species density of oribatids in number of species/gram of dry soil (top) and mean
 2 abundance in number of individuals/gram of dry soil (down) showed under different soil
 3 depths (from dark to light grey; 0, 5, 10, 15, 25, 35, 45 cm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



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



4