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Dynamics of soil fauna after plantation of perennial energy crops on polluted soils

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ABSTRACT

Growing demand for alternative energy sources has led to an increased production of biomass crops. In order to limit the use of fertile agricultural land for producing bioenergy, low quality agricultural land, as well as degraded or drastically disturbed soils have been proposed for the cropping of bioenergy cultivars. Our work aimed at assessing the dynamics of soil invertebrate diversity after plantation of perennial energy crops on metal polluted soils. The results were compared with invertebrate diversity dynamics in soils of other plots, representative either for the dominant land occupancies in the study area, or for unpolluted soil situations. We investigated taxonomic, compositional and functional dimensions of diversity in soil- and surface-dwelling communities. Changes in land use from annual crops to perennial energy crops led to a higher number of individuals in soil. No or few changes in taxonomic richness were recorded with an increasing age of energy cropping. Regarding functional diversity, the proportions of resident invertebrates tended to vary with the age of energy cropping, but neither the trophic composition nor the body spectra were modified. Our findings highlighted an increase of soil carrying capacity after perennial energy crop plantation on metal polluted soils, but effects on invertebrate diversity were limited.

1. Introduction

Growing demand for alternative energy sources has contributed to increased biomass production (Fletcher et al., 2011). Under temperate climates of North America and Western Europe, biomass for the production of energy mainly derives from miscanthus, switchgrass and short-rotation coppice cultures of willow and poplar (Ericsson et al., 2009; Qin et al., 2012). To limit the need of fertile agricultural land for the production of bioenergy, the possibility of using land of marginal agricultural potential, but also of degraded or drastically disturbed soils for such purposes was proposed (Lal, 2003). For instance, rehabilitating trace metal contaminated soils by establishing agroenergy plantations would be a win–win strategy (Evangelou et al., 2012), provided that such practices would not lead to increased metal mobility and/or bioavailability.

Several questions have emerged regarding the effects of biomass cropping on soil biodiversity (Fletcher et al., 2011), notably with respect to soil microbes (Liang et al., 2011), arthropods (Landis and Werling, 2010; Robertson et al., 2012; Rowe et al., 2011; Semere and Slater, 2007b), earthworms (Kohli et al., 1999), mammals (Semere and Slater, 2007a) or birds (Robertson et al., 2011; Semere and Slater, 2007a). These studies showed that biomass cropping modifies biodiversity, but in an unpredictable way. Only few of such studies deal with degraded or polluted soils (Hedde et al., in press). One major problem of assessing the effects of perennial biomass production on biodiversity in polluted soil environments lies in the identification and selection of reference or control situations. For a valid assessment, such situations should be representative at one and the same time for soil pollution aspects, for soil nature and for current land use in the area. Such different representativeness aspects are often ignored in broad literature.

Biodiversity assessments are generally based on the structure and the composition of communities. Although useful, those indices do not satisfactorily inform on the mechanisms by which biota respond to environmental stress, since biodiversity is a multidimensional concept (Purvis and Hector, 2000). Functional traits of species relate to characteristics of organisms that affect their individual fitness and govern their impacts and responses to their environment (Violle et al., 2007).

The objectives of the present work were (i) to quantify the dynamics of soil invertebrate diversity during four years after plantation of energy crops on agricultural soils contaminated by heavy metals due to long-term waste water irrigation practices and (ii) to compare these diversity values with those determined for plots representative of the main land occupancies within the wastewater irrigation perimeter, as well as unpolluted situations located outside that perimeter. We aimed at unraveling the modifications of abundance, structure, composition and functions of soil invertebrate communities after biomass crop establishment. We hypothesized that biodiversity would increase with the age of

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energy cropping and tend to reach values observed under perennial land occupancies on comparable soils.

2. Materials and methods

2.1. Sampling plots

Our sampling strategy consisted in selecting different soil situations within a ~1200 ha-wide agricultural area close to Pierrelaye (France, 49°01′N; 2°10′E). This area has been used during the 20th century for spreading of raw wastewater of the Paris urban region and most of the soils have been managed under market gardening. Large amounts of organic matter, dissolved salts (carbonates, phosphates) and metal pollutants, mainly zinc, lead, copper and cadmium accumulated in the surface horizons of these soils (Bourennane et al., 2006; Lamy et al., 2006; van Oort et al., 2008) from wastewater spreading practices. The irrigation doses were high, with average rates estimated at 2000 mm y^{-1} for more than 100 years (Védry et al., 2001). The soils, Orthic and Albic Luvisols (IUSS Working Group WRB, 2006), were sandy textured in the A and E horizons, and sandy-clay textured in the Bt horizon (van Oort et al., 2008).

Two series of plots have been studied. First, an agricultural trial, referred to as "Biomass trial", was designed within the irrigation perimeter in 2007 to assess the impact of energy crops on contaminated soils. Here, we selected six plots that were sampled annually from 2008 to 2011: an annual wheat crop (considered as the Biomass trial control), a Miscanthus crop (Misc1) and a poplar short-rotation coppice (SRC) planted in 2007, as well as three Miscanthus crops planted in 2009 (referred to as Misc2, Misc3 and Misc4) (Table 1). Second, we compared soil biodiversity dynamics in the Biomass trial to levels of biodiversity in five other situations in the study area, representative for the main land occupancies and for unpolluted soils, developed under the same geo-topographical conditions. They are hereafter referred to as 'representative plots' (Table 1): within the irrigation perimeter, three polluted soils represented a panel of land occupancies ranging from perennial land use (a deciduous mixed wood with a well-developed understory, referred to as pol_wood), to pluriannual land use (fallow land referred to as pol_fallow) and annual land use (a wheat crop, referred to as pol_wheat). Outside the irrigation perimeter, two unpolluted reference situations were selected: a deciduous mixed wood with a well-developed understory (unp_wood) and a wheat crop (unp_wheat). All these 11 plots were located within a small area, less than 5 km^2.

2.2. Soil sampling and chemical determinations

In all plots, four sampling sites of 0.5 m^2 were designed, at least distant by 10 m. At the corners of each sampling site, 0–10 cm soil cores were taken using a stainless steel auger (7-cm in diameter) and carefully pooled for analyses.

The grain size distribution, pH and total organic C and N contents of soils were determined according NF X31-107 (2003), ISO 10390 (2005) and ISO 10694 (1995), respectively. Total Zn, Pb, Cu, and Cd concentrations were determined by inductively coupled plasma mass spectroscopy (ICP-MS) performed after tri-acid HF–HCl–HNO_3 digestion (NF X31-147, 1997). In addition, metal availability in soils was determined using two extracting reagents. The ammonium nitrate (NH_4NO_3) extraction was used to estimate the easily exchangeable pool of metals (Prüess, 1992). For that, the soil was homogenized in a solution of NH_4NO_3 1 mol l^{-1} (1/2.5 m/v) (DIN 19730, 1997). In addition, the diethylenetriamine-pentaacetic acid (DTPA) extraction aims at quantifying exchangeable metals but also metals associated with organic matter and with Mn, Fe and Al oxy-hydroxides (Baize and Tomasson, 2003). Extractions were performed using triethanolamine–$CaCl_2$–DTPA (0.005 M) solution at pH = 7.3 (NF X31-121, 1993). After extraction, total, NH_4NO_3- and DTPA-extractable metal concentrations in solution were determined by ICP-MS.

2.3. Invertebrate sampling and identification

From 2008 to 2011, in the first half of April, two soil habitats were sampled by a combination of standard methods for each plot in the center of the 0.5 m^2 sampling sites. Ground-dwelling macroinvertebrates were sampled using pitfall traps. The traps (7-cm diameter) were partly filled with vinegar used as a preservative

Table 1
Location, land occupancy and soil characteristics of the eleven studied plots in the Pierrelaye plain. In addition to total concentration, two pools of available trace metal were determined (extracted by NH_4NO_3 and by DTPA).

			Biomass trial plots						Representative plots				
			Polluted by irrigation						Polluted by irrigation			Not irrigated	
			Wheat control	*Miscanthus x giganteus*				Poplar SRC	Wood	Fallow	Wheat	Wood	Wheat
				Misc1	Misc2	Misc3	Misc4		pol_wood	pol_fallow	pol_wheat	unp_wood	unp_wheat
pH			7.4	7.4	7.6	7.6	7.7	7.2	4.3	7.8	7.4	4.8	8.2
C	Organic	g kg^{-1}	65	57	46	40	42	19	60	34	18	96	11
N	total	g kg^{-1}	2.8	2.4	2.0	1.8	1.9	1.1	3.5	1.3	1.3	4.3	0.9
C/N			23	24	23	23	22	17	17	25	14	23	13
Cu	Total	mg kg^{-1}	233	167	159	136	181	92	14	81	115	16	11
	NH_4NO_3	μg kg^{-1}	793	660	522	510	762	430	83	459	864	140	81
	DTPA	mg kg^{-1}	47	35	33	28	42	18	5	15	40	5	1
Pb	Total	mg kg^{-1}	401	379	288	307	516	176	104	181	173	50	30
	NH_4NO_3	μg kg^{-1}	18	19	12	11	14	17	3118	17	11	856	5
	DTPA	mg kg^{-1}	43	43	34	30	50	18	107	21	29	39	5
Zn	Total	mg kg^{-1}	706	580	559	449	561	361	28	282	421	49	43
	NH_4NO_3	mg kg^{-1}	1.3	0.9	1	0.9	1.1	1.3	5.7	0.5	1.1	12.1	0.1
	DTPA	mg kg^{-1}	139	107	115	95	121	69	14	50	101	29	4
Cd	Total	mg kg^{-1}	3.0	2.5	2.4	1.9	2.6	2.9	0.1	1.6	2.3	0.3	0.3
	NH_4NO_3	μg kg^{-1}	16	13	15	14	17	31	35	15	28	83	4
	DTPA	mg kg^{-1}	1.2	0.9	0.9	0.8	1	1.1	0.1	0.6	1.1	0.3	0.1

medium. Traps were left for 1 week and the collected contents preserved in ethanol (70%) for ulterior identification. On the same sampling sites, soil-dwelling macroinvertebrates were hand sorted from a 50-cm × 50-cm × 20-cm monolith of soil and were also stored in ethanol (70%). In the laboratory, all invertebrates were identified at the family level. In addition, earthworms (Lumbricidae), woodlice (Isopoda), centipedes (Chilopoda), ground beetles (Carabidae), scarab beetles (Scarabaeidae and Aphodiidae), clown beetles (Histeridae), sap beetles (Nitidulidae), ant-like beetles (Anthicidae), crickets (Gryllidae), earwings (Forficulidae) and spiders (except for Linyphiidae, Theridiidae and Gnaphosidae) were identified at the species level. Larvae were attributed to six morphological groups (campodeiform, elateriform, onisciform, melolonthoid, caterpillar, apode) or to families as possible. Other invertebrates were determined as precisely as possible. The Fauna Europaea (2011) work was used as standard for invertebrate taxonomy.

2.4. Community indices

2.4.1. Abundance

Abundance was assessed by invertebrate density in soil and activity at the soil' surface. The density of soil-dwelling invertebrates, expressed as individuals per m^{-2} was calculated on the basis of the abundance obtained by hand sorting. The activity of invertebrates at the soil's surface, expressed as individuals per trap, corresponded to the number of ground-dwelling individuals collected by traps.

2.4.2. Structural diversity

The punctual richness (the number of identified taxonomic units per sample) at the species and family level was calculated as an indicator for taxonomic diversity from the soil- and ground-dwelling datasets. The median values and the standard errors were calculated for each plot and year. For all plots of the energy crop trial, the effect of energy crop age was tested by the Kruskal–Wallis test. *Post hoc* multiple comparison tests were carried out using the 'kruskalmc' procedure of the pgirmess library for R (Giraudoux, 2011).

The significance of differences in results with respect to the Biomass trial control was tested using the Mann–Whitney test. Furthermore, taxa rarity was measured by means of frequencies of singletons and doubletons, *i.e.*, taxa with at most 1 or 2 individuals per sample, and the number of unique and duplicate taxa, *i.e.*, taxa that occurred in only 1 or 2 samples.

2.4.3. Compositional diversity

The compositional diversity is estimated from the similarity in invertebrate assemblages between plots (β-diversity) or within plots (assemblage heterogeneity related to the age of miscanthus cropping). We computed Bray–Curtis dissimilarity indices between taxa pools (α-diversity) of each plot to assess the β-diversity. Assemblage heterogeneity due to the duration of miscanthus cropping was tested by permutational multivariate analysis of variance on dissimilarity matrices. Such an approach partitions the variability in the dissimilarity matrix according to the selected factor(s), with tests of individual terms obtained using permutations (McArdle and Anderson, 2001). We used a multiple analysis of variance approach (1000 permutations) on pairwise Bray–Curtis dissimilarities and calculated pseudo-*F* ratio tests for testing the significance of the age of energy cropping on each plot. For composition diversity computations, we used the 'adonis' procedure of vegan library for R software (Oksanen et al., 2011).

2.4.4. Functional diversity

Determining the functional diversity aimed at reflecting the biological complexity of communities. The establishment of perennial biomass crops implies large modifications of agricultural practices and soil conditions (no ploughing, no use of pesticides), but also a modified carbon cycling (perennial root system, formation of litter at the soil' surface) and a changed plant phenology (winter harvesting). Such modifications may lead to (i) a modification of the body size spectrum of invertebrates, (ii) a change in their trophic guild distribution, (iii) an increase in the proportion of resident invertebrates. Consequently, we focused on three related functional traits: body size, food and dispersal ability. Food was divided in 9 attributes (animals feeding on soil, plant detritus, feces, dead animals, living animals, aerial vegetative plant material, plant fruit/weeds, roots or fungi), the body size in 9 attributes (<2.5 mm, 2.5–5 mm, 5–10 mm, 10–15 mm, 15–20 mm, 20–30 mm, 30–40, 40–50 mm and >50 mm) and the wing morphology in 3 attributes (apterous = wingless invertebrates; brachypterous = invertebrates with reduced or non-functional wings; macropterous = invertebrates with fully functional wings).

Information on functional traits derived from about 180 sources from West-European studies, giving access to the fundamental trait profile of each taxa. Information was pursued, as possible, at the most precise taxonomic resolution. All information was stored in a database called BETSI (for "Biological & Ecological functional Traits of Soil Invertebrates") (Hedde et al., 2012a).

For the analysis of this abundant information, qualitative, semi-quantitative or quantitative data were implemented in a single numerical format by fuzzy coding (Chevenet et al., 1994). A full description of the adaptation of this fuzzy coding to soil invertebrates communities is available in Hedde et al. (in press). We obtained a data set of 188 taxa × 21 attributes, with a global filling rate of about 91%. In addition, we calculated the mean value of community for each attribute of the 3 traits. The community-weighted mean (CWM) value is the sum of the taxa affinities for a trait attribute, weighted by the taxa relative abundance (log transformed) in the community (Garnier et al., 2004; Hedde et al., 2012b).

The CWM food were gathered at broad levels, *i.e.*, fungi, plants (aerial vegetative plant material, plant fruit/weeds, roots), animals (living animals), detritus (soil, plant detritus, feces, dead animals) to illustrate the pathways of energy flows in soil macrofauna communities. Because of its very low proportion (3.3 ± 0.6%), fungi was omitted. Patterns were represented in a triangle plot.

Insight into the body size response in communities can be gained by examining the mean distribution of sizes of individuals, *i.e.*, the body size spectrum. The body size generally interacts with other correlated traits (Henle et al., 2004). For instance, in water streams, large body sizes were associated with a long life span and less than one reproductive cycle per year. Such associations represent an indication for relatively stable habitats with a low frequency and intensity of disturbances (Townsend and Hildrew, 1994). In our work, the mean value of each CWM body size was calculated, log transformed and the body size spectra were constructed yearly for each plot.

Finally, we considered that apterous individuals represented the less mobile, resident invertebrates. We compared the CWM apterous individuals in surface-dwelling invertebrate communities over the plots. For each trial plot, the effect of age of energy crop plantation was tested by Kruskal–Wallis test and *post hoc* multiple comparison test were realized. Significance of difference from the trial control was examined using the Mann–Whitney test. All indices and figures were obtained using ade4 package included in R software (Dray and Dufour, 2007).

3. Results

3.1. Soil characteristics

The grain size distribution was dominated by the sand content (74–84%) and soils contained less than 10% of clay. Soil pH (Table 1) varied from 4.3–4.8 in wood soils to 7.2–7.8 in irrigated agricultural soils and 8.3 in non-irrigated agricultural soil. By contrast, the soil organic C and total N contents clearly differed between the plots (Table 1). Remarkable diverging amounts of soil organic matter were observed in non-irrigated soils, *i.e.*, the wood soil (C_{org} = 96 g kg^{-1}; N_{tot} = 4.3 g kg^{-1}; C/N = 23) and the wheat crop soil (C_{org} = 11 g kg^{-1}; N_{tot} = 0.9 g kg^{-1}; C/N = 13). Intermediate values were obtained in irrigated soils (C_{org} = 18–65 g kg^{-1}; N_{tot} = 1.1–3.5 g kg^{-1}; C/N = 14–25). In polluted reference plots, these values increased for soils under wheat crop to fallow soils and to soils under wood stand.

Concentrations of total Zn, Pb, Cu, and Cd were higher in plots located within than outside the irrigated perimeter, except for the mixed wood (Table 1). Other irrigated soils exhibited 81–233, 173–516, 282–706 and 1.6–3.0 mg kg^{-1} in Cu, Pb, Zn and Cd, respectively. The lowest contamination levels were recorded in soils under the poplar short rotation coppice.

The concentrations of Cd, Cu and Zn extracted by DTPA were strongly correlated (R^2 > 0.90) with total metal contents (Table 1). Similar results was recorded for Pb extracted by DTPA (R^2 = 0.93) and for Cu, Zn, Cd extracted by NH_4NO_3 (R^2 > 0.6), if woods were omitted. Finally, Pb extracted by NH_4NO_3 did not depend on total content (Table 1). Highest value was recorded in polluted and unpolluted woods (3.1 and 0.9 mg kg^{-1}, respectively) and the lowest in unpolluted wheat crop (5 μg kg^{-1}). Values observed in the other plots were comprised between 11 and 19 μg kg^{-1}.

3.2. Abundance and activity of soil invertebrates

Overall, 4496 individuals were collected and 188 taxa were identified, including 94 species, 63 families and 23 orders. In the reference plots, the density of soil-dwelling invertebrates ranged from 2 to 210 individuals per m^2 (polluted wheat crop and unpolluted wood, respectively) (Table 2). In the Biomass trial plots, these values ranged from 0 to 84 individuals per m^2 during the 1st year of poplar SRC and the 3rd year of Misc1, respectively. While the densities increased with the age of energy cropping, this effect was significant only for Misc1 (χ^2 = 8.72, p = 0.033), Misc3 (χ^2 = 6.20, p = 0.045) and poplar SRC (χ^2 = 11.32, p = 0.010), but not for Misc2 and Misc4 (χ^2 > 0.500). All densities recorded in energy crop plots differed from the trial wheat control, except for the 1st year in Misc2 and Misc4.

The activity of soil-dwelling invertebrates in the reference plots ranged from 3 to 24 individuals per trap for polluted fallow and unpolluted wood, respectively. In the Biomass trial plots, these values ranged from 1 to 60 individuals per trap during the 1st and the 3rd year of Misc3, respectively (Table 2). An effect of age of energy cropping was detected for Misc1 (χ^2 = 9.83, p = 0.020), Misc2 (χ^2 = 7.38, p = 0.025), Misc3 (χ^2 = 7.45, p = 0.024) and for poplar SRC (χ^2 = 10.43, p = 0.012) but not for Misc4 (χ^2 > 0.500). However, the surface-dwelling invertebrate activity did not always increase with the age of energy cropping. For instance, maximum values were obtained for the 1st year and the 3rd year in Misc1 and poplar SRC, respectively. For Misc1, the activity differed from the Biomass trial control by a higher value only in the 1st year. For Misc2, Misc3 and Misc4, values lower than for the biomass control were recorded for the 1st year (1–3 individuals per trap) but they were higher for the 2nd and the 3rd year (45–60 individuals per trap). Poplar SRC presented a higher activity than the biomass control only for the 3rd year (47 individuals per trap).

Table 2
Median values (±standard deviation) of density, activity and diversity (species and family number) in soil- and surface-dwelling invertebrate communities. Plot codes are detailed in Table 1 For soil under energy crops (Misc1–4 and SRC), different letters indicate significant differences between years for a given energy crop plot and asterisks indicate significant difference with Biomass trial control (under wheat).

Plot	Energy crop age	Soil-dwelling invertebrates			Surface-dwelling invertebrates		
		Total density (individuals m^{-2})	Species diversity (species m^{-2})	Family diversity (family m^{-2})	Total activity (individuals per trap)	Species diversity (species per trap)	Family diversity (family per trap)
unp_wheat		88 (29)	2.0 (1.0)	1.0 (0.5)	9 (3)	3.5 (1.7)	3.5 (1.0)
unp_wood		210 (79)	6.0 (1.8)	10.5 (2.4)	24 (19)	5.0 (3.5)	5.5 (3.7)
pol_wood		126 (55)	7.0 (3.1)	8.5 (2.6)	16 (12)	3.0 (2.7)	4.0 (2.2)
pol_fallow		58 (224)	2.0 (0.5)	2.5 (1.0)	2 (9)	0.0 (1.5)	2.0 (3.6)
pol_crop		2 (4)	0.0 (0.0)	0.0 (0.0)	16 (7)	3.0 (2.6)	4.0 (1.5)
Trial wheat control		8 (5)	0.0 (0.6)	1.0 (0.6)	24 (11)	4.0 (2.5)	6.5 (3.0)
Misc1	1 year	26 (2) b*	1.0 (1.5) a*	2.0 (0.5) b*	51 (12) a*	7.5 (2.5) a	7.0 (1.3) a
Misc1	2 years	48 (31) ab*	1.5 (2.2) a*	3.0 (0.5) ab*	14 (8) b	4.5 (2.2) a	5.5 (3.7) a
Misc1	3 years	84 (132) a*	2.5 (2.1) a*	3.0 (2.6) ab*	26 (9) ab	7.5 (1.3) a	10.0 (2.5) a*
Misc1	4 years	50 (40) ab*	3.5 (1.9) a*	6.5 (3.3) a*	22 (11) ab	5.5 (2.2) a	10.0 (2.8) a*
Misc2	1 year	10 (20) a	0.5 (1.4) a	1.5 (1.0) b*	2 (4) b*	1.0 (0.5) b*	1.5 (2.0) b*
Misc2	2 years	48 (19) a*	2.0 (1.3) a*	5.5 (1.0) a*	50 (19) a*	13.5 (1.0) a*	7.0 (1.5) a
Misc2	3 years	58 (66) a*	3.0 (3.6) a*	5.0 (3.0) a*	45 (18) a*	6.0 (3.6) ab	9.5 (2.0) a
Misc3	1 year	28 (22) b*	1.0 (1.5) a	2.0 (0.0) b*	1 (4) b*	0.0 (0.0) b*	1.0 (1.0) b*
Misc3	2 years	40 (5) ab*	3.0 (2.4) a*	3.5 (1.0) ab*	34 (29) ab	9.0 (5.3) a	9.0 (5.0) a
Misc3	3 years	70 (28) a*	3.5 (1.0) a*	5.5 (1.3) a*	60 (24) a*	6.0 (1.8) a	10.5 (2.8) a*
Misc4	1 year	18 (25) a	1.5 (1.0) a*	2.0 (0.8) a*	3 (5) b*	0.0 (0.5) b*	2.0 (2.6) b*
Misc4	2 years	74 (72) a*	2.5 (2.1) a*	6.5 (3.9) a*	40 (21) a*	7.5 (6.1) a	9.5 (4.5) a
Misc4	3 years	54 (59) a*	3.5 (2.4) a*	3.5 (2.1) a*	49 (15) a*	5.0 (1.7) a	7.5 (2.9) ab
SRC	2 years	42 (20) ab*	1.5 (1.0) a*	2.5 (0.6) b*	28 (32) ab	6.5 (2.4) a	5.0 (1.3) b
SRC	3 years	36 (16) b*	1.0 (1.3) a*	2.0 (1.3) b*	47 (21) a*	8.5 (2.4) a	9.0 (2.8) a
SRC	4 years	64 (21) a*	3.5 (1.7) a*	6.5 (0.6) a*	17 (7) b	5.5 (1.7) a	6.0 (1.7) ab

Table 3
β-Diversity (Bray–Curtis dissimilarities) between macroinvertebrate communities in energy crop (Misc1–4 and poplar SRC) soil and plots representative of the main land occupancies in the Pierrelaye plain. β-Diversity was reported for each crop age, and for soil- and surface-dwelling invertebrate communities. Plot codes are detailed in Table 1.

Plot	Age	Soil-dwelling communities						Surface-dwelling communities					
		unpol_wood	pol_wood	fallow	pol_wheat	unpol_wheat	trial_control	unpol_wood	pol_wood	fallow	pol_wheat	unpol_wheat	trial_control
Misc1	1	0.92	0.92	0.96	1.00	1.00	0.69	0.85	0.78	0.81	0.85	0.85	0.71
Misc1	2	0.83	0.80	0.93	0.97	1.00	0.84	0.82	0.72	0.82	0.95	0.87	0.82
Misc1	3	0.85	0.84	0.90	0.95	1.00	0.91	0.86	0.76	0.8	0.91	0.79	0.60
Misc1	4	0.82	0.79	0.91	0.91	1.00	0.83	0.79	0.81	0.81	0.93	0.73	0.65
Misc2	1	0.90	0.89	0.98	0.90	0.98	0.63	0.72	0.78	0.85	0.96	0.64	0.79
Misc2	2	0.83	0.79	0.91	0.88	0.98	0.77	0.79	0.82	0.90	0.98	0.9	0.76
Misc2	3	0.85	0.84	0.94	0.95	1.00	0.85	0.85	0.78	0.84	0.96	0.85	0.55
Misc3	1	0.86	0.82	0.93	0.92	1.00	0.67	0.97	0.95	0.97	1.00	0.93	0.93
Misc3	2	0.84	0.80	0.94	0.95	0.98	0.75	0.66	0.72	0.86	0.91	0.85	0.67
Misc3	3	0.86	0.85	0.95	1.00	0.99	0.88	0.81	0.80	0.86	0.98	0.88	0.65
Misc4	1	0.89	0.85	0.94	0.94	1.00	0.71	0.75	0.78	0.84	0.96	0.70	0.78
Misc4	2	0.85	0.80	0.93	0.93	0.99	0.87	0.66	0.77	0.89	0.92	0.87	0.71
Misc4	3	0.86	0.83	0.93	0.95	1.00	0.82	0.83	0.81	0.84	0.98	0.86	0.70
SRC	2	0.93	0.93	0.92	0.88	1.00	0.85	0.92	0.82	0.91	0.99	0.94	0.85
SRC	3	0.92	0.94	0.93	0.90	1.00	0.78	0.88	0.79	0.89	0.97	0.91	0.71
SRC	4	0.86	0.88	0.93	0.92	1.00	0.83	0.82	0.60	0.79	0.93	0.76	0.64

3.3. *Structural diversity*

Among the 72 species collected in the Biomass trial plots, about half can be considered as satellite species, 44% of species were found in less than 3 individuals (22 singletons and 10 doubletons) and 47% in less than 3 samples (25 in one sample and 9 duplicates). Core species (*i.e.*, species present in more than 10% of the samples) were the ground beetles *Amara aenea* (De Geer 1774), *Harpalus affinis* (Schrank 1781), *Harpalus distinguendus* (Duftschmid 1812), *Harpalus tardus* (Panzer 1797), *Bembidion (Metallina) lampros* (Herbst 1784) and *Poecilus lepidus* (Leske 1785), the beetle *Onthophagus ovatus* (Linnaeus 1767), and the wolf-spiders *Pardosa agrestis* (Westring 1861) and *Trochosa ruricola* (De Geer, 1778).

In soils of the representative plots, the median punctual species richness varied from 0 (the two polluted wheat crop soils) to 2 (the polluted fallow and the unpolluted crop soils) and to 6–7 (wood soils) (Table 2). The punctual family richness presented a rather similar ranking of plots. All wheat crops presented 0–1 families, the polluted fallow 2 families and woods 8.5–10.5 families (median values). All energy crops hosted more numerous species (1.0–3.5) and families (1.5–6.5) than the trial control (0.0 and 1.0, respectively). The increase of the median punctual species richness with the age of energy cropping was not supported by statistical analyses. Contrarily, the median punctual family richness increased with the age of energy cropping in Misc1, Misc2, Misc3 and in the poplar SRC, reaching up to 6.5 families.

At the soil' surface of representative plots, the median punctual species number significantly varied from 0 (polluted fallow) to 3–5 (other plots) (Table 2). The median punctual family number ranged from 2 (polluted fallow), to 3.5–4 (unpolluted and polluted wheat crops and the polluted wood), and to 5.5–6.5 (unpolluted wood and trial control). The plots Misc2, Misc3 and Misc4 hosted a significantly lower punctual species number in the 1st year than observed in the trial wheat control, and a low significant increase in species (Misc2 in the 2nd year) and in family (Misc1 in the 3rd and the 4th year and Misc3 in the 3rd year).

3.4. *Composition diversity*

The results of β-diversity are presented in Table 3. The Bray–Curtis dissimilarities were large, with average values of 0.90 (±0.08) and 0.82 (±0.10) for soil- and surface-dwelling invertebrates. The smallest dissimilarities with energy crops were found for the Biomass trial control (0.79 and 0.72, respectively). The dissimilarities with respect to the Biomass trial control tended to increase with the age of energy cropping.

The age of energy cropping significantly acted on assemblage heterogeneity in soils under Miscanthus (Table 4), both at the species and family levels, and for soil and surface communities. Family heterogeneity was significantly influenced, or tended to be ($p < 0.08$), except for surface communities in the poplar SRC plot. Patterns in species heterogeneity within plots were less clear with a non-significant result for Misc2 (soil invertebrates) and Misc4 and the poplar SRC plots (surface invertebrates).

3.5. *Functional diversity*

3.5.1. *Trophic guilds distribution within invertebrate communities*

The reference soils presented three types of soil-dwelling communities (Fig. 1). The unpolluted arable soil was dominated by detritivores (62% of geophages, 37% of saprophages). Woods and fallow hosted 55–64% of detritivores, 20–31% of zoophages and 13–16% of phytophages. Finally, polluted soils under wheat crops presented a low proportion of phytophages (13–20%) and detritivores (30%), but a high proportion of zoophages (50–56%). The

Table 4
Significance of heterogeneity in taxa composition within plot due to energy crop aging. It corresponds to significance of pseudo-*F* ratio tests for aging effect on Biomass trial plots after pairwise Bray–Curtis dissimilarities and MANOVA approach with 1000 permutations. Plot codes are detailed in Table 1.

		Family	Species
Soil-dwelling	Misc1	0.081	**0.003**
	Misc2	**0.022**	0.388
	Misc3	0.057	**0.008**
	Misc4	0.064	**0.017**
	Poplar SRC	**0.033**	**0.024**
Surface-dwelling	Misc1	**0.003**	**0.004**
	Misc2	**0.001**	**0.018**
	Misc3	**0.002**	**0.020**
	Misc4	**0.006**	0.156
	Poplar SRC	0.348	0.133

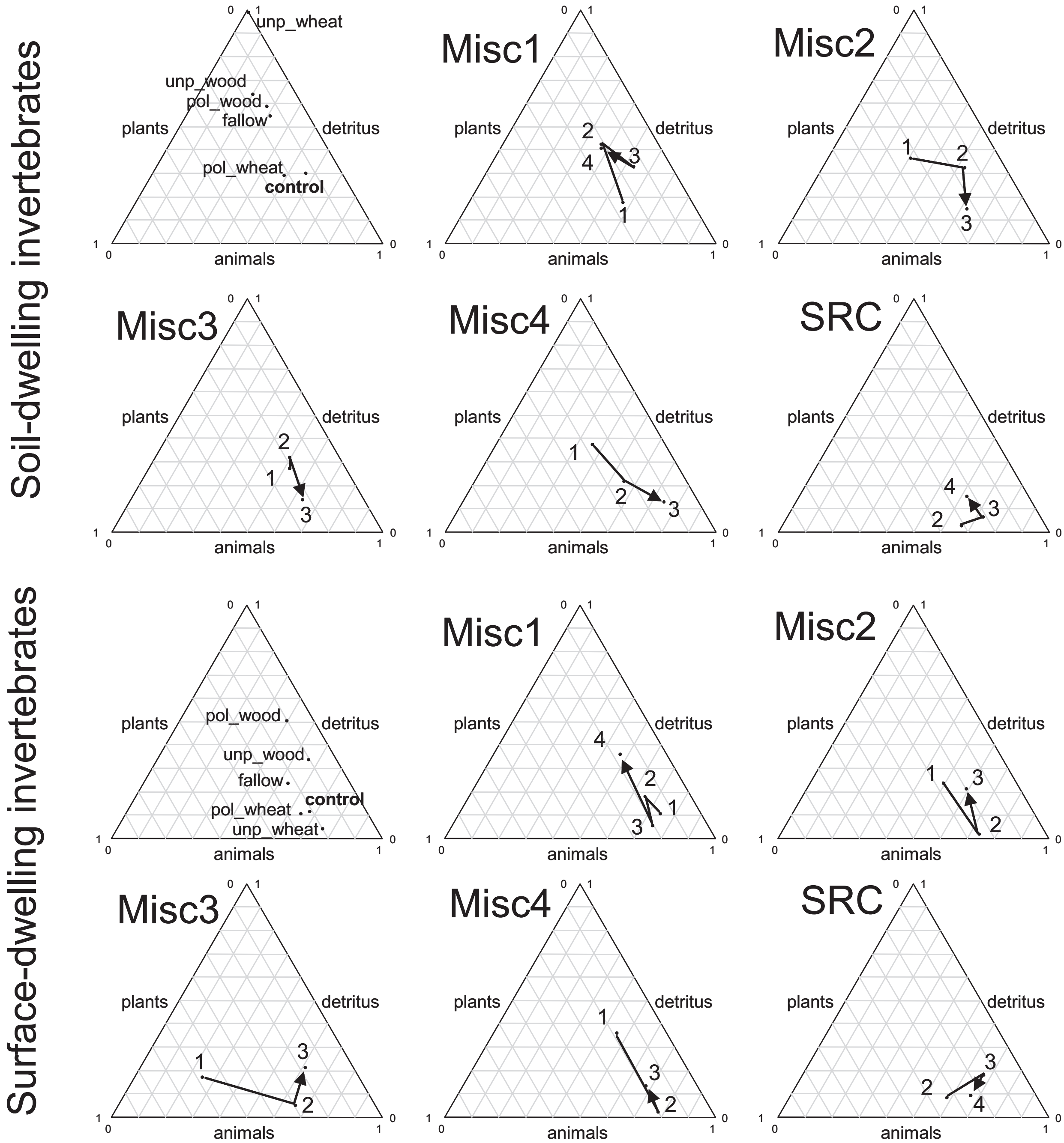

Fig. 1. Triangle representation of community-weighted mean (CWM) indice for food preference in soil- and surface-dwelling invertebrate communities. Upper-left triangle of each series (soil and surface communities) displayed the values obtained in plots representative of the main land occupancies in Pierrelaye plain (codes are detailed in Table 1). The five remaining triangle representations displayed the CWM changes in each Biomass trial plot (Misc1–4 and poplar SRC) over 3 or 4 years.

distribution in soils under Miscanthus was comparable to that of polluted soils under wheat crop, with a dominance of zoophages (30–74%) and low proportions of detritivores (13–37%) and phytophages (12–32%). Under poplar SRC, very low proportions of detritivores (3–15%) were recorded.

The surface-dwelling communities had large proportions of zoophages, with a median value of 65% and a minimum value of 50%, except in the 1st year for Misc3 (24%) (Fig. 1). The proportion of detritivores increased from crop soils (contaminated or not) to fallow, and to forest soils, with values of <10%, 23% and >33%, respectively. No clear common dynamic was identified after plantation of energy crops, their communities being similar to those of the Biomass trial wheat control.

3.5.2. Body size spectra of invertebrate communities

The body size spectra of soil-dwelling communities in the representative soils presented different shapes (Fig. 2). Unimodal distributions were recorded with a peak either for very small, <10 mm sized invertebrates (in the polluted wheat plot, in the Biomass trial wheat control and the unpolluted wood), or for larger invertebrates (in the unpolluted wheat plot). Bimodal distributions were observed for communities under fallow land and in wood soils located in the irrigated perimeter, with a similar minimum peak corresponding to 5–10 mm invertebrates. Consequently, arable contaminated plots tended to have larger proportions of small invertebrates, whereas uncontaminated and/or perennial plots had the highest proportion of large invertebrates. No clear modification

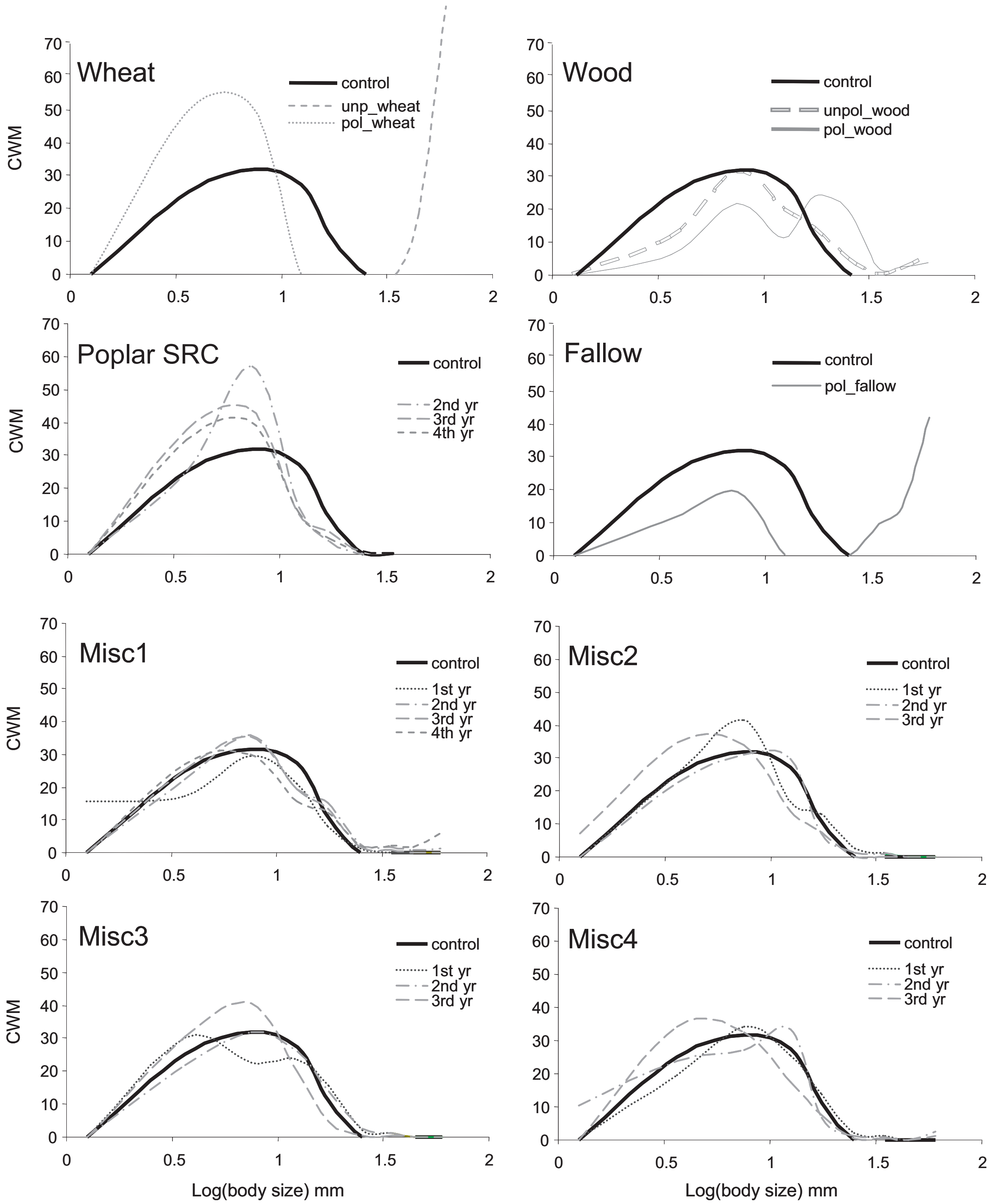

Fig. 2. Comparison of body size spectra for soil-dwelling invertebrate communities. It corresponds to the distribution of community-weighted mean (CWM) over increasing body size (log-transformed). The spectrum of Biomass trial control was displayed on each sub-figure to facilitate visual comparison. Sub-figures dedicated to energy crops showed body size spectra for 3 or 4 years after energy crop establishment. Plot codes are detailed in Table 1.

of the body size spectrum of invertebrates was observed under Miscanthus when compared to the trial wheat control. Only a slight modification was noted under poplar SRC with a narrower spectrum peaking for 5–10 mm individuals.

The body size spectrum of surface-dwelling communities in the soils of the reference plots also presented different shapes (Fig. 3). The distributions for woods, fallow and the unpolluted wheat plots tended to be bimodal, contrarily to unimodal distribution patterns observed for the trial control and polluted wheat plot. Three main peaks were observed. A peak for 2.5–5 mm individuals was shared by communities of the trial control, the unpolluted wheat and the polluted fallow plots. Similarly, a second mode was observed for 7.5–10 mm individuals in communities collected in the fallow, the polluted wheat and the unpolluted wood soils. Finally, a peak for largest invertebrates (20–30 mm) was recorded in woods and fallow communities. In Miscanthus crops, the body size spectrum was always unimodal, with a slight tendency toward larger individuals, but no influence of the age of Miscanthus cropping was observed. Similarly, in the poplar SRC plot, communities tended to present a higher proportion of larger individuals. Additionally, a

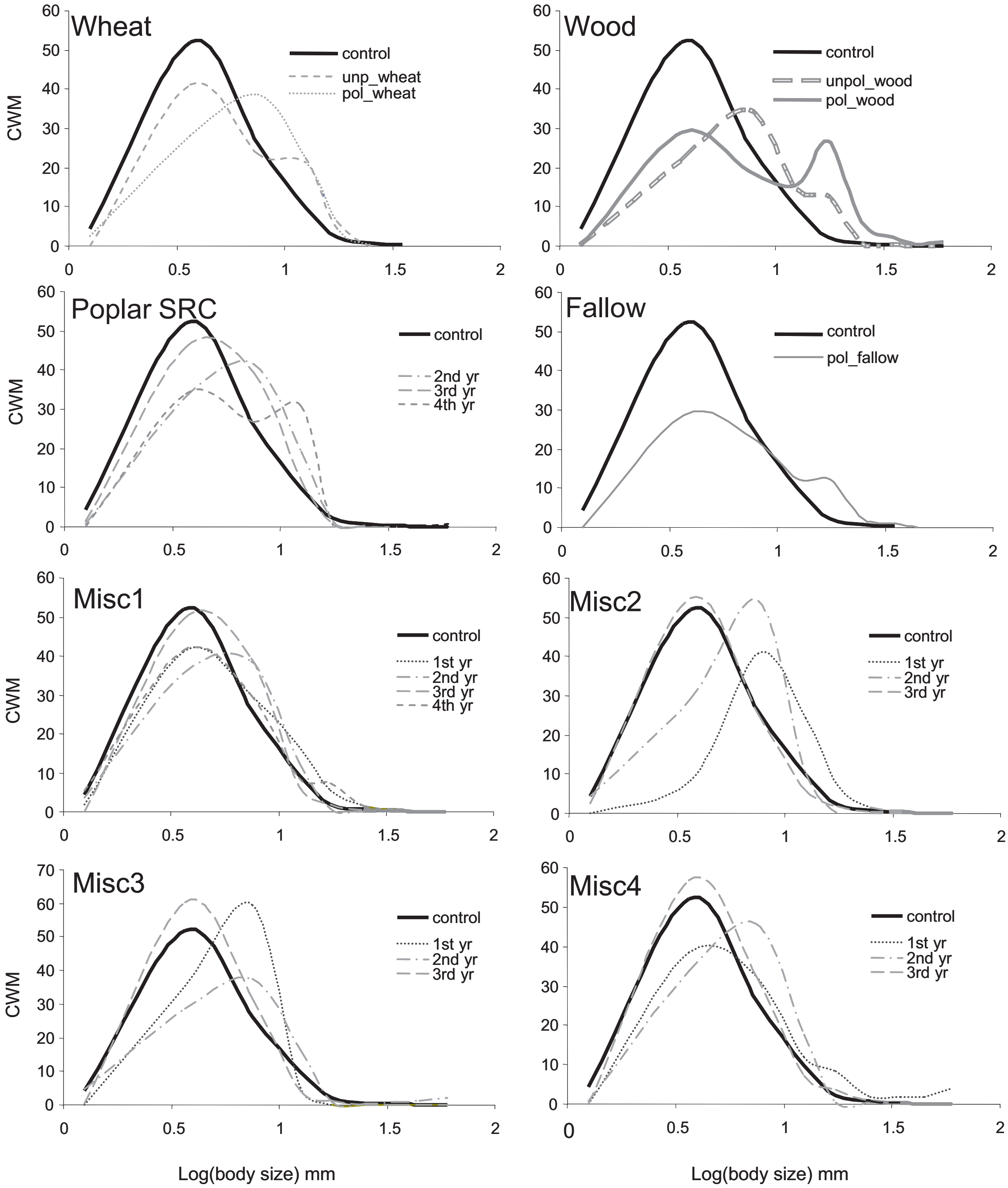

Fig. 3. Comparison of body size spectra for surface-dwelling invertebrate communities. It corresponds to the distribution of community-weighted mean (CWM) over increasing body size (log-transformed). The spectrum of Biomass trial control was displayed on each sub-figure to facilitate visual comparison. Sub-figures dedicated to energy crops showed body size spectra for 3 or 4 years after energy crop establishment. Plot codes are detailed in Table 1.

bimodal distribution was observed, 4 years after poplar plantation, with peaks for 2.5–5 and 10–15 mm individuals.

3.5.3. Dispersal ability

In the reference plots, CWM apterous individuals discriminated perennial land use (woods and fallow, CWM > 66%) and annual wheat crops (CWM = 18–38%) (Fig. 4). In Misc2, Misc3 and Misc4, very few CWM apterous individuals were observed in the early years (20–43%), while in Misc1 they corresponded to a maximum value (76%). In these plots, CWM apterous individuals reached 40–63% after 2–4 years. The poplar SRC plot showed comparable values than Misc1 during the period from 2 to 4 years. After 3 years, all energy crops presented significantly higher CWM apterous individuals than in the Biomass trial wheat control plot.

4. Discussion

4.1. Soil characteristics

In non-irrigated areas, different land occupancy coincided with highly diverging pH values and total organic C and N contents, from poor, alkaline agricultural soils to rich, acidic soils under woods (Table 1). Within the irrigation perimeter, one century of high wastewater spreading (Védry et al., 2001) led to soils with

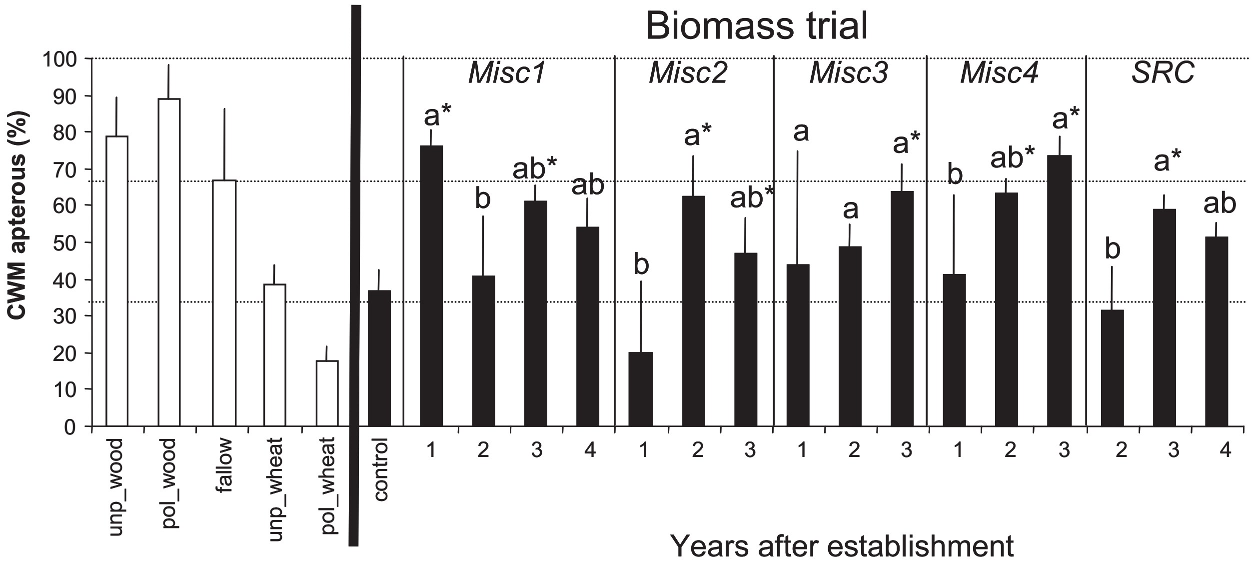

Fig. 4. Mean (and standard deviation) of community weighted mean (CWM) apterous individuals in the studied plots. White barplots correspond to plots representative of the main land occupancies in Pierrelaye plain, black barplots represent energy crop plots. Plot codes are detailed in Table 1.

outstanding high organic matter contents for such sandy soils and a soil pH of about 7.5. The small variation in pH values observed for irrigated agricultural soils was ascribed to the buffering action of large amounts of organic matter added by wastewater irrigation, despite the presence of secondary carbonates (van Oort et al., 2008). Concentrations in total trace metals were higher in plots located within than outside the irrigated perimeter, except for the mixed wood. Surprisingly, this mixed wood soil showed low metal concentrations, except for Pb. This probably resulted from high metal mobility in relation with the low soil pH in this soil under wood stand, as was reported for other acidified forest soils (van Oort et al., 2009). The variability of total metal concentrations in the selected plots was consistent with the more large-scale spatial metal distribution patterns reported by Bourennane et al. (2006) for this wastewater irrigation perimeter. Available trace metal concentrations were strongly correlated with total metal contents, at least if woods were omitted, except for Pb extracted by NH_4NO_3 that did not depend on total content.

4.2. Invertebrate abundance

Our results showed that the cultivation of perennial energy crops on trace metal contaminated soils led to an increase of both the density and the activity of invertebrates. After two years of miscanthus implantation, the density of soil-dwelling invertebrates increased by a 3- to a 9-fold, when compared with the annual wheat Biomass trial control. It is generally stressed that biomass plantations have longer rotation periods, low fertilizer and pesticide requirements which provide an improved soil protection and a greater richness of microhabitats. Furthermore, harvesting is carried out in winter causing less impacts on soil biota (Dauber et al., 2010). For instance, Felten and Emmerling (2011) found earthworm densities in soils under 12-years old miscanthus to be intermediate between annual crops (rapeseed, maize, cereals) and perennial vegetation (fallow, grassland). Similarly, the values of soil-dwelling communities in miscanthus cropped soils (28–84 individuals per m^2) were comparable to those found for the unpolluted wheat crop and the polluted fallow but did not reach densities generally reported for woods. All modifications of habitat favored the establishment of more numerous soil-dwelling invertebrate communities, when compared with the Biomass trial control, but had no significant effect on surface-dwelling communities.

4.3. Invertebrate structural diversity

We found at least the same level of structural diversity in soils under biomass energy crops than for the Biomass trial control and in some cases, this diversity was enhanced. Our results did not fully corroborate the statement that the biodiversity hosted under biomass plantations is generally larger than in comparable arable land soils (Landis and Werling, 2010; Semere and Slater, 2007b). Regarding the age of the energy crop plots, our results showed that the number of invertebrate families slightly increased in the first years after changing land occupancy. The number of soil-dwelling families rapidly increased to 6.5, a value which was intermediate between the polluted fallow (2.5) and the woods (8.5–10.5). Changing soil conditions following biomass plantations (longer rotation periods, low fertilizer and pesticide requirements) are mentioned to promote diversity (Dauber et al., 2010). As for densities, Felten and Emmerling (2011) found earthworm species richness under miscanthus to be intermediate between annual crops and perennial land uses.

Although a trend was observed in the present work, the species number was not found discriminant with respect to the age of energy crop plantation. Even if a lot of invertebrates were identified at species level (94 species in total), communities were constituted by high proportions of life forms that are difficult to identify accurately, *e.g.*, juveniles, larvae or pupae. However, new opportunities arise from DNA barcoding to overcome this well-known frontier in soil ecology (Richard et al., 2010). In the present work, rare species (*i.e.*, sum of singletons and doubletons) represented about 44% of identified species. Although high, this value is consistent with data obtained by Auclerc et al. (2012) in the French Vosges Mountains (34–40%) and were even lower than values reported by Rossi et al. (2006) in Brazilian pastures (57–67%). In theory (Novotny and Basset, 2000), the prevalence of rare species may be ascribed to sampling artifacts (transient or inadequately sampled species), suffusive rarity (specialists with genuinely low population) or diffusive rarity (species present in the plot but whose preferred conditions occurred in a plot in the proximity). Our results did not permit to hierarchize those mechanisms. Studies at a larger scale and including a higher number of pseudo-replicates per plot are needed to assess the respective role of each mechanism on species rarity in biomass energy crops.

4.4. Composition of invertebrate communities

Species and family heterogeneity in energy crops showed high changes over years, revealing that the composition of communities was unstable over the study period. Our results on β-diversity showed that communities in perennial energy crop plots greatly differed from the plots representative of the main land occupancy of the plain. High levels of β-diversity resulted at least partly from the high proportion of rare species we discussed previously. The dynamics of β-diversity between energy crops and Biomass trial control varied between soil- and surface-dwelling invertebrates. Hence, soil community β-diversity increased with the age of energy cropping, implying that changes in soil conditions led to the selection of a different pool of taxa. The surface community β-diversity presented an opposite trend. Such a result was counterintuitive since miscanthus crops and poplar stands modify soil surface habitat, notably by litter production.

4.5. Functional diversity

4.5.1. Trophic guild distribution

Energy crop establishment favored an increasing proportion of detritivore animals. Paoletti et al. (2007) assumed that detritivores are good indicators of landscape stress and soil degradation. These animals play a crucial role in soil behavior, notably for organic carbon turn-over, soil aggregation and nutriment supply (Lavelle and Spain, 2001). However, communities still include significant proportions of zoophages. These animals probably fed on mesofauna (springtails or mites) but intra-guild predation was also likely to occur (Polis et al., 1989).

4.5.2. Body size spectra

Energy crop establishment had little or no effect on body size spectra of soil-dwelling community, the latter being comparable to those of the Biomass trial control. However, during some years, surface-dwelling communities presented higher proportions of larger individuals. This can result from at least two main mechanisms: the reduced use of pesticide and the development of a litter layer at the soil's surface (Dauber et al., 2010; Semere and Slater, 2007b). We can note that the collected invertebrates in such sandy soils are rather small, with few individuals larger than 30 mm in the soil, or larger than 15 mm at the soil' surface. It would be interesting to test if such limits are site-dependent or have a more general application. Regarding the representative soils, both soil- and surface-dwelling communities tended to present bimodal spectrum under perennial land occupancy (woods and fallow). Contrarily, contaminated annual crops were characterized by communities with unimodal distribution. Recently, Thibault et al. (2011) stated that relatively few body size spectra have been characterized in terrestrial systems and that they reveal substantial variability in structure. Based on broad literature, these authors identified three major shapes: monotonically decreasing, unimodal and multimodal. Our study showed that body size distribution of soil and surface macroinvertebrate communities are likely to correspond to the two latter shapes. Allen et al. (2006) proposed community interaction and textural discontinuity hypotheses as mechanisms for body size distribution acting from local to regional scales over decades to a century. Among these two hypotheses, our results more supported the textural discontinuity hypothesis since it predicts that the distribution will be resistant to temporal change until a threshold is reached, in spite of probable shifts in the abundance and identities of species. Once this threshold is exceeded, the system likely evolves to a new system state.

4.5.3. Resident invertebrates

Annual crops exhibited higher proportions of mobile individuals, whereas the woods, and to a lower extent the fallow, had more resident ones. This demonstrates that soil plowing, rather than soil pollution, is the driving force for the proportion of resident individuals in macro-invertebrate communities in soils of the study area. After plantation of miscanthus, the proportion of apterous individuals tended to increase with the age of energy cropping, but no clear unique pattern was shared by all plots. Maximal values were still lower than those observed in woods and fallow. These findings are consistent with results demonstrating the relationships between disturbances and invertebrate dispersal abilities (Feio and Dolédec, 2012; Gerisch et al., 2012; Uys et al., 2009). For instance, Ribera et al. (2001), Sadler et al. (2006) and Venn (2007) showed that the proportion of macropterous ground beetles was greater in the populations of disturbed sites.

5. Conclusion

The establishment of perennial energy crop on contaminated soil had neutral to positive effects on soil invertebrate communities, either at the soil's surface or within the soil. The used sampling strategy of metal polluted soils under energy crops and a series of selected reference situations including annual and perennial land occupancies as well as polluted/unpolluted gave a balanced view on the role of land use and soil pollution on changes in biodiversity. Our findings on the effect of plantation of biomass crops on soil invertebrates must be viewed in the habitat template concept. This concept relates trends in species functional traits to patterns in habitat variation and disturbance, which are recognized as filters for traits. In the present study, energy crop establishment in metal contaminated area led to a higher soil carrying capacity (in terms of number of individuals and of taxa), and to a stabilization of taxonomic diversity after 3–4 years. The communities were characterized by a taxonomic heterogeneity of biologically equivalent taxa (in terms of size, food and mobility) resulting in a high β-diversity with the reference plots. Such results highlighted that biomass crop establishment did not modify current environmental filtering. We demonstrated how functional traits can improve taxonomic and compositional assessment of biodiversity that often represent misleading parameters for the interpretation of the effect of anthropogenic changes on soil fauna. Nevertheless, much work remains to be done before such a trait-based approach will become a more universal biomonitoring tool.

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