Spatio-temporal determinants of arthropod biodiversity across an agro-ecosystem landscape

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Abstract

Arthropod communities globally are declining while undergoing taxonomic and functional homogenization, with agricultural activity being a strong contributory factor. Here we use DNA metabarcoding to quantify how variation in climate, agricultural intensity, and plant community composition shape spatiotemporal variation in a metacommunity of > 10,000 arthropod species sampled from 29 Malaise traps across 15 sites in southern Ontario, Canada. Local variation in plant community composition and canopy cover best explained arthropod community dissimilarity. Climatic variables followed closely as explanatory factors, driven primarily by seasonal variation in temperature. The proportion of agricultural land at the landscape scale had no detectable effect. Our results suggest that plant community composition, microclimate, and seasonality structured the arthropod metacommunity to considerable degree, factors that are rarely incorporated into assessments of biodiversity loss due to agriculture. We conclude that habitat restoration on marginal lands is likely an effective strategy for promoting arthropod biodiversity in agroecosystems.

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ABSTRACT

Arthropod communities globally are declining while undergoing taxonomic and functional homogenization, with agricultural activity being a strong contributory factor. Here we use DNA metabarcoding to quantify how variation in climate, agricultural intensity, and plant community composition shape spatiotemporal variation in a metacommunity of > 10,000 arthropod species sampled from 29 Malaise traps across 15 sites in southern Ontario, Canada. Local variation in plant community composition and canopy cover best explained arthropod community dissimilarity. Climatic variables followed closely as explanatory factors, driven primarily by seasonal variation in temperature. The proportion of agricultural land at the landscape scale had no detectable effect. Our results suggest that plant community composition, microclimate, and seasonality structured the arthropod metacommunity to considerable degree, factors that are rarely incorporated into assessments of biodiversity loss due to agriculture. We conclude that habitat restoration on marginal lands is likely an effective strategy for promoting arthropod biodiversity in agroecosystems.

INTRODUCTION

Rapid declines in arthropod abundance and species diversity across the globe have received a great deal of recent attention (Gossner *et al.* 2016; Hallmann *et al.* 2017; Sánchez-Bayo & Wyckhuys 2019; Seibold *et al.* 2019; Wagner *et al.* 2021). These drastic changes in arthropod communities are a major cause for concern, given their enormous taxonomic and functional diversity, critical role in maintaining ecosystem stability, and provisioning of vital ecological services such as pollination, pest control, and nutrient cycling (Kremen *et al.* 1993; Stork *et al.* 2018). Although the causes of arthropod declines are surely complex and multi-faceted, habitat loss and external inputs associated with agriculture are frequently identified as primary drivers (Gossner *et al.* 2016; Sánchez-Bayo & Wyckhuys 2019; Seibold *et al.* 2019; Wagner 2020). Recent work suggests, however, that arthropod declines are not universal (Crossley *et al.* 2020; van Klink *et al.* 2020), raising fundamental questions about the regulation of arthropod biodiversity in agroecosystems (Wagner 2020).

Metacommunity theory should provide a useful framework to understand how arthropod communities are regulated in agricultural landscapes. On ecological timescales, this framework describes how species are distributed in space and time according to the relative importance of species sorting, dispersal, and ecological drift (Leibold *et al.* 2004; Vellend 2010). These processes can be expected to interact significantly but are rarely quantified in the context of agroecosystems. Under a species sorting paradigm, differences in environmental conditions should be the primary driver of differences in community composition. However, wide compositional differences may be caused by stochastic-based dispersal limitation, an effect exacerbated by patch isolation and especially affecting groups with limited dispersal ability (Vellend 2010). As well, ecological drift can cause species abundances to vary stochastically and

thus community composition can shift through time independently of environmental conditions. In these cases, it is pure distance, not environmental differences, that regulates distribution, abundance, and organismal fitness (Bell 2001; Hubbell 2001). Much more is known about spatial dynamics than temporal dynamics for arthropods in agricultural systems, and few studies have examined this problem from a metacommunity perspective. This is especially significant given the potential for rapid generation times in many arthropod taxa, such that the influences of temporal and spatial processes on community divergence can be powerful even within a single growing season (Kingsolver 1989; Chown & Gaston 1999).

Most of these community-shaping processes have been investigated from a spatial perspective, but temporal factors can also play a central role (Grøtan *et al.* 2012). Seasonality is particularly important in many systems, stemming from an interplay of species-specific responses to abiotic conditions, such as temperature and precipitation, biotic conditions, such as plant resource availability, and stochastic variation through time (Stinson & Brown 1983; Wolda 1988; Grøtan *et al.* 2012; Hatosy *et al.* 2013). Previous studies have found that the effect of habitat composition and the configuration of those habitats in the landscape on arthropod communities can vary across the growing season (Bertrand *et al.* 2016) and that landscape composition can modulate phenological diversity (Sydenham *et al.* 2014). Even in tropical systems with less pronounced seasonality compared to temperate regions, arthropod community composition in both natural forests and rubber plantations shows high seasonal turnover (Beng *et al.* 2018). Seasonality in agroecosystems can unfold by climatic seasonality and associated management-based seasonality of factors such as plowing, planting, and pesticide application. Neither form of seasonality, nor their interaction, are well understood in terms of their impacts on arthropods.

Previous work has suggested that the quantity, quality, and spatial arrangement of habitat can all simultaneously impact the composition of arthropod communities. Variation in arthropod community composition has been linked to local attributes, including plant biomass, structural complexity, and plant community composition (Stinson & Brown 1983; Schaffers *et al.* 2008; Borer *et al.* 2012; Prather & Kaspari 2019) as well as measures of habitat diversity, land-use intensity, landscape connectivity, and the configurational complexity of the landscape (Hendrickx *et al.* 2007; Fahrig *et al.* 2011; Gossner *et al.* 2016; Seibold *et al.* 2019). It is less clear, however, whether these factors are comparable in magnitude to the effect of local variation in agricultural land use. Using DNA metabarcoding to analyze Malaise trap samples of arthropods, we evaluated this proposition across a network of 15 Canadian farms and conservation areas that span a range of agricultural intensity at the landscape scale and varying degrees of natural land and ecological restoration under a novel land management initiative termed the Alternative Land Use Services (ALUS) program (https://alus.ca).

METHODS

We used structural equation modelling to tease apart the contributory impact of (a) climatic variation, (b) plant community attributes (plant community composition, richness, and canopy openness) and (c) agricultural intensity on spatio-temporal variation in β diversity across an agroecosystem landscape in southern Ontario. In each model variant, we predicted a positive relationship between arthropod community dissimilarity and environmental distances. That is, larger differences in environmental conditions should generate more dissimilar communities for environmental variables representing climate, plant community attributes, or agricultural intensity. In keeping with our metacommunity perspective, we incorporate spatial and temporal

distances as covariates in analyses with environmental variables, independent effects of which may tie to dispersal limitation and ecological drift (Jabot *et al.* 2020).

In late April/early May 2019, 29 Townes style malaise traps were placed at 15 farms and conservation areas in Southern Ontario, Canada (SI Appendix, Figure S1). Most of the study region is intensely farmed with crop monocultures typically of corn (*Zea mays*), soybean (*Glycine max*), and winter wheat (*Triticum aestivum*) covering ~90% of the landscape. Mean annual temperatures are 8°C, with precipitation averaging 1035.8 mm (http://climate.weather.gc.ca). Traps were separated by 48,551m on average (range = 71.2 – 142,343, sd = 30,533) and traps on the same farm were separated by 371m on average (sd = 282.1). Malaise traps are well suited for large-scale monitoring as they are easily standardized, time and cost effective, and sample a wide array of arthropod taxa, though they preferentially trap flying insects (D'Souza & Hebert 2018; deWaard *et al.* 2019). The placement of Malaise traps on the sites represented four broad habitat types in varying proportions: woodland, grassland/meadow, aquatic edge, and crop edge. Typically, traps were placed on the edge of two of these habitat types. Sites varied in agricultural intensity, including conventional farms, conventional farms with a higher proportion of natural land (mid-impact), ALUS-supported farms with restored habitat on their marginal lands, and conservation areas. By "conventional", we mean non-organic farms practicing industrialized input-intensive cropping. These farms are fertilized, periodically sprayed with pesticides, but not irrigated. We defined "agricultural intensity" as the proportion of agriculture in the landscape within a 2 km radius of each trap. "Marginal" lands on the ALUS farms were determined based on lack of crop profitability, with soils that were nutrient poor, hydrologically constrained (either under- or over- drained), or difficult to cultivate because of slope. Restored lands on ALUS farms were plowed and seed-

156 planted with native tallgrass prairie species, including C4 perennial grasses and diverse mixtures
157 of forbs (Paterson *et al.* 2019). By “natural land”, we refer to unrestored areas without crops,
158 typically forest or old-field pasture. Edge areas adjacent to aquatic habitat or crop fields refer to
159 narrow unmanaged buffer strips, that are unsuitable for cultivation but can act as refugia for
160 some arthropods on farms otherwise largely dominated by cultivated fields (Paterson *et al.*
161 2019).

162 Arthropods were collected in 500mL plastic bottles filled with 95% ethanol attached to
163 the trap heads. The bottles were collected and replaced biweekly from May through mid-October
164 2019. With few exceptions (damaged samples or early trap takedown), 12 two-week samples
165 were collected at each trap site. All of the collected samples were accessioned and are stored at
166 the Centre for Biodiversity Genomics (CBG) (http://biodiversitygenomics.net). Every other two-
167 week sample was sent for metabarcoding at the CBG’s sequencing facility (http://ccdb.ca/).

168 The metabarcoding analysis targeted a 462 bp amplicon of the mitochondrial cytochrome
169 *c* oxidase subunit I (COI) gene which was PCR amplified from each bulk sample using the
170 forward primer AncientLepF3 (Prosser *et al.* 2016) and the reverse primer cocktail C_LepFo1R
171 (containing LepR1 and HCO2198) (Hebert *et al.* 2004). Detailed laboratory methods are
172 provided in SI Appendix. Sequences recovered from eight replicates from each sample were
173 uploaded to the mBRAVE platform (Ratnasingham 2019; http://www.mbrave.net/) where they
174 underwent the analytical steps (see protocols described in SI Appendix) required to allow their
175 assignment to a Barcode Index Number (BIN) that serves as a species proxy (Ratnasingham &
176 Hebert 2013) based on queries between sequences and reference libraries for chordates, insects,
177 non-insect arthropods, non-arthropod invertebrates, and bacteria. BIN assignments and the
178 taxonomic assignments associated with them are dynamic because they are impacted by the

179 continual expansion of sequence records on BOLD. The taxonomic assignments reported in this
180 study are those current in November 2019. Only arthropods and non-arthropod invertebrates
181 were included in the final BIN table, although arthropods constituted 99.8% of these BINs.

182 Floristic surveys of ground vegetation were conducted monthly on a four-week rotating
183 schedule between May and September, given the importance of non-crop plant resources for
184 food, shelter, and nesting for many arthropods of farm landscapes. Two plant survey techniques
185 were used over the course of the sampling period. For the first three weeks, five quadrats
186 measuring 1x1m were randomly placed on each side within 25 m of the Malaise trap for a total
187 of ten quadrats per trap. From week four onward, two 25 m transects were placed
188 perpendicularly to each side of trap, or as close to perpendicular as possible if there were large
189 waterbodies next to the trap, and 1x1m quadrats were placed every 5 m along the transects for a
190 total of ten quadrats per trap.

191 The identity and percent cover of each plant as well as overhead canopy openness were
192 measured in each plot. Overhead canopy openness was measured given the importance of canopy
193 on microclimate, to which ectothermic arthropods can be highly sensitive. Openness was
194 determined using a convex spherical densiometer (Forestry Suppliers), averaged from four points
195 perpendicular to each side of each plot. Only canopy openness from the second set of plant
196 surveys was used in the analyses as tall vegetation could obscure canopy measurements in later
197 months. Given some uncertainty about field identification of closely related forbs and grasses
198 that were not in flower, all plant data were analysed at the genus level or higher. Because of
199 uncertainty in the identification of some non-native C3 pasture grasses (e.g. *Poa* or *Festuca*),
200 some of these grasses were classified into the tribe *Festuceae.*

Given that arthropods can be influenced by climatic conditions either via physiological mechanisms or by influences on dispersal, weather data were sourced from the Government of Canada Historical Weather Database (https://climate.weather.gc.ca/) from five weather stations closest to the sampling sites as well as temperature loggers attached to each Malaise trap. The loggers recorded temperature (°C) hourly throughout the entire sampling period for each trap. Six loggers malfunctioned; in these cases, temperature data were taken from another trap on the same farm (five traps) or from the nearest site (one trap). Hourly relative humidity (%), hourly wind direction (10s deg), and hourly wind speed (km/h) were obtained from four weather stations. Total daily precipitation (mm) was only available for three stations, so in one case these data were taken from another nearby station. All variables were averaged, and the coefficient of variation was calculated for the temperature data to match the two-week sampling periods of the nearest traps throughout the season. Since the climate variables were expected to be correlated, principal components analysis (PCA) was used to extract the main axes of variation before analysis. 4 axes were retained, representing 88% of the total variation.

Landcover data were obtained from the 2019 Annual Crop Inventory, which classifies landcover types from satellite images with 30m spatial resolution using decision tree algorithms (Agriculture and Agri-Food Canada 2020). All landcover types were reclassified into cropland (excluding pasture/forage and fallow land), semi-natural, and urban categories prior to analysis. Since the percentages of seminatural and agricultural land were highly correlated and we were primarily interested in the effects of agriculture, only the percentage of the landscape that is agricultural within a 2000 m radius was used in the analysis. This scale better represents landscape-level processes including dispersal limitation and spatial turnover in habitat quality, with strong effects of landscape factors on arthropods at 1000-2000 m scales previously

observed (Gámez-Virués *et al.* 2015; Siebold *et al.* 2019). These metrics were calculated using the *landscapemetrics* R package (Hesselbarth *et al.* 2019).

To explore general patterns of spatiotemporal β diversity, a dissimilarity approach based on the Sørensen index was taken where arthropods were grouped by trap across all time periods, by time period across all traps, and for all samples. In the first case, there were 29 units for comparison (traps), in the second there were 6 (time periods) and in the third there were 172 (samples; trap by time combinations). Since the temporal extent of the arthropod and plant data differed slightly (six months and five months, respectively), the arthropod data were filtered to the samples that were closest in time to the plant data for all analyses that involve environmental effects. This meant that either the first arthropod sample or the last arthropod sample was omitted, depending on the plant survey schedule. This left 144 samples available for analysis.

We conducted a distance-based path analysis based on the framework proposed by Jabot *et al.* (2020). The structure of the path model was as follows: arthropod Sørensen dissimilarities between samples were linked to eight environmental distance variables. Three represented plant community attributes, one represented agricultural intensity, and four represented climatic variation (see SI Appendix, Table S1 for a full list). Based on hypothesized relationships between the plant community attributes, plant richness and canopy cover were allowed to have both a direct effect on arthropod community dissimilarity as well as an indirect effect through plant community composition. Arthropod Sørensen dissimilarities were directly linked to spatial and temporal distances, and each environmental distance was also linked with spatial and/or temporal distance depending on whether it showed spatial variation, temporal variation, or both (SI Appendix, Table S1). Environmental distances were calculated as Euclidian distances except for differences in plant communities, which were calculated as Bray-Curtis dissimilarities of the

plant cover data. Spatial distances were calculated as the distance in meters between individual traps using the *geodist* R package (Padgham & Sumner 2020) and temporal distances were calculated as the Euclidian distance between sampling periods.

The importance of each significant path in the model was assessed based on standardized path coefficients (SPC). Model fit was assessed based on a combination of the Standardized Root Mean Square Residual Index (SRMR), the Root Mean Square Error of Approximation Index (RMSEA), and the Comparative Fit Index (CFI). Values typically indicating acceptable to perfect model fit for each index range between 0.09 – 0, 0.08 – 0, and 0.90 – 1, respectively (McDonald & Ho 2002; Fan *et al.* 2016). The significance of parameters was determined using the permutation method of Fourtune *et al.* (2018) to account for non-independence between dissimilarity values and the Benjamini-Hochberg procedure was used to correct P-values for multiple comparisons (Benjamini & Hochberg 1995; Jabot *et al.* 2020). Values of paths to and from groups of environmental distances were calculated by summing the absolute values of significant standardized path coefficients of individual environmental variables, including both direct and indirect effects (Jabot *et al.* 2020). All aspects of model fitting were conducted by modifying scripts provided in the supplementary material of Jabot *et al.* (2020) using the R packages *lavaan* (Rosseel 2012) and *MASS* (Venables & Ripley 2002). We additionally used a variance partitioning approach based on multiple regression (Tuomisto *et al.* 2012) to investigate the unique explanatory power of time and space while controlling for environmental variation and vice versa. Analyses were carried out using R statistical software version 3.6.3 (R Core Team 2020) at a significance level of $\alpha = 0.05$.

RESULTS

The number of BINs varied strongly among samples (mean = 347, sd=151; range = 51 – 792). There also was substantial variation in the number of BINs identified among traps and time periods (SI Appendix, Figure 1 B-C). The greatest number of BINs identified in a single trap over the course of the season was 1,851 while the lowest was 983, and the average was 1,335. Arthropod diversity was highest in July with 5,059 BINs and lowest in May with just a total of 1,761 BINs. The average number of BINs per sampling period was 3,748. Many BINs were uncommon with 39% of BINs represented by only a single sample. 53% of BINs belonged to the order Diptera, 17% to Hymenoptera, 10% to Lepidoptera, 6% to Coleoptera, 6% to Hemiptera, and 2% to Araneae (SI Appendix, Figure 1 A). A total of 144 samples had temporally matching arthropod and plant samples available for analysis. A subset of 44 samples where every specimen was counted yielded a mean of 3,009 individuals. This result suggests that our total collection of 144 samples provided >400,000 individuals for genomic identification. Among this total, 10,359 BINs were identified, with representatives from 34 orders and 428 families.

Arthropod Sorensen dissimilarity among traps across all time periods was very high (mean = 0.73, sd = 0.09), as was dissimilarity among time periods across all traps (mean = 0.61, sd = 0.12). The highest pairwise dissimilarity among months was 0.80, involving comparisons between May and August, while the lowest was 0.44, both between July and August and between August and September.

Arthropod community dissimilarity was significantly related to both environmental distances and temporal distance (SPC = 0.27) (Table 1, Figures 2 – 3; SI Appendix, Figure S2), but there was no significant effect of spatial distance among traps. The total R^2 for the effect of all variables on arthropod dissimilarity was 0.49. Spatial and temporal distances were both significantly related to environmental distances ($P < 0.05$), demonstrating that environmental

292 variables were both spatially and temporally structured. Of the eight environmental variables
293 considered, six had a significant direct effect on arthropod community dissimilarity after
294 Benjamini-Hochberg correction (Table 1, SI Appendix, Figure S2). In order of importance, these
295 included a positive effect of changes in canopy openness (SPC = 0.31), a positive effect of plant
296 community dissimilarity (SPC = 0.26), a positive effect of changes in climate PC1 (SPC = 0.23),
297 a positive effect of changes in climate PC4 (SPC = 0.14), a positive effect of changes in climate
298 PC2 (SPC=0.07), and a positive effect of changes in climate PC3 (SPC = 0.07). Temperature and
299 the coefficient in variation of temperature loaded most strongly on PC1 (0.49 and -0.50,
300 respectively), wind speed and average precipitation loaded most strongly on PC2 (0.72 and -
301 0.43, respectively), relative humidity and wind direction loaded most strongly on PC3 (-0.89 and
302 -0.33, respectively), and temperature and the coefficient of variation in temperature loaded most
303 strongly on PC4 (-0.60 and -0.66, respectively). There was no significant effect of changes in the
304 proportion of agriculture in the landscape nor was there a significant direct effect of plant
305 richness. Plant richness and canopy openness both had indirect effects through compositional
306 dissimilarity among plant communities (SPC with plant community dissimilarity = 0.09 and
307 0.39, respectively).

308 When environmental effects were lumped into variable groups of climate, plant
309 community attributes, and agricultural intensity (Figure 2), plant community attributes had the
310 strongest effect ($\Sigma|SPC| = 0.70$), followed by climate variables ($\Sigma|SPC| = 0.51$), with no
311 significant effect of agricultural intensity. Plant community attributes and agricultural intensity
312 both showed spatial structure ($\Sigma|SPC| = 0.29$ for both), while plant community attributes showed
313 weak temporal structure ($\Sigma|SPC| = 0.04$), and climatic variables showed both temporal and
314 spatial structure ($\Sigma|SPC| = 0.97$ and 0.27, respectively). Corroborating the results of the path

analysis, variance partitioning (Figure 4) showed that most of the variation in arthropod community composition could be explained purely by environmental distances (adjusted R^2 = 0.32). Environmental and spatial distances shared a small fraction of variation (adjusted R^2 = 0.01), while spatial distance retained no unique contribution. Environmental and temporal distances shared a larger fraction of variation (adjusted R^2 = 0.12), indicating that temporal variability in environmental conditions across the sampling season played an important role in structuring arthropod communities. Temporal distance retained a unique but small contribution (adjusted R^2 = 0.05). After accounting for the effects of space and time, most of the variation that was explained by environmental distances was due to plant community attributes (adjusted R^2 = 0.21), followed by climate (adjusted R^2 = 0.06), and no unique effect of agricultural intensity. These results indicate that much of the effect of local plant communities was trap-specific (independent of spatial and temporal distances), whereas climate variables were largely collinear with temporal distance.

DISCUSSION

Taken together, our results suggest the dominance of species-sorting dynamics in both space and time, a possible effect of ecological drift, and no evidence of dispersal limitation in this system. We found arthropod communities to be highly variable among localities and across time periods. Plant community attributes best explained this variation, the effects of which were much stronger than agricultural intensity despite variation in the percentage of agriculture in the landscape ranging from 11% to 78%. Climatic variability across the sampling season also played an important role. Environmental variables demonstrated both temporal and spatial structure and significant effects of temporal distance on arthropod dissimilarity remained even after accounting for environmental variables, while spatial distance did not retain a significant effect.

Canopy openness had a significant direct effect while plant richness did not, though both had indirect effects through plant community composition. These results highlight that arthropod communities tend to be strongly specialized on specific plant communities. This could be due to species-specific preferences for food (either directly on plants or other organisms that depend on those plants), nesting, shelter, and mating resources, or because plant community composition also acts as a reliable index of other environmental factors such as light availability or soil type (Schaffers *et al.* 2008). It is noteworthy that plant richness alone did not have a significant direct effect in our analyses, as many studies have shown this to be an important determinant of arthropod community composition (Borer *et al.* 2012; Ebeling *et al.* 2018). Combined with the indirect effect through plant community composition, this means that the identities of plants mattered more than their richness for the arthropod communities studied here (e.g., Harvey & MacDougall 2015).

Researchers seldom demonstrate these relationships in agroecosystems (but see Boutin *et al.* 2009), tending instead to place emphasis on remote sensing data that show effects of agricultural intensity at larger spatial scales (Schweiger *et al.* 2005). Contrary to these results, we did not find a significant effect of agricultural intensity at the landscape scale. Our findings demonstrate that restoration of multiple habitat types with compositionally distinct plant communities at a local scale is likely to be an effective method for sustaining arthropod diversity in agroecosystems, provided that the landscape contains enough functionally connected habitat to maintain the species pool (Scheper *et al.* 2013).

Variation in climatic conditions, particularly across the growing season, also played an important role in determining arthropod community composition. This could be explained by several mechanisms. The first is that climate has a direct effect on arthropod survival and

reproduction. Arthropods are generally constrained to narrow optimum ranges of temperature and humidity and taxa differ widely in their tolerances for climatic conditions, with some species being specialized for early emergence (Høye & Forchhammer 2008). Such differences in the phenology of emergence due to climatic conditions results in compositional turnover throughout the season. It could also partially explain why strong differences were observed with forest canopy, as turnover of arthropods between shaded cool forest and warmer and often drier herbaceous plant communities tends to be high (Yekwayo *et al.* 2017). A related explanation could be resource limitation. Many arthropods depend on specific feeding and nesting/shelter resources, and many of those resources are not available early in the season due to plant phenology in the case of herbivores (foliage) and pollinators (flowers), and the phenology of prey in the case of predators (Høye & Forchhammer 2008).

Climate change is predicted to have major impacts on seasonal systems, particularly regarding increases in temperature and the timing of seasonal events (Hoegh-Guldburg *et al.*, 2018). Our results suggest that arthropod communities in seasonal agroecosystems are likely susceptible to shifts in seasonal norms, either directly via physiological mechanisms or indirectly due to changes in the resource phenology (Høye & Forchhammer 2008). Studying the interactive effects of temporally varying agricultural practices, especially pesticide application, and variation in climate on the plant and arthropod communities in farm landscapes will be critically important to understand the effects of multiple stressors on these dynamic communities. This is an area that warrants further research.

Much of the variation in arthropod community composition could be explained by environmental factors, indicating a strong role for species sorting in both space and time in these communities. The effect of spatial distance on community dissimilarity was not significant after

384 accounting for environmental variation. This finding is especially interesting, given the large
385 extent of our study region and its ~90% cover of crop monoculture, the fact that many non-crop
386 areas with natural or restored plant cover can be highly spatially isolated, and that this habitat
387 isolation has been in place for many decades given that this region has been intensely farmed
388 since at least the 1930s (Riley 2013; McQuarrie 2014). This degree of habitat transformation
389 over the last century might imply acute species turnover by spatial distance but this was not the
390 case. That being said, single individuals of many flying arthropods such as some species of bee
391 have foraging ranges upwards of 5km (Greenleaf *et al.* 2007) and are likely to travel much
392 further in windy conditions (Pasek 1988), resulting in many transient individuals being caught in
393 the traps and high dispersal potential. This could explain the high incidence of singleton
394 occurrences observed here as well as the weak effect of spatial distance. The effect of temporal
395 distance, however, did remain significant after controlling for environmental variation.
396 Theoretical and empirical work has shown that ecological drift should result in directional
397 turnover through time that is independent of environmental variability (Hubbell 2001; Hatosy *et*
398 *al.* 2013; Jabot *et al.* 2020). We find evidence of this here, though it should be interpreted with
399 care as our study was observational and could not control for all potentially relevant factors.
400 Either way, the discovery of such strong temporal turnover (far exceeding the magnitude of
401 turnover with spatial distance) over a growing season was a novel finding that emphasizes the
402 important but often neglected role of seasonality.

403 Despite the limitations on inferring process from pattern, the use of a metacommunity-
404 based framework is useful for investigating which mechanisms may be most important for
405 shaping arthropod communities in agroecosystems. Several recent studies have shown that
406 ecological drift may play a stronger role in determining the composition of local communities

than commonly thought (Gilbert & Levine 2017; Sydenham *et al.* 2017; Jabot *et al.* 2020; Siqueria *et al.* 2020), but this remains an infrequent subject of empirical investigation. Attaining a better understanding of these mechanisms has implications for the management of agricultural landscapes. If species sorting mechanisms primarily govern the composition of arthropod communities then the focus of conservation efforts might be placed on ensuring that a diverse set of habitat types and local resources are represented in the landscape (Economo 2011). If ecological drift and dispersal limitation primarily govern the composition of arthropod communities then more focus might be placed on the size and spatial arrangement of habitat patches (Economo 2011; Gilbert & Levine 2017). Finally, the powerful role of seasonal turnover on arthropod diversity that we observed, deriving from unprecedentedly frequent sampling intervals, implies that the timing of pesticide application could have large impacts on arthropod communities, with the potential to more closely target application windows to avoid overlap with non-target species including those with high functional benefit.

Our ability to examine the composition of arthropod communities with such broad taxonomic coverage at a large spatiotemporal scale was mainly due to the combined use of metabarcoding and Malaise traps, both of which are highly scalable methodologies (deWaard *et al.* 2019). Metabarcoding has many advantages over morphological identification. It provides a standardized method for species assignment even when a species has not been formally described, allows finer taxonomic resolution, speeds sample processing time, and is very cost-effective (Cristescu 2014; Bush *et al.* 2020). Using barcoding rather than morphological identification can also increase estimates of species richness and beta diversity by revealing cryptic species (Brehm *et al.* 2016; D'Souza & Hebert 2018), which allows for a more robust

assessment to be made about the factors that drive variation in community composition (Bush *et al.* 2020).

Habitat restoration is commonly promoted as a useful land management strategy to prevent or reverse biodiversity loss in agroecosystems (Tilman *et al.* 2002; Green *et al.* 2005 Fahrig *et al.* 2011; Ekroos *et al.* 2016). Under a land-sharing framework, agricultural systems should be managed to retain and/or enhance habitat heterogeneity to ensure that a wide array of organismal needs can be met, resulting in more abundant and taxonomically diverse communities, enhanced ecosystem services, and improved ecological stability (Borer *et al.* 2012; Tscharntke *et al.* 2012). Results from our study suggest that enhancement of local habitat heterogeneity, particularly restoration of woody cover and local vegetation composition, should be a particularly useful means of managing agro-ecosystems to better conserve arthropod biodiversity and the critical ecosystem services that flying arthropods provide. Given the immense challenge that the world faces in feeding a large and growing human population, it is critical that we continue to better understand and implement the strategies that can work alongside agriculture to maintain viable habitat for the benefit of human and non-human communities alike.

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TABLES

Table 1. Results from path analysis and associated standardized path coefficients. Entries in bold are significant ($P < 0.05$ with Benjamini-Hochberg correction). The fit statistics of the path model were as follows: SRMR = 0.055, RMSEA = 0.08, CFI = 0.889.

GROUP	PATH	STANDARDIZED PATH COEFFICIENT
	SØRENSEN DISSIMILARITY ~ Δ TIME	**0.27**
	SØRENSEN DISSIMILARITY ~ Δ SPACE	0.02
PLANT COMMUNITY ATTRIBUTES	**SØRENSEN DISSIMILARITY ~ Δ CANOPY OPENNESS**	**0.31**
	SØRENSEN DISSIMILARITY ~ Δ PLANT COMMUNITY	**0.26**
	SØRENSEN DISSIMILARITY ~ Δ PLANT RICHNESS	0.04
	Δ CANOPY OPENNESS ~ Δ SPACE	**0.06**
	Δ PLANT COMMUNITY ~ Δ TIME	**0.04**
	Δ PLANT COMMUNITY ~ Δ SPACE	**0.16**
	Δ PLANT RICHNESS ~ Δ TIME	0.03
	Δ PLANT RICHNESS ~ Δ SPACE	**0.07**
	Δ PLANT COMMUNITY ~ Δ CANOPY OPENNESS	**0.39**
	Δ PLANT COMMUNITY ~ PLANT RICHNESS	**0.09**
CLIMATIC	**SØRENSEN DISSIMILARITY ~ Δ PC1**	**0.23**
	SØRENSEN DISSIMILARITY ~ Δ PC2	**0.07**
	SØRENSEN DISSIMILARITY ~ Δ PC3	**0.07**
	SØRENSEN DISSIMILARITY ~ Δ PC4	**0.14**
	Δ PC1 ~ Δ TIME	**0.40**
	Δ PC1 ~ Δ SPACE	0.02
	Δ PC2 ~ Δ TIME	**0.14**
	Δ PC2 ~ Δ SPACE	**0.27**
	Δ PC3 ~ Δ TIME	**0.36**
	Δ PC3 ~ Δ SPACE	-0.03
	Δ PC4 ~ Δ TIME	**0.07**
	Δ PC4 ~ Δ SPACE	0.00
AGRICULTURAL INTENSITY	SØRENSEN DISSIMILARITY ~ Δ % AGRICULTURE	0.02
	Δ % AGRICULTURE ~ Δ SPACE	**0.29**

FIGURES

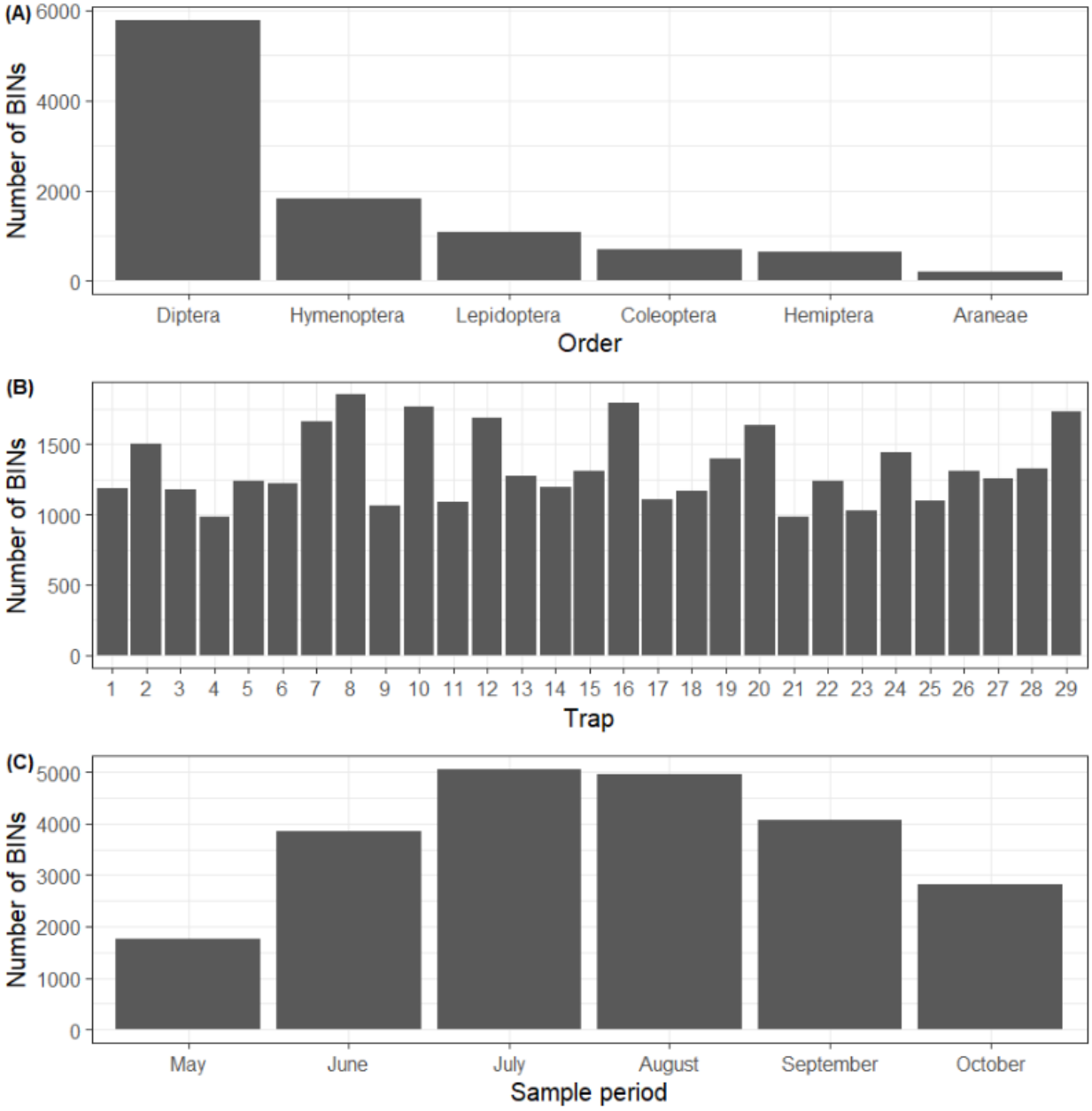


Figure 1. Distribution of arthropod BINs (Barcode Index Number; a species proxy) for (A) the top six most species-rich orders of arthropods across all samples, (B) among traps pooled across all time periods, and (C) among sample periods pooled across all traps.

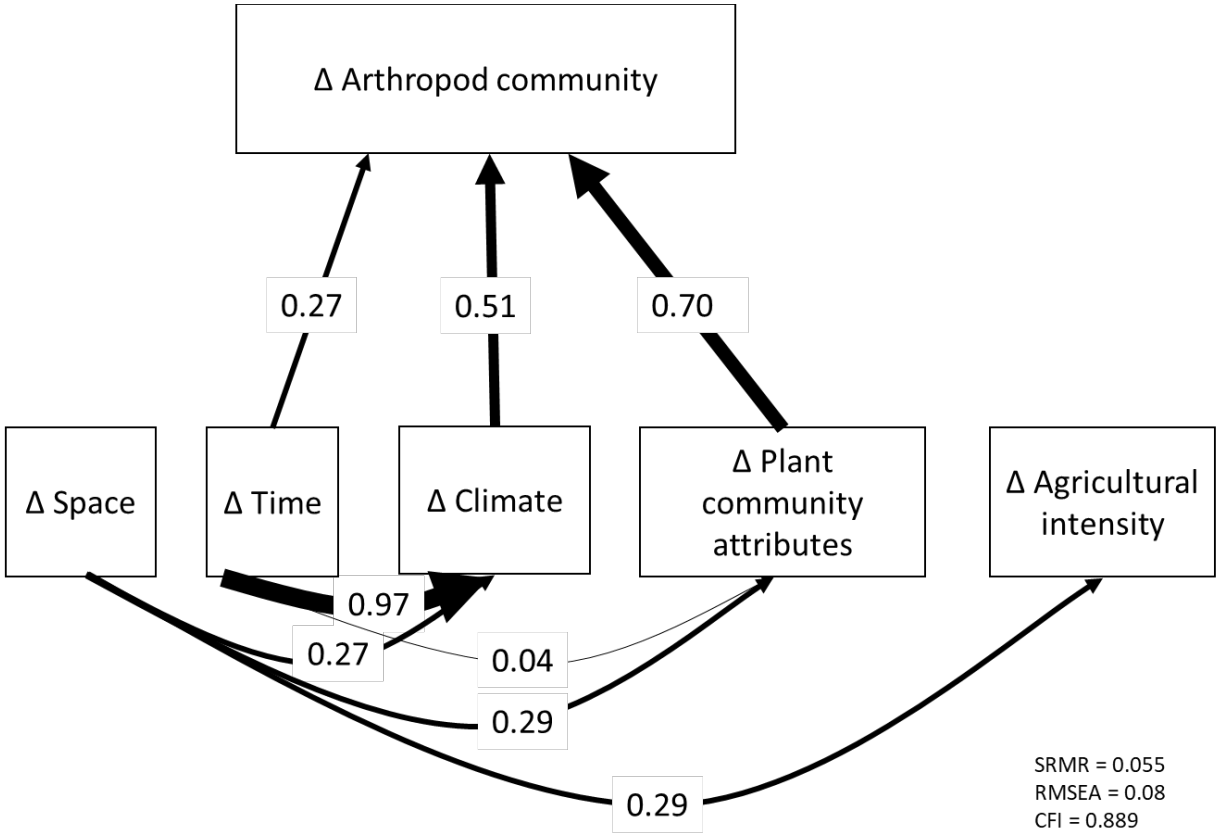


Figure 2. Path analysis of factors influencing spatiotemporal Sørensen dissimilarity of arthropod community composition. Only significant paths are shown. Values correspond to standardized path coefficients, and values towards or away from variable groups (e.g. climate) represent the sum of the absolute values of standard path coefficients for each variable within those groups (including direct and indirect effects). Arrow thickness is proportionate to the magnitude of the standardized path coefficients.

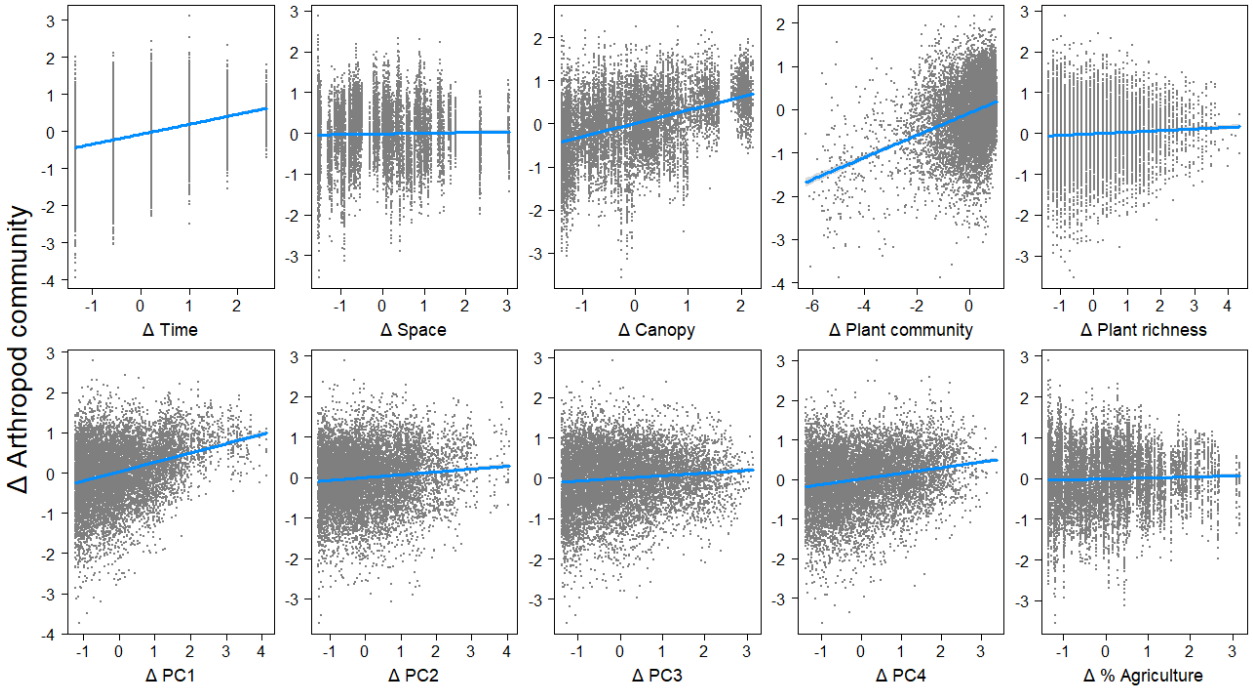
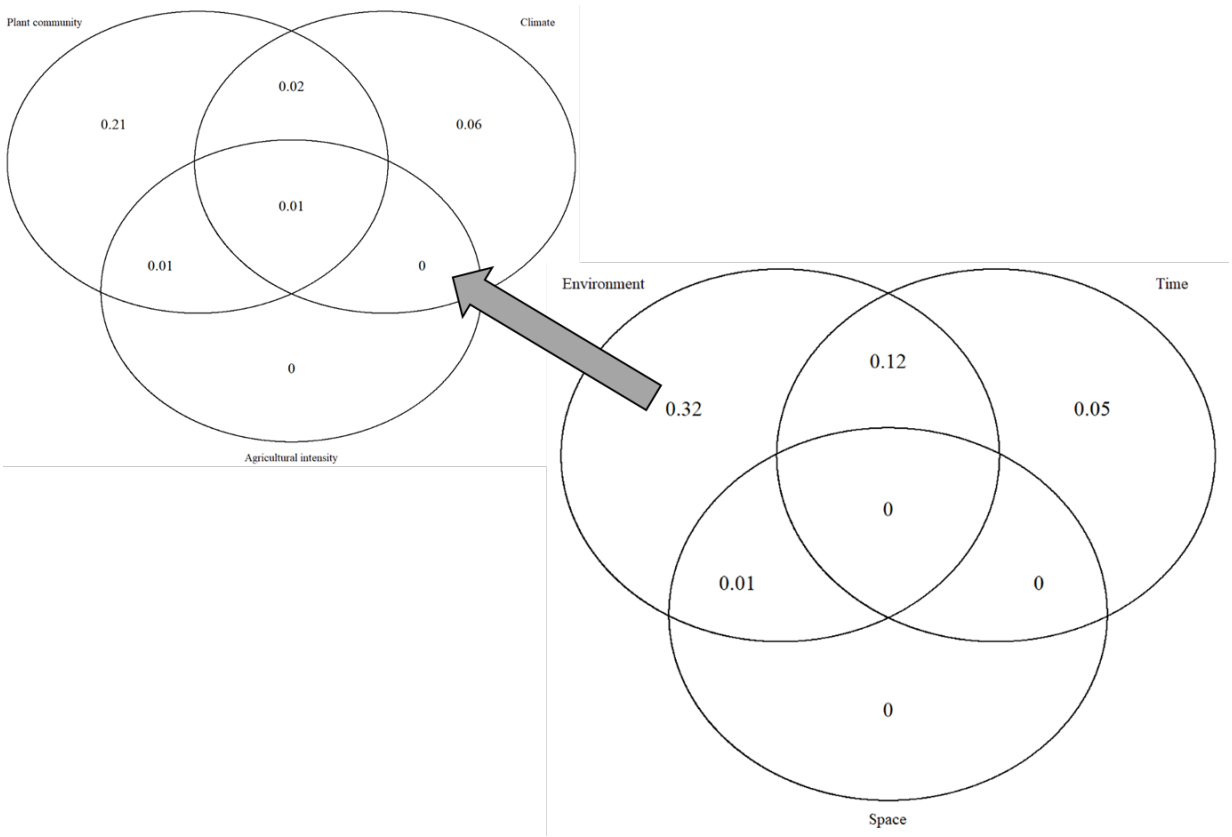


Figure 3. Partial regression plots for the effect of all variables in the path analysis on spatiotemporal arthropod Sørensen dissimilarity. PC1 – 4 are principal components of the climatic variables. All variables are standardized.



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669 Figure 4. Regression-based variance partitioning results for the effect of distances in
670 environment, space, and time on arthropod Sørensen dissimilarities. Numbers are adjusted R^2
671 values and the results showing the relative effects of plant community attributes, climate, and
672 agricultural intensity are conditional on the effects of space and time.

673 **SUPPORTING INFORMATION**

674 **APPENDIX**

675 *METABARCODING METHODS*

676 Bottles sent for metabarcoding first had their ethanol filtered off using a sterile
677 Microfunnel 0.2 uM Supor membrane filter (Pall Laboratory) using a 6-Funnel Manifold (Pall
678 Laboratory). The filters were then weighed to measure wet arthropod biomass. DNA extractions
679 employed a membrane-based protocol (Ivanova *et al.* 2006). Wet biomass was used to
680 standardize the amount of arthropod lysis buffer added to each bottle (~10 ml buffer per g of
681 biomass). After lysis buffer was added, each bottle was incubated at 56°C overnight on a shaker.
682 After lysis, technical replicates were created by taking 300 µl of lysate from eight locations in
683 each bottle. 50 µl from each of these technical replicates were placed into a separate well in a 96-
684 well microplate along with 8 negative controls (no DNA) and 8 positive controls (known
685 community DNA sample: public dataset at http://dx.doi.org/10.5883/DS-AGAKS) per plate. 100
686 µl of binding mix was added to the lysate which was then transferred to a 3.0 µm Pall Supor
687 Membrane glass fiber plate and centrifuged at 5000g for 5 minutes. The resultant DNA extracts
688 were purified in three wash steps: 180 µl of protein wash buffer centrifuged at 5000g for 2
689 minutes followed by two washes with 600 µl of wash buffer centrifuged twice at 5000g for 5
690 minutes. The filter plate was then transferred onto a sterile 96-well microplate and incubated at
691 56°C for 30 minutes. DNA elution was carried out by adding 60 µl of 10 mM Tris-HCl pH 8.0
692 followed by centrifugation at 5000g for 5 minutes.

693 A 462 base-pair amplicon of cytochrome *c* oxidase subunit I (COI) was PCR amplified
694 using the forward primer AncientLepF3 (Prosser *et al.* 2016) and the reverse primer cocktail

C_LepFo1R (containing LepR1 and HCO2198) (Hebert *et al.* 2004). The PCR cocktail included: 1.25 µl of 10x Platinum Taq reaction buffer (Invitrogen), 6.25 µl of 10% trehalose (Fluka Analytical), 0.625 µl of 50 mM $MgCl_2$, 0.0625 µl of 10 mM dNTPs (KAPA biosystems), 0.125 µl of each primer (1 µM), 0.06 µl of Platinum Taq (5 U/µl), 2 µl of DNA extract, and 2µl of Hyclone ultra-pure water (Thermo Scientific). PCR employed the following cycling regime: initial denaturation at 94°C for 2 minutes, 20 cycles of denaturation at 94°C for 40 seconds, annealing at 51°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The resultant PCR products were diluted by 2x before a second round of PCR with fusion primers to attach a different pair of unique molecular identifiers (UMIs) to the amplicons from each well along with sequencing adaptors that are required for IonTorrent S5 libraries. The resultant PCR products were pooled, standardized to 1 ng/µl, and the sequence libraries were prepared on the Ion Chef™ system (Thermo Fisher Scientific) for characterization on a 530 Chip according to manufacturer instructions.

The reads derived from the eight technical replicates for each sample were separately uploaded to the mBRAVE platform (Ratnasingham 2019; http://www.mbrave.net/). Sequences were only retained if they had a mean quality value (QV) > 20, a minimum length of 350 bp, less than 25% of bases with QV <20, and less than 5% of bases with QV <10. Reads were trimmed 30 bp at the front with a trim length of 450 bp. Reads were queried against mBRAVE reference libraries for chordates, insects, non-insect arthropods, non-arthropod invertebrates, and bacteria. Reads were assigned to a Barcode Index Number (BIN) that serves as a species proxy (Ratnasingham & Hebert 2013). The BIN system uses the Refined Single Linkage (RESL) algorithm to designate OTUs and then match them to BINs in the Barcode of Life Data System (BOLD; http://boldsystems.org) based on a predefined distance threshold (Ratnasingham &

718 Hebert 2013). Thus, BIN assignments are dynamic and depend on the continual updating of
719 sequence information in BOLD; the taxonomy reported in this study is current as of November
720 2019. During a second denoising process, BINs were discarded under the following
721 circumstances: (1) they had less than 5 sequence reads summed across all technical replicates,
722 (2) their read count was less than the mean read count for the run in at least 75% of the technical
723 replicates, or (3) their read count was less than 1% of the maximum read count for the run with
724 less than 10 total reads. BINs that showed up in negative controls would have been removed in
725 this process and if the noise could not be removed through these steps, the run was excluded
726 and/or rerun. Only arthropods and non-arthropod invertebrates were included in the final BIN
727 table, though arthropods constituted 99.8% of these BINs.

728 Table S1. Environmental variables considered in the path analysis and their groupings into either
729 plant community attributes, agricultural intensity, or climatic variables. Type of variation
730 indicates whether the explanatory variable exhibits spatial (S) temporal (T) or spatiotemporal
731 (S+T) variation. *Limited spatial variation based on closest weather stations. Climatic variables
732 were subject to principal components analysis prior to use in path analysis and 4 axes were
733 retained, explaining 88% of the variation.

GROUP	VARIABLE	TYPE OF VARIATION
PLANT COMMUNITY ATTRIBUTES	CANOPY OPENNESS	S
	PLANT GENUS RICHNESS	S+T
	BRAY-CURTIS PLANT COMMUNITY COMPOSITION	S+T
AGRICULTURAL INTENSITY	% AGRICULTURE IN 2KM RADIUS	S
CLIMATIC	AVERAGE TEMPERATURE	S+T
	AVERAGE RELATIVE HUMIDITY	S+T*
	CV TEMPERATURE	S+T
	AVERAGE WIND SPEED	S+T*
	AVERAGE WIND DIRECTION	S+T*
	AVERAGE PRECIPITATION	S+T*

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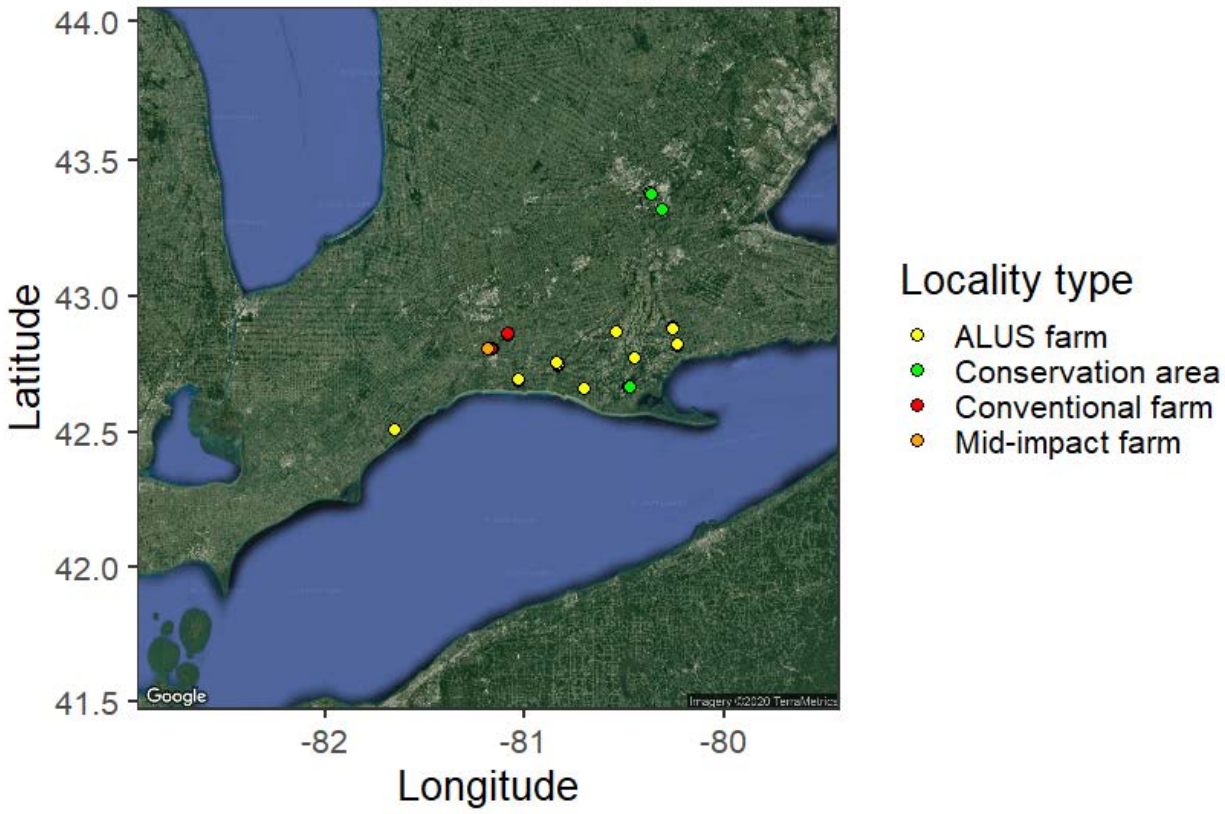

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736 Figure S1. Map of Southern Ontario, Canada showing the study sites and their respective

737 management types.

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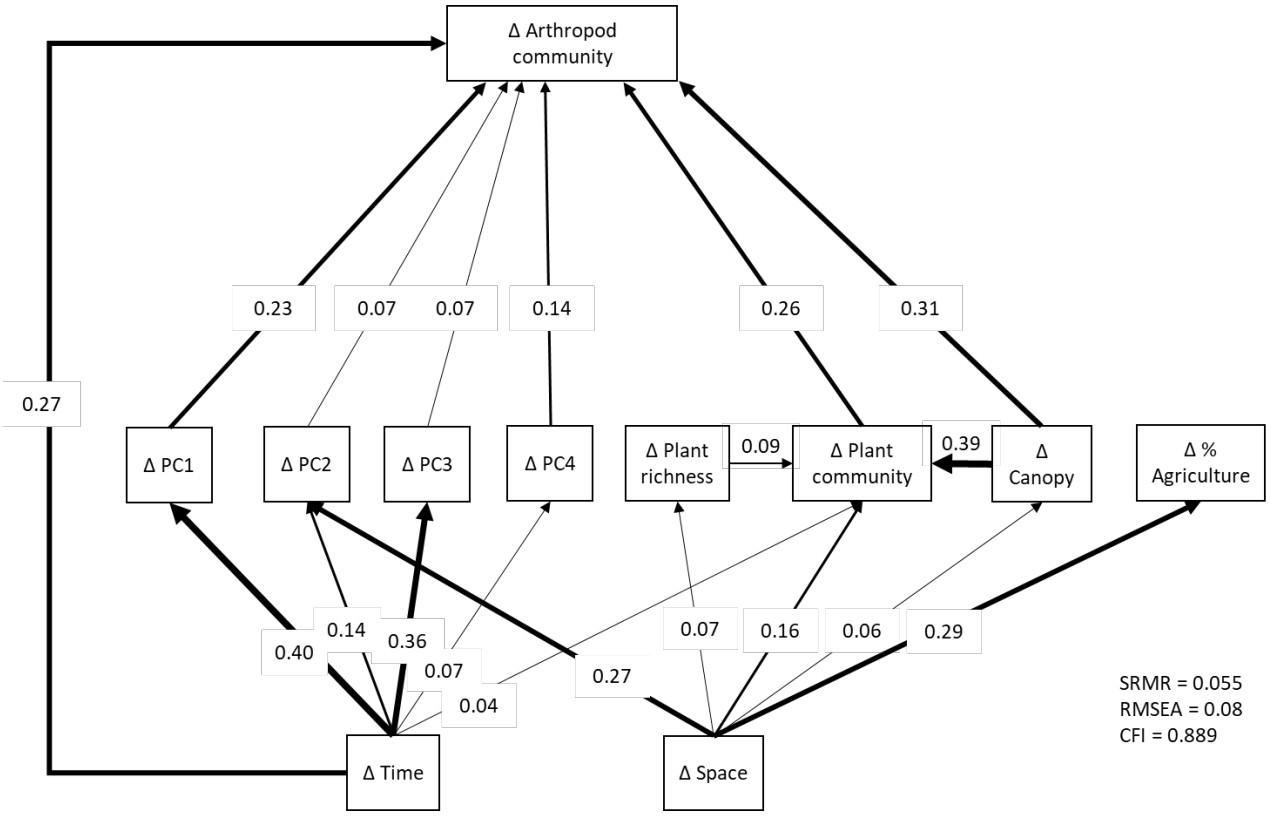

Figure S3. Path analysis of factors influencing spatiotemporal Sørensen dissimilarity of arthropod community composition. PC1 – 4 are principal components of the climatic variables. Values correspond to standardized path coefficients. Only significant paths are shown. Arrow thickness is proportionate to the magnitude of the standardized path coefficients.

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