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ORIGINAL ARTICLE

Perennial alternative crops for biogas production increase arthropod abundance and diversity after harvest – results of suction sampling and metabarcoding

Johannes BURMEISTER[1], Bernd PANASSITI[1], Franziska HEINE[2], Sebastian WOLFRUM[1] and Jerome MORINIERE[3]

[1] Bavarian State Research Center for Agriculture (LfL), Lange Point 6, 85354 Freising, Germany;
e-mails: johannes.burmeister@lfl.bayern.de, bernd.panassiti@lfl.bayern.de, sebastian.wolfrum@lfl.bayern.de

[2] School Center Wangen – Agricultural Schools, Jahnstraße 19, 88239 Wangen im Allgäu, Germany;
e-mail: franziska.heine94@web.de

[3] Advanced Identification Methods – AIM GmbH, Leipzig, Germany; e-mail: jerome.moriniere@aim.science

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Abstract. The effects of novel perennial energy crops on arthropod abundance and diversity were tested using three field trials in Bavaria. Established cup plant (*Silphium perfoliatum*), Virginia mallow (*Sida hermaphrodita*) and tall wheat grass (*Thinopyrum ponticum*) plots were sampled in autumn using a modified leaf blower and their arthropod faunas compared with that recorded in maize plots. At the time of sampling, maize, cup plant and Virginia mallow had already been harvested and samples from the remaining stubble were compared with those from open ground. Collembola were the most abundant group. The highest abundance and diversity of arthropods was recorded in tall wheat grass, with Hemiptera and Aranea especially benefitting from the green and taller vegetation. Post-harvest cup plant and Virginia mallow plots harboured a more diverse and more abundant arthropod assemblage than maize plots. Stubble was identified as a suitable habitat for arthropods in agricultural fields. In conclusion, the integration of perennial energy crops into existing biogas production systems could play an important role in supporting arthropods in agricultural landscapes by providing crucial structures and resources, such as the grassy vegetation of tall wheat grass in autumn and the long-lasting stubbles of cup plant and Virginia mallow.

1. INTRODUCTION

Land use intensity is acknowledged to be one of the main drivers of biodiversity loss in temperate agroecosystems (Kleijn et al., 2009). In arable landscapes, land use intensity is defined in terms of pesticide use, tillage, application of fertilizers, crop selection and rotation. In addition, the presence of hedgerows and other unproductive habitats in these landscapes needs to be considered. Increasing intensity may reduce the habitat quality at both field and landscape scales (Tscharntke et al., 2021; Rosenfield et al., 2021). Several recent studies report a serious decline in insect biodiversity and abundance in agricultural landscapes (Hallman et al., 2017; Seibold et al., 2019; Wagner et al., 2021). But on the other hand, agricultural landscapes harbour a great number of arthropods that contribute to the high local biodiversity recorded over many centuries in temperate regions. Many of these arthropods are thought to provide ecosystem services (Isaacs et al., 2009; Salman et al., 2019). Therefore, sustainable land use that maintains or restores biodiversity and maximize ecosystem services should be implemented.

In addition to the challenge to conserve biodiversity and produce food, farmland is increasingly needed to produce renewable energy. To reduce greenhouse gas emissions the German Renewable Energy Sources Act (EEG) fostered bioenergy production. Consequently, the number of biogas plants in Germany increased from about 850 in 2000 to 9,311 in 2017 (Yang et al., 2021). The associated, increase in cultivation of maize (*Zea mais* L., Poaceae) for the biogas plants and resultant loss of set aside land and field borders due to increasing land costs and management intensity lead to a controversy over the environmental and socio-economic effects of this policy (Dauber et al., 2010; Gevers et al., 2011; Robertson et al., 2012).

When compared to oilseed rape, potatoes, or even cereals, maize requires fewer pesticide treatments per year (Roßberg, 2016), because yields are less dependent on fungal diseases and insect pests. Yet, insect diversity in maize crops is often negatively associated with high nitrogen input, erosion, shading, and hence, in combination with the use of herbicides, the complete elimination of weeds (Stuart et al., 2018). Nevertheless, these effects are expected to be species specific (Gevers et al., 2011). As 37% of the 2.64 Mio ha used for growing maize in Germany in 2019 was used for growing maize for bioenergy production (Fachagentur für Nachwachsende Rohstoffe, 2022) it clearly altered the spatial and temporal heterogeneity of landscapes, and thus, potentially species richness and diversity (Fahrig et al., 2011; Bertrand et al., 2016; Marja et al., 2022). Therefore, alternative crops are of interest for a sustainable future production of biogas (Theuerl et al., 2019). Perennial energy crops in particular have the potential to decrease management intensity by reducing the need to apply agrochemicals and disturb the soil (Uellendahl et al., 2008; Chmeliková & Wolfrum, 2019). Use of perennial grasses may reduce nitrate leaching (Grunwald et al., 2020), erosion (Grunwald et al., 2020; Scordia & Cosentino, 2019) and threats to biodiversity (Lask et al., 2020). Given the absence of tillage and annual reestablishment, reduced use of pesticides and fertilizers, overall, perennial energy crops require less intense management compared to annual crops.

In Germany, the cup plant (*Silphium perfoliatum* L., Asteraceae) and tall wheat grass (*Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang, Poaceae) have been cultivated as alternative biogas substrates, since 2015 and 2012, respectively. Other perennials are still being tested, such as Virgina mallow (*Sida hermaphrodita* (L.) Weakley & D.B.Poind., Malvaceae) (Cumplido-Marin, 2020). Recent studies have shown that cup plant cultivation positively affects the below ground fauna (Schorpp & Schrader, 2016, 2017; Emmerling et al., 2021), flower visitors (Mueller et al., 2016; Chmeliková & Wolfrum, 2019) and also ground dwelling arthropods (Platen et al., 2017; Burmeister, 2021). In contrast, Radzikowski et al. (2020) report that arthropod diversity assessed by pitfall traps and yellow bowls is lower in cup plant crops than fallow land and crops of *Miscanthus x giganteus* and woody energy plants. An investigation of the fauna in fields of tall wheatgrass and maize verified that earthworms are more abundant in the former than the latter (Emmerling et al., 2021). Another study reports the association of a greater diversity and abundance of spiders and ground beetles with the complex vegetative structure of tall wheatgrass (Platen et al., 2017). Whereas, Burmeister (2021) reports no differences in the diversity of ground beetles in tall wheat grass and the common crop rotation (maize – winter wheat – whole plant rye silage). As the results are often dependent on sampling method and especially the species studied, further research on the ecological effects of perennial energy crops is needed (Lask et al., 2020). This is particularly relevant considering the large area used for biogas substrate production in Germany. Thus, even the replacement of a small proportion of the hectarage currently used to produce maize for biogas production by biodiversity enhancing crops may have a considerable effect (Chmelikova & Wolfrum, 2019). As the biodiversity associated with native plants tends to be higher than that associated with non-native plants (Isaacs et al., 2009), the extent of the potential effect of novel non-native energy plants such as cup plant and Virginia mallow should be of special interest to policy makers.

Soil surface and vegetation cover after harvest are altered by remaining residues of the crop and resprouting of perennial crops. In absence of tillage, organic material accumulates on the surface of the soil. The physical structure of soil affects food webs and soil arthropods (Erktan et al., 2020). A structurally rich soil surface (complex pore space, presence of litter and particulate organic matter in different states of decomposition) is likely to stimulate the abundance and activity of soil arthropods. In addition, the soil fauna is damaged by mechanical disturbance (Boström, 1987; Fadl et al., 1996). In autumn many arthropods prepare for overwintering and hibernation. Those overwintering in fields have to cope with the habitat conditions of either harvested or freshly sown cultures. This is also of agricultural importance as many pests hibernate in dead stalks of their host, for example, the corn borer (*Ostrinia nubilalis* Hübner, 1796) and hessian fly (*Mayetiola destructor* Say, 1817), which can be controlled by appropriate stubble management. However, little is known about the suitability of the habitats provided by the stalks and stubble of the cup plant, Virginia mallow and tall wheatgrass for non-pest species. Especially, as they form long lasting rootstocks and tussocks.

Using a modified leaf blower epigeous arthropods were collected from three different perennial energy crops (cup plant, tall wheat grass, Virginia mallow) at three sites in autumn. A metabarcoding analyses of the samples was used to measure the relative abundance and diversity of the arthropod community. The main aim of this study was to evaluate the beneficial effect post-harvest for arthropods in perennial energy crops rather than silage maize. Specifically, the following questions were addressed:

Q (1) does the abundance of arthropods in total or the abundance of selected taxa differ in maize and perennial energy crops,

Q (2) is stubble an important habitat for ground dwelling arthropods and

Q (3) does metabarcoding of suction samples collected post-harvest in autumn reveal differences in arthropod richness between perennial energy crops and maize?

2. MATERIAL AND METHODS

2.1. Sites and cultivation

Two field trials were established using a randomized block design in southern Germany in 2014 at Rosenau and Aholfing and another one in 2015 at Wolferkofen, to test site-specific suitability for cultivating different perennial energy crops. Each field trial consisted of four replicates of each energy crop at each site. The size of the plots was about 100 m^2. Each site differed in soil and climate characteristics (Table 1). Rosenau has a humous soil that originated from degraded fen, Aholfing a rather sandy and shallow soil on alluvial material in the Danube valley. Wolferkofen is in the Gäu lowland in Bavaria, a landscape shaped by intense

Table 1. Characteristics of the field sites studied.

	Aholfing	Rosenau	Wolferkofen
Altitude (m a.s.l.)	322	346	322
Mean temperature (°C)*	8.6 (8.9)	8.8 (8.8)	8.6 (8.7)
Precipitation (mm)*	760	780	760
Soil type	cambisol/luvisol	histic gelysol	luvisol/cambisol
Texture	loamy sand	histic silty loam	silty loam
Organic carbon (mg/g)**	8.3	90.7	10.2
pH soil**	6.5	7.4	6.9
Year of establishment	2014	2014	2015

* Data from DWD Climate Data Centre (CDC): Multi-annual means (1981–2010) of grids of air temperature (2 m) and precipitation recorded in Germany 1981–2010, version v1.0.

** Results of soil analysis done before the field trials.

arable farming. Soils in this area originated from loess sediments and are highly fertile.

Cup plant (*Silphium perfoliatum*) was planted between the end of May and the first week of June. Virgina mallow (*Sida hermaphrodita*) and tall wheatgrass (*Thinopyrum ponticum*) were sown in July, except at Wolferkofen where Virginia mallow was sown in May and tall wheat grass in June. A crop rotation consisting of maize, winter wheat and winter silage rye was established as an annual energy crop reference. Cup plant and Virginia mallow were harvested for the first time in the second year after establishment, whereas tall wheat grass was cut for the first time in the autumn of the year of establishment. In the following years Virginia mallow and tall wheat grass were harvested twice a year, Virginia mallow at the beginning of July and in October, tall wheat grass between the end of June and beginning of July and a second harvest in October. Cup plant was harvested together with silage maize in September. All plots received mineral fertilizers based on nutrient removal, soil mineralization and plant demand. Soil tillage with a cultivator was done once before the establishment of the perennial energy crops and yearly in the reference plots. Weeds in perennial crops were controlled using herbicides during crop establishment in the first year and yearly in tall wheat grass crops. The crop rotation with maize, winter wheat, silage winter rye was treated with pesticides according to conventional practice.

2.2. Suction sampling

Suction samples were used to evaluate the abundance and richness of arthropods in each plot three to four years after the establishment of the crops. Suction sampling was used as it gives a rough estimate of the abundance and species richness of arthropods on the surface of the soil and vegetation (Sanders & Entling, 2011). Sampling was done at the end of September after a period without rain of at least 48 h (Aholfing 20.09.2017; Wolferkofen 20.09.2017; Rosenau 28.09.2017), eight (Rosenau) or 15 days (Aholfing, Wolferkofen), respectively, after the harvesting of the different crops. For suction sampling, an insect net with mesh size < 0.5 mm was attached inside the nozzle of the suction sampler. The surface of the area sampled was 104 cm². Arthropods were collected by placing the suction sampler on the surface of the soil for 5 s while the engine was running at full speed. To collect a sample this procedure was repeated 10 times at distances of at least one meter along a transect across the plot and then 10 times back across the plot, resulting in a total area sampled of 0.208 m². At each plot two samples were collected: one from the surface of the soil and another from the stubble of the crop. As tall wheat grass had already sprouted again after the first cut in June, suction samples were collected from the tussocks and the space between the tussock rows, respectively.

In total this resulted in 96 samples which were individually put into zip plastic bags and stored cooled during transport to the laboratory where they were frozen at –30°C. Prior to examination the samples were sieved through 4-, 2- and 1-mm sieves. The material passing through the 1-mm sieve was not analysed. Arthropods were separated from soil, plant matter and detritus and stored in 70% ethanol. Based on their morphology the arthropods were classified as: Nematocera, Brachycera, Hymenoptera (except Formicidae), Formicidae, Coleoptera, Heteroptera, Auchenorryncha, Sternorrhyncha, Orthoptera, Lepidoptera, holometabolean Larvae, Psocoptera, Collembola, Acari, Araneae, Opiliones, Isopoda, Chilopoda or Diplopoda. A rough population density of the seven most abundant arthropod orders was estimated by dividing the number of individuals recorded by the area of ground sampled (Narimanov et al., 2021).

The arthropod material from two sites (Wolferkofen, Aholfing) was assigned to metabarcoding. In addition, only two out of the four replicates were used and for each replicate the sample was pooled for the two sites sampled.

2.3. Molecular and bioinformatic analyses (Metabarcoding)

DNA extraction

First, the ethanol was carefully removed and then the vials containing the samples were placed in an oven at +65°C for at least eight hours in order to dry them. Completely dried samples were then mixed with sterile steel beads and homogenized in a FastPrep96 (MP Biomedicals) homogenizer. Equal amounts were transferred into sterile 25 ml plastic vials for tissue lysis. Tissue lysis was performed in 10 mL of a premixed insect lysis buffer containing 10% of Proteinase K for eight hours at 56°C. Genomic DNA was then extracted in 50 µL of elution buffer following the manufacturer's instructions of the DNEasy Tissue plate kit (Qiagen, Hilden, Germany).

DNA amplification and sequencing

Initial PCR reactions were carried out using the MyTaqTM Plant-PCR Kit (Bioline GmbH, Germany) with Illumina-ready fusion primers derived from the primer pair of Leray et al. (2013).

dgLco	5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'
mlCOIntF	5'-TAAACTTCAGGGTGACCAAARAAYCA-3'

Amplification success and correct fragment length of all samples were determined using gel electrophoresis in a 1% TAE gel using GelRed (Genaxxon bioscience GmbH, Ulm, Germany). Amplified DNA for each sample was cleaned and resuspended in 50 µL molecular water before proceeding. Illumina Nextera XT (Illumina Inc., San Diego, USA) indices were ligated to the samples in a second PCR reaction using the same annealing temperature as for the first PCR reaction, but with only seven cycles. Ligation success was again confirmed using gel electrophoresis. DNA concentrations were measured using a Fluorsokan plate reader (Life Technologies) along with Qubit fluorometer dsHS chemicals (Life Technologies, Carlsbad, USA) and samples were combined into pools containing equimolar concentrations of 100 ng each. Library pools were purified and size selected using Next Generation Sequencing (NGS) magnetic beads (MagSi-NGSPrep Plus, Magtivio) for downstream sequencing applications. The final library was then checked again for DNA concentration and amplicon size using Qubit fluorometer (Life Technologies) and Bioanalyzer (Agilent Technologies). High-Throughput Sequencing (HTS) was carried out on an Illumina MiSeq using v3 (2*300 bp, 600 cycles, maximum of 25 mio paired-end reads) chemistry.

Pre-processing and clustering of sequence data

Raw FASTQ files were combined and sequence processing was done using VSEARCH v2.4.3 suite (Rognes et al., 2016) and cutadapt v1.14 (Martin, 2011), which screened for correct fusion primer adaptor sequences and complete primer removal. All the sequenced samples yielded reverse reads of high enough quality to enable paired-end merging. Quality filtering was carried out using the "fastq_filter" program in VSEARCH with the following options fastq_maxee = 2 and minimum bp length = 100. Sequences were dereplicated with "derep_fulllength", first at the sample level and then concatenated into one fasta file, which was then dereplicated. Chimeric sequences were filtered out from the large fasta file using "uchime_denovo". Remaining sequences were clustered into OTUs at 97% identity with "cluster_size", and an OTU table created using "usearch_global". To reduce the incidence of false positives, a cleaning step was employed, which excluded read counts in the OTU table of less than 0.01% of the total. OTUs were blasted against a custom database downloaded from BOLD in late 2019, including taxonomy and BIN information of Central European animals, by means of Geneious (v.10.2.5 – Biomatters, Auckland – New Zealand) using methods previously described (Morinière et al., 2016, 2019). The resulting csv file, which included the OTU ID, BOLD Process ID, BIN, Hit-%-ID value (percentage of overlap similarity (identical basepairs) of an OTU query sequence with its closest counterpart in the database), length of the top BLAST hit sequence, phylum, class, order, family, genus and species information for each detected OTU was exported from Geneious and combined with the OTU table generated by the bioinformatic pipeline. Entries with identifications below 97% and total read numbers below 0.01% of the summed reads per sample were not removed from the analysis, as low hit-% matches are expected for local faunas (Morinière et al., 2019). OTUs were then assigned to the respective BIN. In addition, the API provided by BOLD was used to retrieve BIN species and BIN countries for every OTU, and the Hit-%-IDs were aggregated over OTUs that found a hit in the same BIN and shown in the corresponding column as % range. To validate the BOLD BLAST results, a separate BLAST search was carried out in Geneious (using the same parameters) against a local copy of the NCBI nucleotide database downloaded from ftp://ftp.ncbi.nlm.nih.gov/blast/db/ in late 2019. We furthermore applied an annotation of OTU sequences using a CO1-trained RDP classifier as described in Porter & Hajibabaei (Porter & Hajibabaei, 2018). Resulting taxonomies of BOLD, NCBI and RDP classifier were then concatenated within a consensus taxonomy taking into consideration the highest consensus of taxonomic overlap.

2.4. Data and statistics

To determine the influence of crop type and site on the number of arthropods sampled, negative binomial mixed models with a log link function in R (v. 4.1.1 R-Core Team, 2021) and the lme4 R-package (v. 1.1.27.1 Bates et al., 2015) were used, because the study design included repeated measures, nested effects (sites, replications) and count data. Random effects were individual parcels nested within sites. Fixed effects were crop type, sampling position and their interactions. Likelihood ratio tests were used to determine whether the fixed terms and their interaction resulted in a significant decrease in model deviance at the 0.05 significance level (Zuur et al., 2009). Interactions were excluded if they did not explain a significant part of the variation. Assumptions of models were checked visually by using residual plots (R-package DHARMa, v. 0.4.4 Hartig, 2021) and calculation of dispersion parameters. To check the overall fit of the model, conditional and marginal pseudo r squared values were calculated (R-package piecewiseSEM, v. 2.1.2 Lefcheck, 2016). Treatments (crop type) were compared by using estimated marginal means with Tukey's adjustment for multiple testing with $\alpha = 0.05$ (R-package emmeans, v. 1.7.0 Lenth, 2019).

Using arthropod metabarcoding data the following diversity indicators were calculated: (1) total number of observational taxonomic units ("OTU_richness"); (2) the number of corresponding BINs (from BOLD database) for arthropods with a hit-identity higher than 97% ("BIN richness"); and (3) an estimate of the number of species ("Species richness"). Species richness was derived using a combination of the taxonomy of the blasts against BOLD, NCBI and the RDB classifier, reduced to the highest taxonomic unit with consensus. Further taxonomic units above species level were counted as species, if the sample did not contain units of this taxon at lower, better determined levels (Burmeister & Panasitti, 2022). The sum of reads ranged between 7,900 and 368,600 per sample before data adjustment. One sample from Virginia mallow at Aholfing had very low reads, consequently, the reads for Aholfing were only 40% of total reads. To create comparable diversity indices, the number (richness) of OTUs and BINs was adjusted to the minimum number of reads using the iNEXT R-package (Chao et al., 2014; Hsieh et al., 2020). Retrieved values were rounded to integers. To test for the effects of treatment on obtained diversity indices, negative binominal models with a random site effect were created and the significance of crop type analysed as stated above.

Phylogenetic implications for kinship and evolution using CO1 alone are limited (Raupach et al., 2019). To test genetic differences between samples, the phylogenetic diversity of the metabarcoding data pooled for each energy crop was analysed. As suggested by Saitou & Nei (1987), the Hamming Distance was calculated for sequence pairs from the aligned OTU sequences, and a phylogenetic tree was constructed using the neighbour-joining tree algorithm (R-package ape, v. 5.5 Paradis & Schliep, 2019). Data preparation and visualization was done using the R-package DECIPHER (v. 2.20.0 Wright, 2016) and ggtree (v. 3.2.1 Yu et al., 2017). From the phylogenetic tree, the phylogenetic diversity of each crop was derived (Faith, 1992) and the nucleotide diversity from aligned OTU sequences computed as the number of differences between pairs of sequences divided by the number of comparisons (R-package pegas, v. 0.14 Paradis, 2010).

3. RESULTS

3.1. Arthropod abundance

In total there were 27,836 arthropods in the suction samples. The most abundant groups were Collembola (n = 11.695, 42%), followed by Acari (n = 5.220, 19%), Auchenorryncha (n = 1923, 7%), Aranea (n = 1.887, 7%) and Coleoptera (n = 1.845, 7%). Averaged over all crops, the approximate abundance of animals m^{-2} of soil surface was

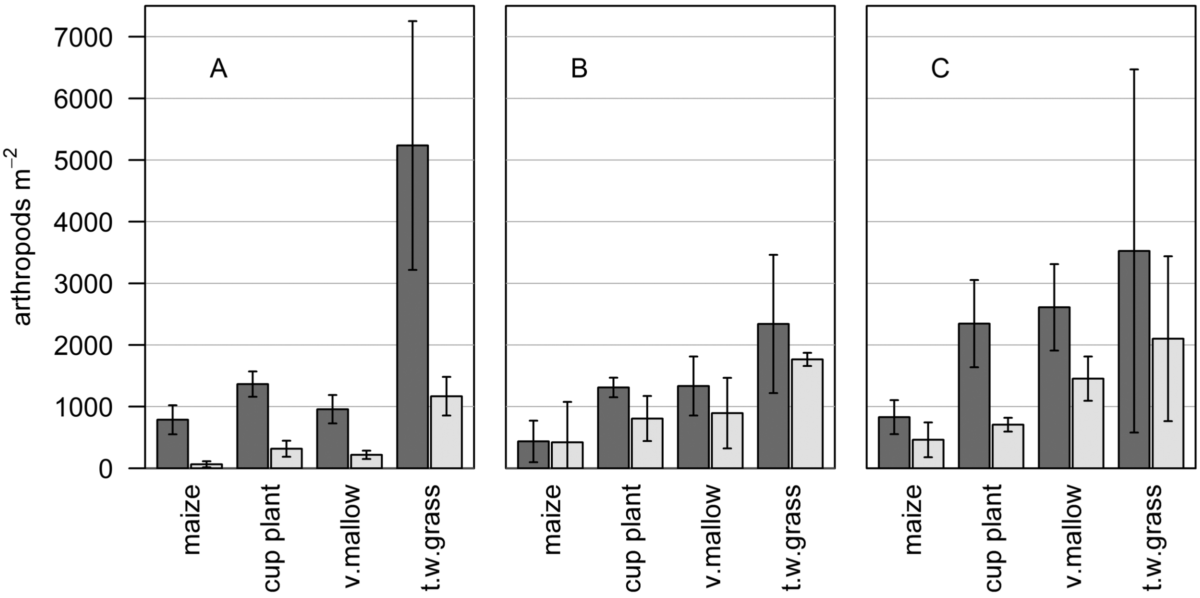

Fig. 1. Arthropod abundance from suction samples on stubbles or within the plant rows (black bars) and on open soil or between plant rows (grey bars). Error bars indicate standard deviations. (A – Wolferkofen, B – Rosenau; C – Aholfing; maize – maize *Zea mais*; cup. plant – cup plant *Silphium perfoliatum*; v.mallow – *Sida hermaphrodita*; t.w.grass – tall wheat grass *Thinopyrum ponticum*).

about 1,300 at Wolferkofen, 1,200 at Rosenau and 1,800 at Aholfing.

The mixed models revealed that crop type and sample site explained a significant part of the variation (56%) in total arthropod abundance (likelihood ratio $\chi^2 = 87.9$, df = 7, $p < 0.001$; Table 2). Interactions between both factors were removed from the model, as they did not improve the fit. The highest variation in arthropod abundance was recorded for maize and the lowest for cup plant. The highest abundance of arthropods was recorded for tall wheat grass

Table 2. Summary of the negative binominal mixed effect models used to determine the association between crop type and arthropod abundance. Model coefficients were back transformed to a response scale. (***p < 0.001, **p < 0.01, *p < 0,05, ·p < 0.1, n.s. – not significant).

Taxonomic Group (response variable)	Likelihood ratio Test vs. Null-model (df = 7)	Likelihood ratio Test vs. Interaction model (df = 3)	Likelihood ratio Test vs. crop type (df = 3)	Likelihood ratio Test vs. sampling position (df = 1)	Coefficients for crop type	Coefficients for sampling position	Coefficients for interaction of crop type and sampling position	Conditional R²	Marginal R²	Dispersion parameter
Total arthropods	$\chi^2 = 87.9$ ***	$\chi^2 = 1,4$ n.s.	$\chi^2 = 50.5$ ***	$\chi^2 = 40.2$ ***	6.0 t.w.grass 2.6 v.mallow 2.4 cup.plant	2.5 crop	(not included)	0.68	0.56	1.03
Collembola	$\chi^2 = 70.1$ ***	$\chi^2 = 4.6$ n.s.	$\chi^2 = 32.3$ ***	$\chi^2 = 33.2$ ***	6.3 t.w.grass 2.0 v.mallow 3.9 cup.plant	2.9 crop	(not included)	0.81	0.27	1.09
Acari	$\chi^2 = 43.6$ ***	$\chi^2 = 2.2$ n.s.	$\chi^2 = 21.0$ ***	$\chi^2 = 19.6$ ***	6.7 t.w.grass 6.4 v.mallow 5.6 cup.plant	2.1 crop	(not included)	0.82	0.25	1.05
Hemiptera	$\chi^2 = 66.7$ ***	$\chi^2 = 0.5$ n.s.	$\chi^2 = 47.2$ ***	$\chi^2 = 14.0$ ***	13.9 t.w.grass 1.8 v.mallow 0.6 cup.plant	2.1 crop	(not included)	0.60	0 .52	1.11
Diptera	$\chi^2 = 53.9$ ***	$\chi^2 = 7.4$ ·			6.8 t.w.grass 3.9 v.mallow 3.1 cup.plant	2.7 crop	0.5 c:t.w.grass 0.5 c:v.mallow 0.7 c:cup.plant	0.79	0.45	1.03
Aranea	$\chi^2 = 126.5$ ***	$\chi^2 = 14.8$ **			24.6 t.w.grass 8.5 v.mallow 6.1 cup.plant	7.3 crop	0.4 c:t.w.grass 0.2 c:v.mallow 0.4 c:cup.plant	0.86	0.75	1.05
Hymenoptera	$\chi^2 = 66.5$ ***	no convergence			7.9 t.w.grass 2.0 v.mallow 2.0 cup.plant	3.5 crop	0.5 c:t.w.grass 1.0 c:v.mallow 0.7 c:cup.plant	0.73	0.42	1.02
Coleoptera	singular fit, no convergence									

Table 3. Mean total arthropod abundance and abundance of the seven most abundant taxonomic groups of arthropods (individuals m^{-2}) recorded in the three energy crops and maize; different letters indicate significant differences at the 0.05- significance level obtained from comparisons of estimated marginal means for models in Table 1 (models without interactions only), Tukey's method for p value adjustment.

	Maize	Virginia mallow	Cup plant	Tall wheat grass
Total arthropods	500 a	1245 b	1142 b	2690 c
Collembola	201 a	446 ab	541 bc	1155 c
Acari	124 a	299 b	213 b	411 b
Hemiptera	32 ab	69 b	19 a	377 c
Diptera	50	140	124	228
Aranea	23	64	60	233
Hymenoptera	23	80	32	120
Coleoptera	37	103	102	128

plots at all sites (Fig. 1, Table 3). Total arthropod abundance was significantly higher for cup plant and Virgina mallow than maize. The average total arthropod abundance was more than twice as high in tall wheat grass plots (2690 individuals m^{-2}) than in cup plant (1140 individuals m^{-2}) and Virginia mallow plots (1250 individuals m^{-2}) and about five times higher than in maize plots (500 individuals m^{-2}). In addition, as expected, suction samples collected from stubble or vegetation (tall wheat grass) had higher arthropod abundances than those collected from bare ground. Mixed Models estimated the difference to be about 2.5 times greater for stubble and vegetation (Table 2). However, this was site specific (Fig. 1). The samples from bare ground yielded an average of about 870 individuals m^{-2} whereas for stubble or crops it was 1930 individuals m^{-2}.

Total abundance of arthropods and the abundance of the seven most frequent arthropod groups can be found in Table 3 and model summaries in Table 2. For all groups except the Coleoptera, for which no appropriate model could be fitted, the abundance was higher by at least a factor of two in stubble and vegetation than on bare ground. Especially for spiders (Aranea) the presence of vegetation was associated with a higher abundance (Table 2). For the Diptera and Aranea the interaction term improved the fit of the model (Diptera: $\chi^2 = 7.4$ df = 3 p = 0.059; Aranea: $\chi^2 = 14.8$ df = 3 p = 0.002). The highest coefficients were recorded for tall wheat grass for spiders and Hemiptera. Tall wheat grass harboured about 380 Hemiptera m^{-2} and 230 spiders m^{-2}, whereas for maize it was only about 30 and 20 individuals m^{-2}. Distinctly lower abundances were also recorded for cup plant and Virginia mallow. Mean abundance was highest in tall wheat grass for all of the seven most abundant taxonomic groups. Mites (Acari) were significantly less abundant in maize than in the perennial energy crops.

3.2. Metabarcoding

Metabarcoding yielded 864 OTUs, which were assigned to 216 BINs of arthropods (BOLD Barcode Index numbers) with a hit-identity higher than 97%. The total number of species was estimated to be 208. The highest number of OTU reads was assigned to three springtail species: *Orchesella villosa* L. (Collembola: Entomobryidae) with 38% of total reads, followed by *Lepidocyrtus paradoxus* Uzel, 1891 (Collembola: Entomobryidae) with 10% and *Isotoma anglicana* Lubbock, 1862 (Collembola: Isotomidae) with 4%.

At both of the sites studied, the highest number of OTUs, arthropod BINs and estimated number of species were recorded in tall wheat grass (114), followed by Virginia mallow (113) and cup plant (112) (Table 4), and the lowest numbers in maize plots (84). The four crops had 25 species in common, 78 were recorded uniquely in tall wheat grass and only 22 in maize (Virginia mallow: 36; cup plant: 34). The mixed negative binominal model of the estimated species numbers revealed a significant association with the type of crop (likelihood ratio χ^2 = 13,3, df = 3, p < 0.001), with a significant higher richness in tall wheat grass than in maize and cup plant plots. However, the explanatory value of the random site factor was very low resulting in a singular fit. Both OTU richness and BIN richness were associated with type of crop (OTU: likelihood ratio χ^2 = 10.7, df = 3, p = 0.014; BIN: likelihood ratio χ^2 = 10.6 df = 3, p = 0.014). The two diversity parameters were significant higher for tall wheat grass plots than maize plots. As indicated by examination of estimated marginal means, in comparison to maize, significantly more OTUs and BINs

Table 4. Biodiversity indicators assessed using metabarcoding, pooled for each crop or means of four replicates (two sites). Different letters indicate statistical differences between crops.

	Parameter	Maize	Cup plant	Virginia mallow	Tall wheat grass
Pooled	OTU richness	238	338	343	399
	BIN richness	68	88	109	116
	Species richness	72	89	107	113
	Nucleotide diversity	0.237	0.223	0.232	0.254
	Phylogenetic diversity	13.6	17.7	18.0	21.3
Mean ± sd	OTU richness	81 ± 26 a	115 ± 17 b	112 ± 18 ab	137 ± 41 b
	BIN richness	27 ± 12 a	34 ± 7 ab	40 ± 2 b	46 ± 10 b
	Species richness	30 ± 11 a	38 ± 6 ab	42 ± 3 bc	52 ± 9 c

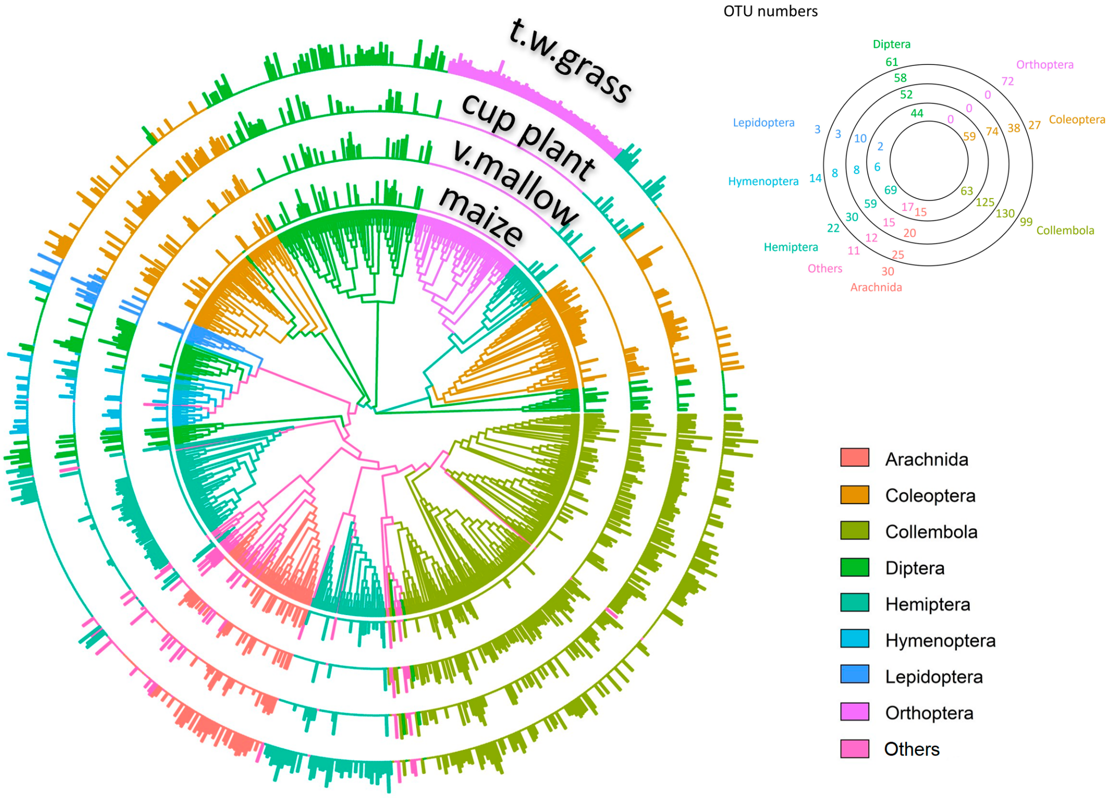

Fig. 2. Phylogenetic tree for metabarcoding OTU sequences attributed to arthropods. Bars indicate log transformed total number of reads of single OTUs for investigated crops (from inner to outer circle; maize – maize *Zea mais*; cup.plant – cup plant *Silphium perfoliatum*; v.mallow – *Sida hermaphrodita*; t.w.grass – tall wheat grass *Thinopyrum ponticum*). Colors show ascribed different taxonomic groups.

were recorded for cup plant and Virginia mallow, respectively.

The phylogenetic tree based on the CO1 barcode sequence reveals the arthropod orders based on the consensus taxonomy as shown in Fig. 2. The computed phylogenetic diversity was lowest for maize (13.6), intermediate for cup plant (17.7) and Virginia mallow (18.0) and highest for tall wheat grass (21.3).

Collembola made up the biggest part of the tree with 188 OTUs. Frequently for this taxon more than one OTU was assigned to the same species, with up to 28 OTUs for *Isotoma anglicana*. It is obvious from the tree, that Orthoptera were only detected in the samples from tall wheat grass (in total 72 OTUs). For the maize plots, the number of OTUS in the Collembola branch was much lower than in the three other energy crops. In total 63 OTUs assigned to Collembola were recorded for maize, 96 for tall wheat grass, 124 for Virginia mallow and 128 for cup plant. A higher diversity of Hemiptera (excluding the Aphrodinae) was recorded in tall wheat grass plots. Aphrodinae were apparently more abundant in the Virginia mallow plots, but a lot of OTUs could only be assigned to the genus or family level.

4. DISCUSSION

Higher abundances and greater diversity of arthropods were recorded in tall wheat grass than in maize, cup plant and Virginia mallow plots in autumn. This result is linked to a higher green vegetation cover, which provides food and habitats for phytophagous insects and arthropods. The abundance of true bugs (Hemiptera) and spiders (Aranea) was clearly higher in perennial tall wheat grass than control plots with maize, indicating a higher availability of food (true bugs) and complex structures for spiders to build their webs. Sanders & Entling (2011) report large variation in the efficiency of suction sampling in vegetation of different densities and for different arthropod groups. In addition, Moomertz et al. (1996) concludes that suction sampling is especially not appropriate for collecting large and heavy individuals. Therefore, the comparison of the catches from stubble and grass plots must be interpreted with care. It was assumed that the findings reported are robust as this study did not focus on one taxonomic group and the plots with maize, cup plant and Virginia mallow were nearly identical in terms of the height and density of the vegetation. Higher and more variable densities of arthropods were recorded in tall wheat grass plots than in the other crops, which is in accord with the high variation in grassland samples reported by Sanders & Entling (2011). Nevertheless, the differences between sown rows and unsown sparse vegetation could be verified by suction sampling. The results presented here agree with Platen et al. (2017) who report that the more complex structure of perennial energy crops, especially tall wheat grass, is positively associated with the diversity of spiders. In this study web building spiders, such as *Pisaura mirabilis* (Clerck, 1757) and *Centromerita bicolor* (Blackwell, 1833), but also non web building, surface living spiders, such as *Pardosa prativaga* (Koch, 1870), were recorded more frequently in tall wheat grass. Another study on spider diversity in field margins reports a positive correlation of spider abundance and species richness with the height of the vegetation in September (Baines et al., 1998). Thus, spiders may benefit from late harvested and fast growing, cool season grasses, such as tall wheat grass. The species that were associated with the tall wheat grass plots in the current study were mostly bugs and cicadas associated with species of grass, for example, *Notostira elongata* (Geoffroy, 1785) and *Euscelis aemulans* (Kirschbaum, 1868). Therefore, crops like tall wheat grass provide habitats for species that either feed on grass or require structured habitats. This may enhance habitat connectivity in mainly arable dominated landscapes for species with poor powers of dispersal that are mainly adapted to grassland habitats. Further, connectivity between semi-natural habitats that were previously cultivated, such as field margins and fallows, can be enhanced by environmentally friendly management of fields. The results of a field study on spiders in France (Nardi et al., 2019) indicates that perennial crops (and meadows) are especially important for connecting habitats in agricultural landscapes. Increasing habitat connectivity can also increase the positive effects of ecosystem service providers such as the natural enemies of pests (Grass et al., 2019). Although the phytophagous insects on perennial energy grasses, native prairie and maize in North America have been studied (Harrison & Berenbaum, 2013), little is known about the usability of non-native energy crops as a food source for insects in Europe. It is known that high plant species richness is associated with more diverse and more complex arthropod communities (Ebeling et al., 2018). In addition, to high plant diversity, which is especially associated with high species richness of phytophagous insects, the abundance of resources, in particular the abundance and availability of prey, is associated with a high diversity of predators (Simons et al., 2014). Management of crops directly influences the density of arthropods, which are the prey of predators. As tall wheat grass is cut only twice a year, it might provide a high amount of food for grass adapted insects throughout the year and especially in autumn. This increased food resources in autumn may support higher trophic levels. Cutting frequency is also known to negatively affect many insects (Steidle et al., 2022). Comparisons between permanent grasslands and energy grasses subject to different cutting regimes could be used to determine the specific effects of cutting, vegetation structure and plant diversity on the arthropod fauna and a better understanding of the trade-off in biodiversity for optimizing the integration of bioenergy cropping in sustainably managed landscapes.

Arthropod abundance and diversity in cup plant and Virginia mallow were lower than in tall wheatgrass and higher than in post-harvest maize plots. This study, therefore, supports the hypothesis, that cup plant grown as biogas crop can enhance the biodiversity in agricultural landscapes if it replaces or supplements silage maize. Schorpp et al. (2016) states that the abundance of euedaphic springtails is two times higher in cup plant than maize plots. The current study based on suction samples supports the above, which is based on soil samples, which reports an increase in springtails in the uppermost soil layer, aboveground and in vegetation. The most commonly recorded springtails, *Lepidocyrtus paradoxus* and *Orchesella villosa*, are epedaphic species (Potapov et al., 2016). Dry mass yields of Virginia mallow were far less than that of silage maize, and at least at one site weeds infested the plots. Thus, the abundance and diversity of arthropods recorded for this crop may not be correct, as less dense stands with gaps and a diverse flora are not comparable with older, dense and weed free plantations (Dauber et al., 2015; Williams et al., 2019). The metabarcoding verified the presence of the mallow flea beetle *Podagrica fuscicornis* (Linnaeus, 1766), which causes visible damage to Virginia mallow leaves and may be a potential future pest of this crop.

Interestingly, a higher estimated abundance of arthropods was recorded for stubble than bare ground. Stubble management is an important phytosanitary measure in corn production, which suppress fungal diseases that infect cereals and the European corn borer (Schaafsma et al., 1996). It is important to note that stubble, however, is not only a source of pests but is also a suitable habitat for other arthropods. For example, stubble provide a source of food for decomposers and shelter and favourable microclimatic conditions for other organisms. Further, stalks connect the soil surface with the soil, which might be important for the overwintering of certain invertebrates, but more research on this aspect is needed. In agricultural landscapes, the long lasting stubbles and stalks of cup plant and Virginia mallow, as well as their rootstocks, may increase the diversity of habitats.

The objective of this study was to assess the effect of different perennial energy crops on the fauna of arthropods. Because the taxonomic knowledge of many groups of arthropods is poor and species are often difficult to identify, metabarcoding was used to obtain a comprehensive list of the arthropods sampled. In addition to the enormous advantages in terms of time, cost and taxonomic width, there are difficulties in estimating diversity indices using this method. The number of OTUs should not be used as a diversity index, as for some taxa, such as orthoptera, many OTUs are recorded for them because of their nuclear mitochondrial pseudogenes (Hawlitschek et al., 2017). In addition, species sharing very similar or identical CO1 sequences may not be distinguished at the species level. As the consensus of the taxonomy used and the resultant derived estimates of species richness is based on different databases and methods the results are meaningful in the context of the current study. To obtain more exact results, it is important to have high quality and complete databases. It was assumed that the estimation of phylogenetic diversity would complement the estimated species richness, as taxonomic position and traits are related. Nevertheless, using phylogenetic diversity as a proxy for functional diversity is controversial (Winter et al., 2013) as it is affected by integrated traits and species groups. For assessing the assembly processes, Xu et al. (2019) recommend the use of phylogenetic diversity, if the dimensions of the traits are limited. As datasets based on metabarcoding include a very wide range of taxa, for which traits are not available or are impossible to compare, phylogenetic diversity still seems to be a promising proxy for functional diversity. For instance, how many and what kind of functional traits are needed to compare a hoverfly with a wolf spider? In addition to the other limits to using metabarcoding, the obstacle that barcoding based on the CO1 gene cannot reveal the details of kinship has to be considered. The results may also be influenced by the abundance of species in the sample, with more abundant species yielding more OTUs. Nevertheless, the visualization of the relations using a phylogenetic tree, helps with the comparisons of the compositions of faunas and in distinguishing ecological relations from metabarcoding artefacts.

5. CONCLUSION

The tackling of two of the most concerning challenges of our times, the conservation of biodiversity and the transition to renewable energy sources, by integrating perennial energy crops into the established bioenergy production systems, is appealing in terms of area and resource efficiency. This study revealed a greater positive effect in terms of the abundance and diversity of ground dwelling arthropods in post-harvest cup plant plots than in maize plots. Chmelikova & Wolfrum (2019) predict a reduction in methane production of 17.8% if the value of species richness is increased by 10%. For similar field trials to those used in this study a methane production of about 75% (dry mass: 82%) of that of silage maize is predicted for tall wheat grass and 60% for cup plant (dry mass: 92%), respectively (Förster et al., 2021). Integrating cup plant and tall wheat grass into multifunctional biogas production may result in a more efficient and sustainable system. The integration of these crops could result in an increase in the autumnal arthropod fauna and connect semi-natural habitats, such as, field margins and remnants of grassland in landscapes dominated by intensive arable farming. Increasing the connectivity of habitats in this way could also positively affect the species richness and composition at landscape scale (Tscharntke et al., 2021). To evaluate the differences between natural grasslands and bioenergy grasses, their specific assemblages of species need to be identified.

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