ASSESSING THE CONSERVATION AND ENHANCEMENT VALUE OF BLOCKS OF REMNANT VEGETATION ON BENEFICIAL ARTHROPOD ASSEMBLAGES IN A PASTURE LANDSCAPE

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Abstract

Investigating the community dynamics of ecosystem service-providing arthropods in non-crop habitats within agroecosystems is crucial in promoting ecological intensification methods that further contribute to engineering sustainable agroecosystems. Arthropods are important as drivers of ecosystem functions and processes, such as pollination and pest suppression. The specific objectives of this study were to compare arthropod assemblages in blocks of native remnant vegetation and exotic pastures, and survey abundances and diversity of beneficial arthropods between remnant vegetation and adjacent pasture in early spring (September 2009) and mid-summer (December 2009 - January 2010) on two farms in southeastern Gippsland, Victoria, Australia. The structure of arthropod communities were significantly different between the core of remnant vegetation and pasture sites in both seasons. Arthropod community assemblages at the edge of remnant vegetation were significantly different compared to assemblages 20 m in adjacent pasture in early spring. However, these assemblages were not distinctly different in mid-summer. Apidae (bees) were of similar abundances in the two habitats. Carabidae (ground beetles) was the only taxa to have significantly higher abundances at pasture sites in early spring. Seven of the 14 ant genera collected, ten spider families and Xylophagidae (awl flies) were found exclusively in remnant vegetation. Bethylidae (bethylids) and Dolichopodidae (long-legged flies) were not found 80 m into adjacent pasture during either season. In early spring, Araneae (spiders), Lycosidae (wolf spiders), Staphylinidae (rove beetles), Bethylidae (bethylids), Syrphidae (hoverflies) and Formicidae (ants) were found to have significantly higher abundances in the core or edge of remnant vegetation compared to pasture sites; in mid-summer the higher taxonomic groups showed no significant differences between sites. These results demonstrate that beneficial arthropods use remnant vegetation as refugia in early spring and that remnant vegetation enhanced invertebrate conservation in pasture systems. Relevance of these findings to improved biological control is also discussed.

Key words: *beneficial invertebrate, remnant vegetation, natural enemy, non-crop habitat, native vegetation*

1. INTRODUCTION

Ecological intensification (EI) is a knowledge-intensive process that harnesses ecosystem services (ES), such as nutrient recycling and water purification, to improve agricultural system efficiency and sustainability (Bommarco *et al.*, 2013, Pretty and Bharucha, 2014). EI aims to increase yields by utilizing agricultural practices which are not environmentally damaging and which require fewer inputs (Petersen and Snapp, 2015). Investigating assemblages of beneficial arthropods in the agricultural matrix is crucial in devising methods to manage agroecosystems in a way that enhances arthropod-driven ES and contributes to resilient farming systems (Altieri, 1999, Crowder and Jabbour, 2014).

Beneficial arthropods provide an array of ES, including pollination and pest suppression (Crowder and Jabbour, 2014). Preserving non-crop habitat in agricultural landscapes has been shown to conserve and enhance populations of beneficial arthropods (Landis *et al.*, 2000, Moron *et al.*, 2014, Dong *et al.*, 2015). Established shelterbelts in agricultural landscapes have been shown to support various groups of beneficial arthropods including ladybeetles (*Propylea japonica* (Thunberg) and *Harmonia axyridis*

(Pallas)) and spiders (Dong *et al.*, 2015) in China; predatory mites (Tsitsilas *et al.*, 2006), bees and long-legged flies (Dolichopodidae) (O'Donnell and Wright, 2021) in Australia. Other research has demonstrated that non-crop vegetation and natural enemies in agricultural production systems can suppress pest species below economic thresholds, increase crop yields and increase quality of grain crops in India (Gowda and Sharanabasappa, 2004) and Africa (Munyuli *et al.*, 2007); and of cotton crops in Australia (Mensah, 2002). Extensive knowledge of the invertebrate community is needed to determine the beneficial arthropod assemblages present and their requirements in the agroecosystem, in order to promote ES (Shackelford *et al.*, 2013).

The essential resources of shelter, nectar, alternative prey and pollen, collectively known as SNAP (Gurr *et al.*, 2017), are not available in many cropping systems during critical periods in the life-cycle of beneficial arthropods (Dong *et al.*, 2015). To enhance the efficacy of beneficial arthropods, habitat manipulations that provide SNAP in the agroecosystem need to be studied (Gurr *et al.*, 2017). Investigating how arthropod communities interact with different flora is important in determining the vegetation required to enhance arthropod numbers (Colley and Luna, 2000). Vegetation structure (i.e., the presence/absence of multiple canopy layers) and composition (i.e., the phenology and floral architecture defining the plant community) are of interest when examining the flora-fauna interaction of beneficials. Certain traits in plants, such as floral supplement accessibility, inflorescence height and the synchronicity of arthropod life-cycle with plant phenology, have been shown to be attractive to beneficial arthropods (Klecka *et al.*, 2018). Vegetation structure and composition can be heavily managed in some kinds of non-crop habitat (MacLeod *et al.*, 2004, Tsitsilas *et al.*, 2011). However, the flora comprising blocks of remnant vegetation is not often intentionally altered by humans due to legal restrictions and societal norms (Malcolm *et al.*, 2010). Nevertheless, studies involving the interactions of native flora and beneficials in remnant vegetation is important in informing on which native plants are best suited to conserve populations of beneficial arthropods by providing resources at critical times.

Even though vegetation in non-crop habitat may provide resources to improve abundances of beneficial arthropods, previous research has demonstrated that this does not necessarily translate into increased pest suppression in adjacent farmland in some agroecological contexts. In New Zealand, for example, many of the natural enemies in non-crop habitat did not emigrate into adjacent fields (Mclachlan and Wratten, 2003). Uncultivated habitat can also have a sink effect on beneficial arthropod populations by luring them away from cropland (Gontijo, 2018). Thus, it is imperative that assemblages of beneficial arthropod and their emigration into adjacent cultivated lands be measured if establishing and protecting native vegetation in farmland is to be widely adopted.

In south-eastern Australia, blocks of remnant vegetation are often used as windbreaks to provide protection to livestock and limit wind erosion (Abensperg-Traun and Smith, 1999, Wainer *et al.*, 2001). These blocks are often fenced off from pasture to prevent livestock from entering (Malcolm *et al.*, 2010). The impact of remnant vegetation on populations of beneficial invertebrates in pasturelands has not been amply studied in Australia (Tsitsilas *et al.*, 2006). As such, there is space for research which measures the effects of remnant native vegetation on the abundances and diversity of beneficial arthropods in Australian pastures. The overarching aim of this research was to examine whether blocks of remnant native vegetation were serving as a refuge for beneficial arthropods. We hypothesized that there would be a higher abundance of beneficial arthropods in the blocks of remnant vegetation than nearby pasture and that beneficials would colonize adjacent pasture from these remnant blocks.

2. MATERIALS AND METHODS

2.1 Study sites

Two field trials were conducted at two beef/sheep farms (FarmDB and FarmDM) located approximately 22 km apart in the Darriman-Giffard area of south-eastern Gippsland, Victoria bounded by the southwest coordinates 38°35'59"S, 146°40'10"E and the northeast coordinates 38°03'17"S, 146°52'34"E (Fig. 1).

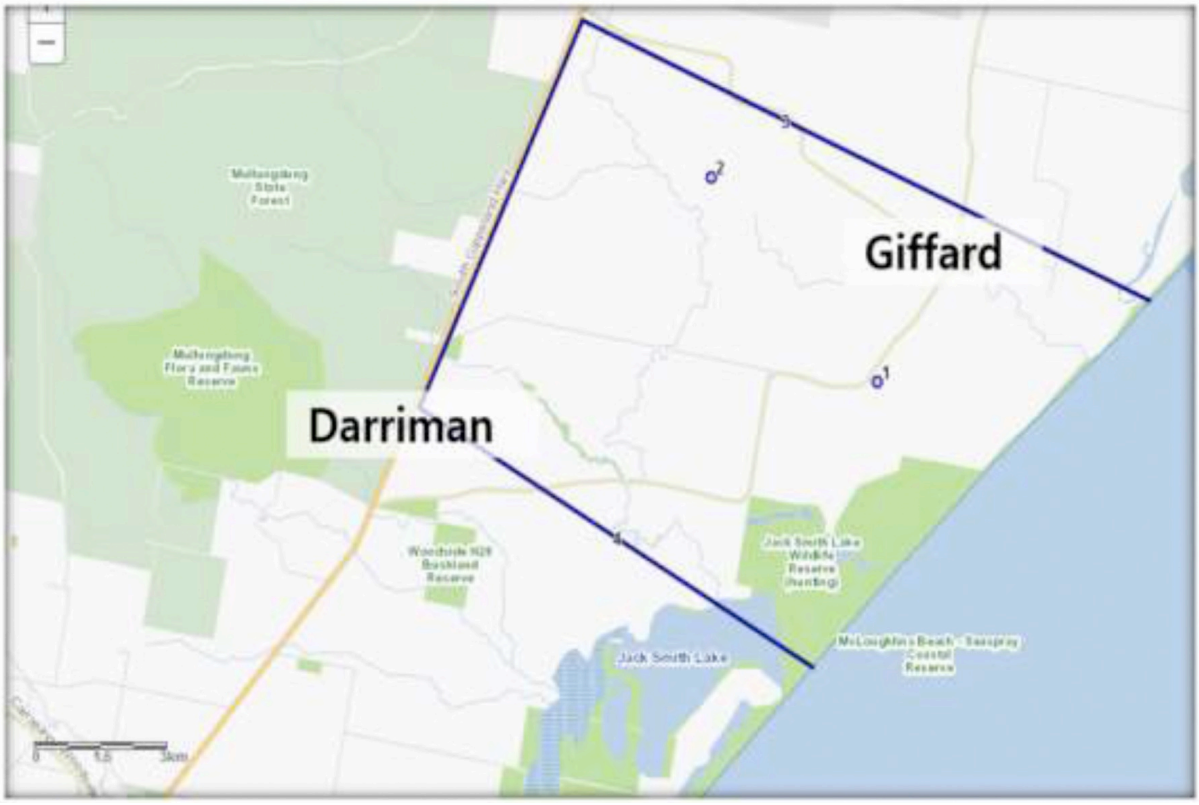


Fig. 1. Black outlined area encompasses the study region, which was located within southeastern Gippsland in Victoria, Australia. Black dots: Location of the two farms where invertebrate surveys were conducted (Department of Environment, 2015).

Farms were located between the Mullungdung State Forest to the west and Ninety Mile Beach to the east. Waterways that bound the farms were the riparian zones of Merrimans Creek to the northeast and Bruthen Creek to the southwest. The research area was a highly modified agricultural landscape with large expanses of cleared land for grazing of livestock. Native vegetation made up less than 18% of vegetation found on either farm. FarmDB and FarmDM each had 2 blocks of remnant native vegetation. These comprised one 32 ha and one 8 ha blocks at FarmDB and one 34 ha and one 2 ha block at FarmDM. The two larger blocks were the focus of this study. FarmDB and FarmDM had four and two strips of revegetated land, respectively. The two remnant vegetation blocks surveyed were more than 1 km from other blocks of remnant native vegetation and from strips of revegetated land.

Farms had comparable management strategies [off-farm input levels (medium), insecticide spraying frequency (low), and stocking regime (set)] and were located in the same bioregion (Gippsland Plains Bioregion). Farms were selected to minimize variation in management and vegetation type, which reduces potential confounding factors but also limits applicability of the results. The Gippsland Plains Bioregion are completely described in O'Donnell and Wright (2021). Rainfall is variable throughout the Gippsland region. Annual average rainfall at the time of this study was 618 mm.

The main Ecological Vegetation Class (EVC) in both blocks of remnant vegetation was lowland forest/heathy woodland mosaic (LF/HWM), which is a merger of two other EVCs: lowland forest and heathy woodland. EVCs are mapping units for native vegetation and discussed thoroughly in O'Donnell and Wright (2021). LF/HWM is classified as 'vulnerable', which indicates that between 10-30% of this vegetation type remains compared to its modelled extent prior to European settlement (pre-1750).

Areas of remnant vegetation were fenced off for more than 6 years, never logged and unaffected by fires for at least 20 years. The overstorey was dominated by narrow-leaf peppermint (*Eucalyptus radiata* s.l. DC.) and rough-barked manna gum (*Eucalyptus viminalis* ssp. *pryoriana* Labill.). Species in the shrub layer included blackwood (*Acacia melanoxylon* R.Br.), prickly tea-tree (*Leptospermum continentale* Thomps.), prickly moses (*Acacia verticillata* L'Her.), common heath (*Epacris impressa* Labill.), and heath tea-tree (*Leptospermum myrsinoides* Schltdl.). The ground layer included dense austral bracken (*Pteridium esculentum* G. Forst.)) in addition to grasses (tussock grass (*Poa australis* spp. *agg*. R.Br.)) and forbs (common raspwort (*Gonocarpus tetragynus* Labill.) and thatch saw-sedge (*Gahnia radula* R.Br.)). The pastures were dominated by perennial ryegrass (*Lolium perenne* L.) and subterranean clover (*Trifolium subterraneum* L.) with some cock's-foot (*Dactylis glomerata* L.), phalaris (*Phalaris aquatic* L.) and Capeweed (*Arctotheca calendula* L.) in early spring, and near complete dominance in mid-summer by *L. perenne*. Ground vegetation was taller in remnant vegetation compared to adjacent pasture in early spring (414 mm vs 99 mm) and mid-summer (315 mm vs 72 mm). There was a higher percentage of organic matter (OM) such as leaf litter, dead grass and woody debris in remnant vegetation compared to adjacent pasture in early spring (56% vs 5%) and mid-summer (61% vs 41%). The OM found on the floor of remnant vegetation was composed of a diverse mixture of materials ranging from bark and sticks to dead bracken and kangaroo scat. The pasture OM was predominately composed of a very thin layer of shriveled, dried blades of grass, especially in mid-summer.

2.2 Arthropod sampling

Arthropod surveys were executed in the same manner as in O'Donnell and Wright (2021), which involved making measurements at points in space and time (Hurlbert, 1984). The treatments were distances from (and including) the edge of remnant vegetation. The replicate samples for treatments were the total sampling transects at each location on the main transects. Transects were placed in early spring (September 2009) and in mid-summer (December 2009 - January 2010). Four main transects were positioned perpendicular to the long axis of each remnant block on both FarmDB and FarmDM. Each main transect was positioned approximately 100 m apart. Main transects extended 80 m into the interior of remnant vegetation (core) to 80 m into the adjacent pasture. Sampling transects, each 15 m long, were established at four positions perpendicular to the main transects (8 main transects × 4 sampling transects = 32 sampling transects). These positions were the core of remnant vegetation (-80 m), the edge of remnant vegetation (0 m), 20 m into adjacent pasture and 80 m into adjacent pasture. Since time and labour were limited, more sampling between the edge of remnant vegetation and 80 m into pasture could not be done. Three pitfall traps (7.5 m apart) and two water traps (10 m apart) were placed along a sampling transect at each main transect position. Thus, a total of 96 pitfall traps and 64 water traps were deployed per season (48 pitfall and 32 water traps, at each farm). During the course of the study, 22 pitfall traps and 14 water traps were not collected due to disturbances: (early spring: 6 pitfall traps in the core and 7 pitfall traps at the edge; 2 water traps in the core and 5 water traps at the edge / mid-summer: 3 pitfall traps in the core, 4 pitfall traps at the edge, 2 pitfall traps at 20 m; 3 water traps in the core, 3 water traps at the edge, 1 water trap at 20 m.)

The dimensions, colouring (in the case of yellow water traps) and contents in pitfall traps and water traps were consistent with those used in O'Donnell and Wright (2021). Pitfall traps were placed in the ground using a soil corer such that the top of the container was level with the soil surface. Water traps were installed at ground level and held in place with thin wire to prevent dislodgement from wind. Pitfall and water traps were deployed for five days within each study site on the same day (early spring: September 12 - 17, 2009 / mid-summer: December 29, 2009 - January 3, 2010), during a forecast period of no rain (Neville *et al.*, 2001, Wainer *et al.*, 2001). Traps were collected on the morning of September 17, 2009 in early spring and January 3, 2010 in mid-summer.

2.3 Beneficial arthropod identification & groupings

Data analysis used the same approach as in O'Donnell and Wright (2021): the hierarchical taxonomic approach. This approach was used to examine the effects of native vegetation on their respective subjects in a pasture landscape.

Analyses at the family and genus level were focused on taxa for which some or all species comprising those taxa are known to perform services in the agroecosystem (e.g., pest suppression or pollination). Only some families of each of the four orders were explored at the family level. The abundances for each of the families were derived from the number of all individuals caught and identified as a member of that family during the study; and includes individuals belonging to genera that were not investigated individually. The Family: Formicidae (ants) was explored at the genus level. Abundances for each ant genus represent the number of individuals caught and identified as a member of that genus.

Contents of pitfall and water traps were pooled at each main transect position and taken to a laboratory for identification of beneficial arthropods (Neville *et al.*, 2001, Wainer *et al.*, 2001).

Beneficial arthropod taxa of interest were identical to the list in Material and Methods in O'Donnell and Wright (2021), except the ant genus Myrmecia was included in this study. Ants were explored at the genus level due to ease of identification and ecological importance (Majer, 1983). They can, however, cause ecosystem disservices by protecting pests from predation (Mgocheki and Addison, 2009) or by directly preying on beneficials (Pereira *et al.*, 2004). Resources used to identify arthropods included Harvey and Yen (1989), New (1996) and CSIRO (2012) for beetle families and ant genera; Hamilton *et al.* (2006) for fly families; Raven *et al.* (2002) for spider families and Stevens *et al.* (2007) for wasp families.

2.4 Statistical analyses

The statistical methods were the same as those used in O'Donnell and Wright (2021).

2.4.1 Multivariate analyses of arthropod community assemblages and emigration into adjacent pasture

All arthropod counts were square root transformed prior to analyses of similarities (ANOSIM) using Primer 6 software package (Clarke *et al.*, 2006) to avoid the statistical analyses being dominated by a few of the most abundant taxa while also avoiding giving sporadic, singleton taxa too much weight (Clarke *et al.*, 2014). Bray-Curtis similarity coefficient (Bray and Curtis, 1957) was used to calculate similarities between sample pairs, within and among the sample group. Two-way ANOSIM was performed to examine effects of distance from the edge of remnant vegetation (an ordered factor) and farm identity (an unordered factor) on abundances of beneficial arthropods. The Global ANOSIM test for overall differences between groups was carried out first. The total number of replicates, and therefore possible permutations, were relatively large, and so the tests were considered reliable and informative (Clarke and Gorley, 2006). If the Global ANOSIM test was not significant, then no further interpretations were made. If the Global ANOSIM test was significant, it is then appropriate to ask where the main between-group differences occur (Clarke and Gorley, 2006).

The ANOSIM *R* Statistic for the ordered factor has an O superscript: R° Statistic, to differentiate it from the plain ANOSIM *R* Statistic for the unordered factor: *R* Statistic. The R Statistic in ANOSIM is a scaled measure of differences between groups bounded between -1 and 1. The closer an R value is to 1, the more the sites within a group are similar to each other, which suggests unique arthropod fauna within these sites (Clarke *et al.*, 2014). An R value closer to 0 indicates that similarity within sites is equivalent to that between sites, which implies arthropod fauna in these sites are not unique. Due to their considerable vagility, an R value ≥0.30 was used to show significant differences in arthropod assemblages between sites. Non-metric multi-dimensional scaling ordination plots (nMDS) (Kruskal, 1964) were developed for viewing patterns among sample groups and community assemblages.

Abundances of the four arthropod orders and of all the collected ant genera were used in the assemblage analysis, but from these groups, only abundance of the five most abundant ant genera (*Aphaenogaster*, *Camponotus*, *Iridomyrmex*, *Myrmecia* and *Rhytidoponera*) were investigated further by comparing their mean abundances along main transects. Mean abundance of each arthropod taxon was calculated for

each main transect position by dividing the sum of individuals of each taxon by the number of traps (three for pitfall traps and two for water traps). This was done separately for each type of trap. The mean abundances of each arthropod taxon and trap type could then be averaged across the number of main transect positions (8) to obtain mean abundances for each position along the transect.

An index of abundance (IA) was calculated when both types of trap were required to capture an arthropod taxon. The family Bethylidae has females that are ground-dwelling, which could fall into pitfall traps, and males that fly and feed on sugary fluids, which could be attracted to water traps. Index of abundance was necessary for Bethylidae. Here, the separate mean abundances for each taxon from each trap type were added together for each main transect position. These mean abundances were then summed for the number of main transect positions of interest and divided by 8, the total number of those transect positions. This index is limited in applicability, since it does not take account of the relative numbers of the two kinds of traps, nor of their differing properties to attract individuals of a taxon.

3. RESULTS

The total number of arthropods sampled from remnant vegetation and adjacent pasture were 16,601 and 13,333, respectively. On FarmDB, 18,444 specimens were collected with 9,916 from remnant vegetation and 8,528 from adjacent pasture. On FarmDM, 11,490 specimens were collected with 6,685 from remnant vegetation and 4,805 from adjacent pasture. The number of arthropods collected in remnant vegetation was plainly larger than those collected in pasture.

3.1 Arthropod community assemblage

There were marked differences (Pairwise $R^o \geq 0.31$) in arthropod community assemblages in both seasons between transect positions located within remnant vegetation to those found in adjacent pasture at all taxonomic levels (family and ant genera) (Table 1). The probability values indicated that these differences were significant ($P \leq 0.05$). These outcomes indicate that sites in remnant vegetation had unique arthropod assemblages compared to pasture sites. These differences were supported by the nMDS plots representing approximations (stress levels below 0.2), which also showed substantial differences in arthropod assemblages sampled within remnant vegetation (core and edge positions) compared to those sampled within pasture (20 m and 80 m positions). In mid-summer, however, arthropod community compositions were not distinct at Edge vs 20 m for all three taxonomic levels, indicated by low Pairwise R^o values ($R^o \leq 0.09$) and no probability values ($P > 0.05$) showing statistical significance (Table 1). At Ant Genus level: Edge vs 80 m, the Pairwise R^o value was low, $R^o = 0.16$, indicating that ant assemblages at these sites were not distinct from each other in early spring; however, the probability value ($P > 0.05$) did not show significance, suggesting that the similarities in ant assemblages at these sites were not large.

In early spring, arthropod assemblages were not unique at Core vs Edge for all taxonomic levels (Pairwise $R^o \leq 0.16$, $P > 0.05$). However, in mid-summer, arthropod assemblages were substantially different at Core vs Edge for all taxonomic levels, indicated by high R^o values (Pairwise $R^o \geq 0.39$) and significant probability values ($P \leq 0.05$) (Table 1). There was no clear pattern observed across taxonomic levels for arthropod community composition at 20 m vs 80 m for the two seasons. These results appear to support other research that showed taxonomic level in an arthropod study can have an impact on trends in the data sets (Neville *et al.*, 2001, Wainer *et al.*, 2001).

Community composition for all taxonomic levels of arthropods demonstrated significant differences (Global R^o Statistics≥ 0.31) across main transect positions, with the probability values indicating significance ($P \leq 0.05$), in early spring and mid-summer (Table 1). There was a farm effect on arthropod assemblages at all taxonomic levels (family and ant genera) for both seasons (R Statistics≥ 0.34, $P \leq 0.05$) (Table 1). The one exception was at Ant Genus level in early spring (R Statistic$= 0.25$, $P \leq 0.05$). The probability value indicates statistical significance, but the low R statistic suggests there was little difference between ant assemblages across the two farms in early spring.

Table 1: Two-way ANOSIM for invertebrate assemblages of transect positions in remnant vegetation and adjacent pasture on two farms in southeastern Gippsland, Victoria, Australia in early spring (September 2009) and mid-summer (December 2009 – September 2010). Global Rº Statistic (ordered) and Pairwise Rº Statistic (ordered) (transect position) and Global R Statistic (unordered) (Farm Effect).

Taxa Level	Global R[oa]/R Statistic[b]		Pairwise Rº Statistic[c]		P-value	
	Early spring	Mid-summer	Early spring	Mid-summer	Early spring	Mid-summer
	R^O =0.45	R^O =0.31			0.001	0.001
FAMILY						
Transect positions	R^O =0.41	R^O =0.43			0.001	0.001
Core vs Edge			0.08	0.44	0.23	0.02
Core vs 20m			0.70	0.53	0.001	0.08
Edge vs 20m			0.49	0.09	0.01	0.20
Edge vs 80m			0.57	0.63	0.002	0.002
20m vs 80m			0.33	0.09	0.01	0.19
Farm effect	R=0.42	R=0.73			0.002	0.001
ANT GENUS						
Transect positions	R^O =0.32	R^O =0.55			0.001	0.001
Core vs Edge			0.16	0.98	0.07	0.001
Core vs 20m			0.48	0.90	0.007	0.002
Edge vs 20m			0.38	0.05	0.006	0.30
Edge vs 80m			0.16	0.70	0.09	0.002
20m vs 80m			0.06	0.34	0.26	0.02
Farm effect	R=0.25	R=0.56			0.002	0.001

[a] Global Rº signify ordered ANOSIM for perpendicular transect types

[b] Global R signifies unordered ANOSIM for farm effect

[c] Pairwise Rº signify ordered ANOSIM for perpendicular transect types

3.2 Arthropod abundances

3.2.1 Family: Lycosidae (wolf spiders)

In early spring, Lycosidae displayed a gradual decrease in abundance from the core of remnant vegetation out into adjacent pasture with significantly higher abundances in the core of remnant vegetation compared to 20 m and 80 m in pasture (Fig. 2). In mid-summer, no clear trends and no significant differences in Lycosidae abundances were observed across main transect positions (Fig. 2).

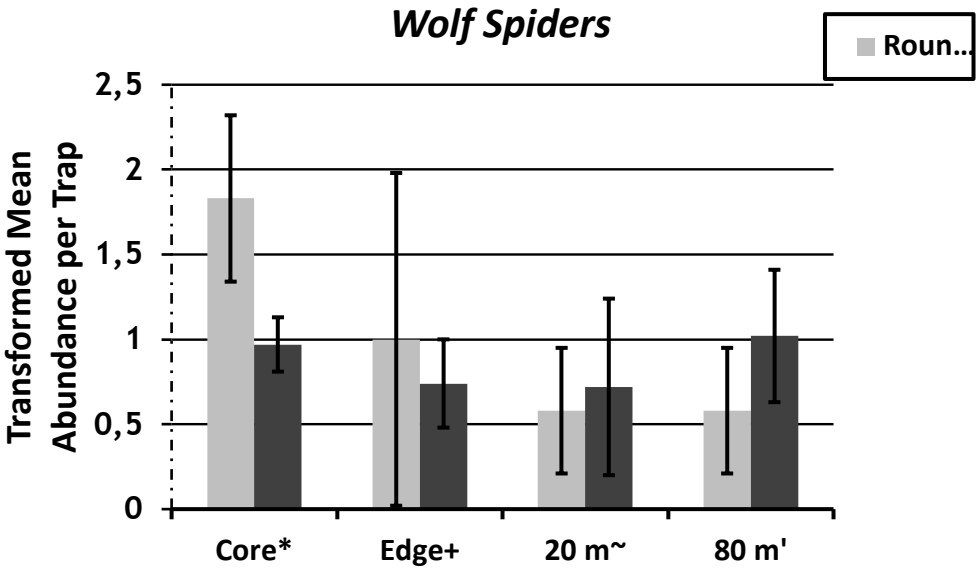


Fig. 2. Transformed mean abundance of Lycosidae (wolf spiders) sampled in early spring (September 2009) and mid-summer (December 2009 - January 2010) in the Darriman-Giffard area of southeastern Gippsland, Victoria, Australia. Samples were collected from pitfall traps at positions along 8 transects from the core of remnant vegetation to 80 m into the adjacent pasture. Error bars represent two standard errors.

3.2.2 Families: Carabidae (ground beetles) and Staphylinidae (rove beetles)

In early spring, Carabidae abundance was significantly higher at 20 m and 80 m in pasture compared to the core of remnant vegetation; in mid-summer, ground beetles displayed no significant differences between main transect positions (Fig. 3). Mean abundances of Staphylinidae were low (≤ 1.06 per pitfall trap) across all transects, especially in mid-summer where only one individual was collected at 80 m in pasture. In early spring, rove beetle abundance was significantly higher in the core compared to 20 m and 80 m in pasture.

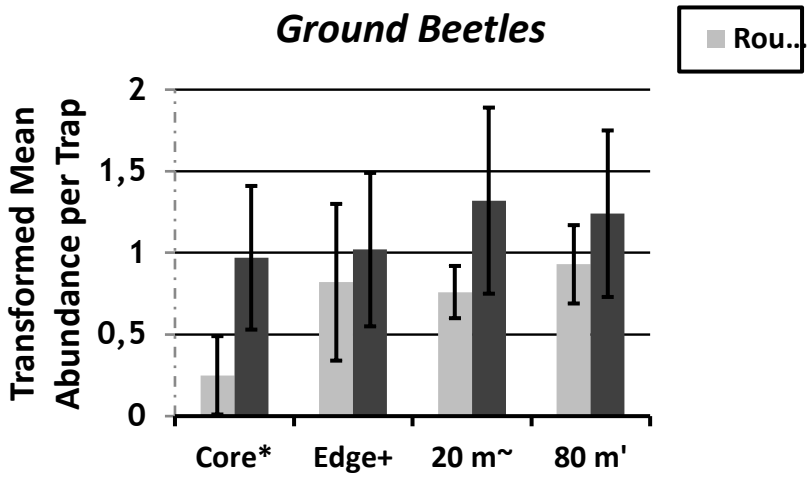


Fig. 3. Transformed mean abundance of Carabidae (ground beetles) sampled in early spring (Spring 2009) and mid-summer (December 2009 - January 2010) in the Darriman-Giffard area of southeastern Gippsland, Victoria, Australia. Samples were collected from pitfall traps at positions along 8 transects from the core of remnant vegetation to 80 m into the adjacent pasture. Error bars represent two standard errors.

3.2.3 Families: Asilidae (robber flies), Dolichopodidae (long-legged flies), Syrphidae (hoverflies), and Tachinidae (tachinids)

The dipteran families that were sampled had low mean abundances (≤ 1.75 per water trap) in early spring and mid-summer. Asilidae abundances were highest in remnant vegetation but displayed no significant differences between main transect positions in either season. Dolichopodid flies were most abundant at the edge of remnant vegetation (mean of 1.48 per trap) in mid-summer, and individuals were not found at 80 m in pasture in either season (Fig. 4). In early spring, abundances of Syrphidae were highest in remnant vegetation with significant differences between the edge and 20 m and 80 m into pasture, while in mid-summer, hoverflies showed no significant differences between main transect positions. Tachinidae abundance was highest along the edge of remnant vegetation in early spring (edge: mean of 0.35 per trap) and mid-summer (edge: mean of 1.09 per trap) but with no significant differences in abundance across main transect positions.

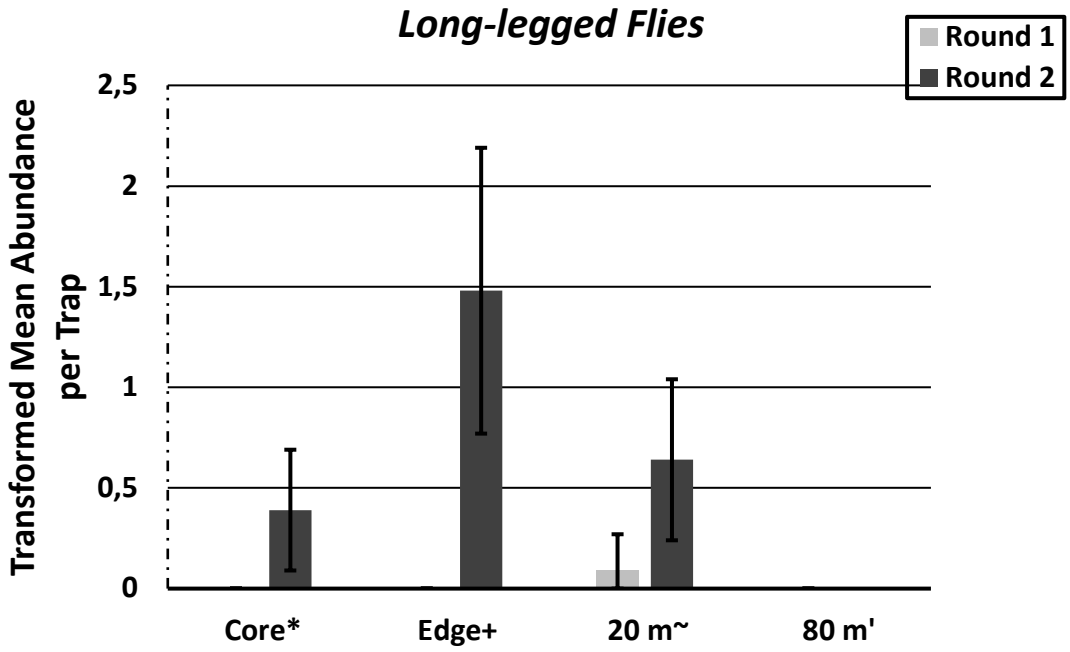


Fig. 4. Transformed mean abundance of flies from Dolichopodidae (long-legged flies) sampled in early spring (September 2009) and mid-summer (December 2009 - January 2010) in the Darriman-Giffard area of southeastern Gippsland, Victoria, Australia. Samples were collected from water traps at positions along 8 transects from the core of remnant vegetation to 80 m into the adjacent pasture. Error bars represent two standard errors.

3.2.4 Families: Apidae (bees) and Bethylidae (bethylids)

Bees had low abundance in early spring (mean of 0.59 per trap) and were most abundant at the edge in mid-summer (mean of 3.63 per trap), but showed no significant differences across main transect positions in either season. Bethylidae were found only in the core in early spring, had the highest abundance at the edge in mid-summer but with no significant differences across main transect positions, and were not collected from 80 m in pasture during either season.

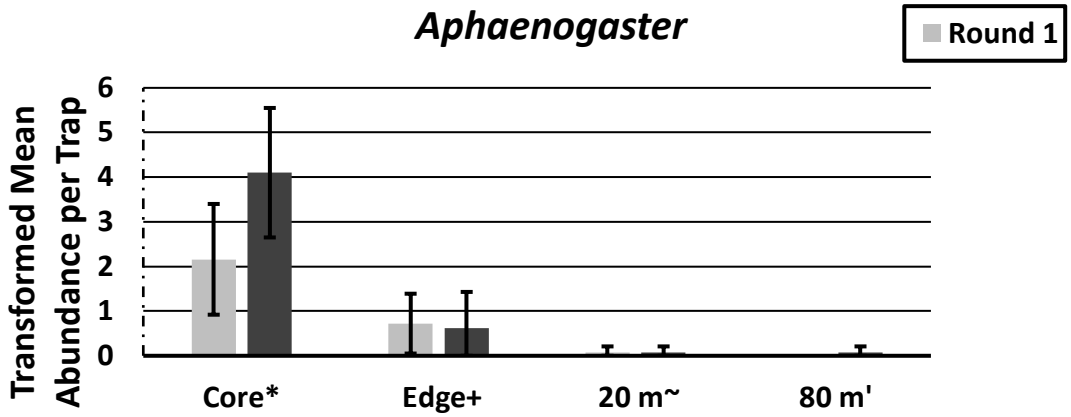
3.2.5 Family: Formicidae (ants) and Ant genera: Aphaenogaster, Camponotus, Iridomyrmex, Myrmecia and Rhytidoponera

In early spring, ants had significantly higher abundances at the edge compared to 20 m and 80 m in pasture and the core and 20 m in pasture. In mid-summer, ants showed a decrease of abundance moving

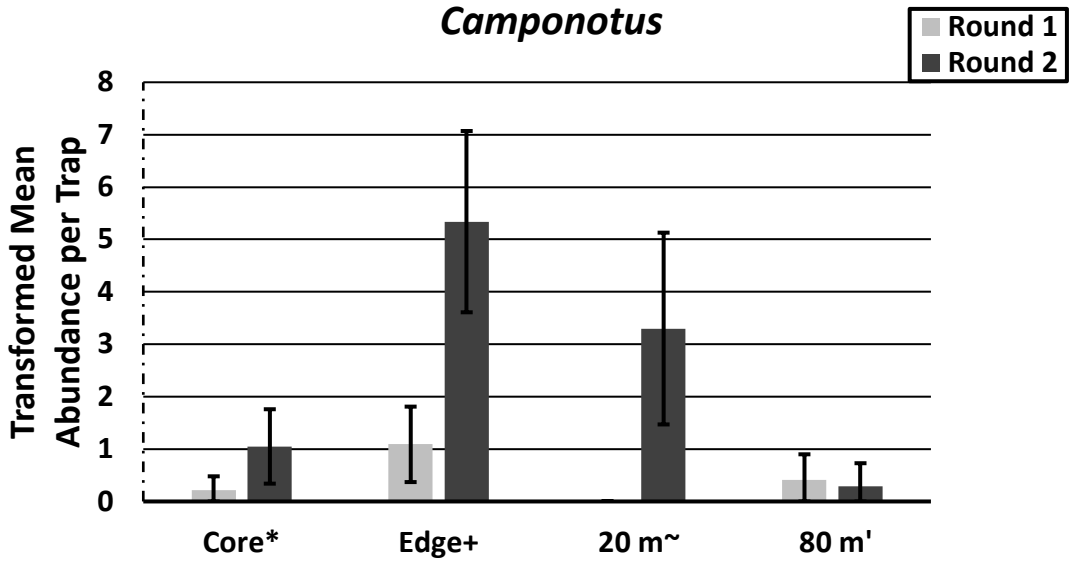
out from the edge of remnant vegetation (mean of 128.42 per trap) but with no significant differences across main transect positions.

The core of remnant vegetation had higher abundances of *Aphaenogaster*, with significant differences between the core (mean of 2.16 per trap) and 20 m (mean of 0.07 per trap) in early spring, and between the core (mean of 4.10 per trap) and the other three transect points (edge: mean of 0.62 per trap; 20 m: mean of 0.07 per trap; and 80 m: mean of 0.07 per trap) in mid-summer (Fig. 5A). Very few individuals of *Aphaenogaster* were found in pasture. In both seasons, *Camponotus* showed a trend of decreasing abundance in either direction out from the edge with significant differences in mid-summer between the core (mean of 1.05 per trap) and edge (mean of 5.34 per trap), the edge and 80 m (mean of 0.29 per trap), and 20m (mean of 3.30 per trap) and 80 m (Fig. 5B). *Camponotus* was most abundant along the edge of remnant vegetation in both seasons (Fig. 5B). *Iridomyrmex* was most abundant at the edge of remnant vegetation in both seasons, with significant differences between the edge (mean of 5.23 per trap) and 80 m (mean of 2.03) in mid-summer. *Myrmecia* was most abundant in remnant vegetation in early spring and mid-summer with significant differences between the core (mean of 0.94 per trap) and 20 m (mean of 0.07 per trap) in early spring (Fig. 5C). No individuals of *Myrmecia* were found 80 m in pasture during the study. There were no clear trend or significant differences in mean abundance among main transect positions for *Rhytidoponera* in either season.

A)



B)



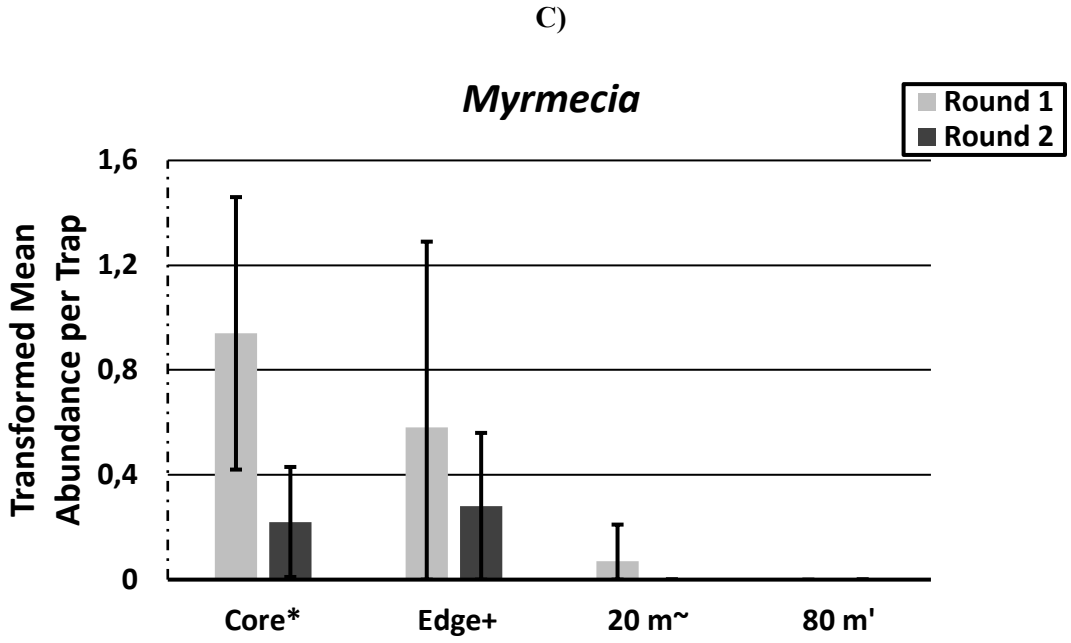


Fig. 5. Transformed mean abundance of ants from A) Genus: *Aphaenogaster*, B) Genus: *Camponotus* and C) Genus: *Myrmecia* sampled in early spring (September 2009) and mid-summer (December 2009 - January 2010) in the Darriman-Giffard area of southeastern Gippsland, Victoria, Australia. Samples were collected from pitfall traps at positions along 8 transects from the core of remnant vegetation to 80 m into the adjacent pasture. Error bars represent two standard errors.

4. DISCUSSION

The findings of this study support a number of other projects that demonstrate non-crop vegetation being used as a faunal refuge and enhancing beneficial arthropod populations (Bianchi *et al.*, 2006, Fukuda *et al.*, 2011, Mkenda *et al.*, 2019a, O'Donnell and Wright, 2021). In both seasons, the arthropod assemblage data clearly demonstrated that distinct differences existed in arthropod communities found in native remnant vegetation compared to adjacent pasture. Wainer *et al.* (2001), using a comparable trapping regime in South Gippsland, Australia, also recorded distinct arthropod communities in remnant vegetation compared to pasture.

In mid-summer, however, significant differences were not observed between arthropod communities at the edge of remnant vegetation compared to 20 m into adjacent pasture, at any taxonomic level. These results show that a change in arthropod communities occurred at the edge-pasture boundary over two seasons. O'Donnell and Wright (2021) observed a change in arthropod communities at the edge of revegetated strips compared to those in adjacent pasture over time. Arthropods may be migrating from distant locales and colonizing the area in mid-summer. However, fragmentation of remnant vegetation was found to negatively impact the dispersal of some orders, such as Hemiptera and Diptera, and all orders researched had species that were restricted to isolated blocks of remnant vegetation (Ingham and Samways, 1996). Remnant blocks in this study were separated by large areas of highly disturbed, intensely grazed pasture, which likely posed a barrier to movement for many invertebrate species (Bromham *et al.*, 1999). And yet, Major *et al.* (2006) concluded pasture may not be hostile to generalist ground dwelling predators such as wolf spiders. This dynamic at the edge of habitats appears to be a cosmopolitan phenomenon being recorded elsewhere in Australia (Tsitsilas *et al.*, 2011), Africa (Mkenda *et al.*, 2019a) and in other locales (Collins *et al.*, 2002). More research at the landscape scale would be crucial in determining the key factors involved in these changes of arthropod assemblages at the boundary of remnant blocks and pasture over seasons.

Local factors may in fact be influencing the changes in arthropod assemblages across the edge-pasture boundary over the seasons. Beneficial arthropods could be overwintering in remnant vegetation and

emigrating into adjacent pasture as temperature warm, or pasture species migrate towards remnant vegetation in search of SNAP in mid-summer (Tsitsilas *et al.*, 2011, Dong *et al.*, 2015). In either situation, remnant vegetation would be acting as a refuge for beneficial arthropods in a landscape dominated by pasture. A refined sampling regime that includes sampling sites at 1 m, 5 m, and 10 m between the edge and 20 m would help in understanding this population dynamic (Mkenda *et al.*, 2019b). Another method to further investigate changes in invertebrate assemblages over time and space is the tracking of individuals using radioisotopes, florescent dyes or radio telemetry (Madeira and Pons, 2015, Ruzickova and Vesely, 2016, Mkenda *et al.*, 2019b). These methods, although time consuming, can measure the movement patterns of individuals, thus providing information about whether local individuals are actually moving out to adjacent pasture from remnant vegetation.

A significant change occurred in arthropod assemblages at the core compared to the edge of remnant vegetation between seasons. In early spring, arthropod assemblages were similar at the core and edge, but in mid-summer these were significantly different at every taxonomic level. These results differed from the findings of a previous survey, where arthropod assemblages were not significantly different between the core and edge of revegetated strips in either season (O'Donnell and Wright, 2021). Revegetated strips were thin lines of trees where the core was 10 m from the edge, whereas remnant vegetation were large blocks of trees where the core was 80 m from the edge. Arthropods seem to have a highly variable response to differences in habitat, which has been shown to be related to habitat selectivity and foraging substrate (Denys and Tscharntke, 2002).

This study provides evidence that remnant areas in pasture landscapes are important in conserving arthropod biodiversity, supporting other research in Africa (Ingham and Samways, 1996, Mkenda *et al.*, 2019b), Europe (MacLeod *et al.*, 2004, Moron *et al.*, 2014), China (Dong *et al.*, 2012), and Australia (Holloway *et al.*, 2008, O'Donnell and Wright, 2021). Araneae, Lycosidae, Syrphidae and Staphylinidae had higher abundances in remnant vegetation in early spring and Bethylidae were found only in the core of remnant vegetation in early spring. Formicidae, Diptera and Araneae had higher taxa richness in remnant vegetation with seven genera of ants (*Crematogaster*, *Dolichoderus*, *Epopostruma*, *Papyrius*, *Philidris*, *Podomyrma*, and *Stigmacros*), one family of flies (Xylophagidae) and ten families of spiders (Amaurobiidae, Amphinectidae, Cyclocentidae, Gnaphosidae, Pisauridae, Prodidomidae, Sparassidae, Thomisidae, Trochanteriidae, and Uloboridae) being found only in remnant vegetation. The distinct environmental differences in remnant vegetation and pasture areas likely account for the substantial differences in arthropod richness and abundance in the two habitats.

Taxa richness and abundance of arthropods in remnant vegetation was in fact underrepresented as the understory and canopy layers were not surveyed in this study. The sampling regime captured ground active arthropods in the case of pitfall traps and a narrow range of flying arthropods attracted to the colour yellow wavelength for water traps. As such, arboreal arthropods, an important component of biodiversity in forested habitat, went unreported in this survey of LF/HWM remnant blocks.

Remnant blocks are crucial in providing shade and shelter to livestock and protecting soils from erosion in pastureland (Malcolm *et al.*, 2010). The foliage of the trees in these shelters need to be in good health to provide maximum protection in adverse weather conditions. Therefore, it is important to have a robust assemblage of beneficial arthropods colonizing stands of remnant vegetation in a pasture landscape (Cunningham *et al.*, 2005). A sampling regime that includes collecting techniques which sample the canopy and shrub understory, such as fogging or beating the flora, should be done to obtain a more complete assessment of the arthropod abundance and diversity residing in treed areas. Using such techniques would add important layers of foliage to future studies that could be valuable in developing habitat management processes which enhance populations of natural enemies while also informing arthropod taxonomy and biodiversity conservation in pasture landscapes.

In early spring, Lycosidae had significantly higher abundances in the core of remnant vegetation compared to adjacent pasture, but in mid-summer there was no significant differences across sites. These results differed with the results found in comparable studies in the region by Wainer *et al.* (2001) and O'Donnell and Wright (2021). Other arthropod taxa also showed different responses in abundance and richness in the remnant vegetation-adjacent pasture survey compared to the revegetated strips-adjacent pasture survey (O'Donnell and Wright, 2021) done in the same area. These results suggest that

ecological factors such as age of vegetation and type of vegetation impact the abundance and richness of arthropod communities differently. The abundance and taxa richness of beneficial arthropod populations were for the most part higher in revegetated strips (O'Donnell and Wright, 2021) and remnant vegetation compared to surrounding pasture. A mosaic of habitats (i.e., habitat heterogeneity) on farmland provides a diversity of ecological niches and microclimates for species to exploit (Fahrig, 2001, Gonzalez *et al.*, 2015). The results of this study lend evidence to the conclusions of Benton *et al.* (2003) that habitat heterogeneity across farmland is associated with increases in taxa richness and the conservation of biodiversity.

Lycosidae and Syrphidae, important natural enemies of pest species, were most abundant in remnant vegetation in early spring. In mid-summer, however, the population distribution of these taxa demonstrated no significant differences between transect points. It appears that these natural enemies were using both habitats over time. Other families also appeared to use remnant vegetation as refugia in early spring. Staphylinidae, general predators of many pest species, had significantly higher abundance in remnant vegetation compared to adjacent pasture in early spring. Dolichopodidae, predators of soft bodied arthropods such as aphids and mites, and Bethylidae, parasitoids of moth and beetle larvae, were found only in remnant vegetation and closely adjacent pasture. It is conceivable that remnant vegetation provides SNAP, such as protection from cold weather by altering wind flow or shelter from rain, in early spring to beneficial arthropods (Brandle *et al.*, 2004), and that some taxa emigrate out in mid-summer, potentially contributing to pest suppression in pasture.

Remarkably, some beneficial taxa, such as Apidae and Tachinidae, showed no significant differences between the two habitats. Carabidae was the only beneficial arthropod to have significantly higher abundances in pasture in early spring, but ground beetle abundance was not significantly different across habitat types in mid-summer. Carabidae may be feeding on springtails (Class: Collembola), soft-bodied invertebrates that consume plant materials, which were plentiful in pasture in early spring due to the cool wet weather (personal observation). MacLeod *et al.* (2004) recorded species of ground beetles that preferred open field while other species were most often found at the interface of cultivated and non-cultivated land. The matrix of the research area, being dominated by large swaths of pasture with relatively small blocks of native vegetation, is likely more suitable for open field ground beetle species. Taking Carabidae down to species would help in understanding the role habitat has in the distribution of ground beetle populations.

Ants are a diverse group that can be beneficial, as soil augmenters and predators, as well as pests, as crop consumers and protectors of other pest species from predators and parasitoids (Buckley, 1992). Ants were taken down to the genus level, and from this, it is clear that remnant vegetation enhanced the genus richness of ants in a pasture landscape. Some of these genera emigrated little from remnant vegetation and thus not likely to augment ecosystem services (ES) in pasture. *Aphaenogaster* and *Myrmecia* are two examples of ant genera that were rarely found in adjacent pasture. To fully appreciate their role in an agroecosystem, ants would need to be taken down to species.

Ants, being extremely abundant and responsive to changing environmental conditions, have been proven to be reliable bioindicators in monitoring ecosystem recovery following land rehabilitation (Majer, 1983). One of the key aims of habitat restoration is to help return areas to a natural state (Majer *et al.*, 2007). Ant community composition in remnant vegetation in this research showed high dissimilarity with ant community composition in revegetated strips in the previous project of O'Donnell and Wright (2021) (data not shown). In remnant vegetation, *Aphaenogaster* and *Myrmecia* were in high abundance. Both genera are specialists and commonly require low disturbance habitat (Gray, 1974, Shattuck, 2008). In revegetated strips, *Myrmecia* were absent and only one individual of *Aphaenogaster* was found, while *Rhytidoponera*, *Camponotus* and *Iridomyrmex* were found in high abundance. These three ant genera are generalists and are often found in degraded or disturbed habitat (Shattuck, 1992). It is suggested that these five genera of ants be studied further in the research area as a quick and efficient means to measure the degree to which restored native vegetation is reaching benchmarks of the natural state.

Research has shown that abundance and diversity of natural enemies can be boosted by field margins, and that beneficials emigrate out providing ES in adjacent fields (Tsitsilas *et al.*, 2006, Mkenda *et al.*, 2019b). However, studies in Australia and New Zealand have shown that beneficial invertebrates, such

as Carabidae, Staphylinidae and Araneae, in non-crop habitat may not emigrate out to adjacent cropland (Mclachlan and Wratten, 2003, Gontijo, 2018, O'Donnell and Wright, 2021). It is thought that the non-crop habitat was supplying sufficient resources year round to reduce the need for beneficials to move out into arable land. Sustainable intensification has real potential to sustainably produce food for a growing population threaten by climate change, and yet there are still challenges to overcome (Pretty and Bharucha, 2014). Non-crop habitat will need to be engineered in ways that enhance populations of beneficials while manipulated in ways that encourage colonization of cultivated lands at critical times during the growing season. Manipulations can be done mechanically, for example by mowing the edges of fields to force beneficials into adjacent crops (Dong *et al.*, 2012); or botanically, by choosing vegetation which offers essential resources (e.g., nectar and pollen) at crucial times in the life cycle of beneficials while not causing a sink effect in which beneficials are attracted to non-crop habitat from arable land and content to stay there during the growing season (Mclachlan and Wratten, 2003). The botanical approach will need to be multidisciplinary since having an intimate understanding of both plants and insects will be crucial for synchronizing the lifecycles of beneficials with plant phenology to promote colonization of cropland (Dong *et al.*, 2012). Further research on new developments in habitat engineering and manipulation, and monitoring the impact of these developments on beneficial arthropod populations and behavior is recommended to improve agriculture sustainably.

The ANOSIM results showed that the study site (farm effect) had a pronounced effect on invertebrate communities even though the farms had similar management styles and were not distant from each other (~22 km). These results lend evidence to one of the inherent difficulties of examining arthropod populations, which is their stochastic nature over relatively short distances.

This study could not clearly determine where beneficial arthropods in pasture adjacent to remnant vegetation originated or whether the presence of beneficials were significantly lowering pest populations in pasture. The results of this study do however support observations that the diversity and abundance of beneficial arthropod populations is enhanced by non-crop habitat (Gardiner *et al.*, 2009, Shackelford *et al.*, 2013).

Future research could investigate whether the presence of beneficial arthropods in an 80 m zone of pasture surrounding remnant vegetation correlates with lower populations of pest invertebrates and reduced damage in pasture. Depending on the results of these future studies, 'no-spray' or buffer zones, where spraying of insecticides is discontinued, could be established ranging from the edge of remnant vegetation to 80 m out into adjacent pasture. This method of sustainable intensification would both conserve communities of beneficial arthropods and lower costs for farmers. No-spray zones would also have the benefit of reducing toxic chemicals in the environment and lower the carbon footprint of agriculture.

Some of the major pests in pasture in the research area were two species of cockchafer beetles (redheaded cockchafer: *Adoryphorus couloni* (Bermeister); blackheaded cockchafer: *Aphodius tasmaniae* (Hope)) and the redlegged earth mite (*Halotydeus destructor* (Tucker)) (Bailey, 2007). The larvae of the cockchafer species are subterranean and the adults have hard elytra and robust bodies. The 3rd instar larva of the blackheaded cockchafer, however, emerges from its burrow to feed on pasture at night, which makes it susceptible to predation by epigeal hunters such as wolf spiders. The redlegged earth mite (RLEM) normally feeds on leaves of plants (Tsitsilas *et al.*, 2011). The long-legged flies (Dolichopodidae) are predators of soft-bodied invertebrates and can be found residing on the surface and underside of leaves. Long-legged flies were found along the edges of remnant vegetation and revegetated strips, and in nearby adjacent pasture. It is therefore suggested that future research analyse the assemblages of Dolichopodidae to determine whether some species could be augmented as biological control agents for RLEM. Determining habitat requirements, such as oviposition sites, larval feeding requirements, and adult flower preference, would be crucial in engineering refugia that substantially increases the abundance of these predatory flies.

This project was done in the same research area, during the same seasons and followed the same methodology as O'Donnell and Wright (2021). As such, the limitations and obstacles of the previous study were mirrored in this project. Arthropod diversity and abundance estimates were very likely underestimated for remnant vegetation since research has shown that heavy foliage cover can reduce

catches in pitfall traps by retarding movement of ground dwelling invertebrates and in water traps by obscuring them from view (Wainer *et al.*, 2001, Scudder, 2008). With its long, cool springs and short, hot-dry summers, many arthropods have only a narrow period of activity to be captured in the research area of southeast Australia. These climatic conditions could be partly responsible for the patchy distribution observed for many of the arthropod populations in this study. This study was done over two season and only ants were taken down to the genus level. It is suggested that future studies be done over longer time frames to provide the consistent trends necessary to clearly evaluate the impact of remnant vegetation on arthropod assemblages. It would also be a significant help if some of these groups of arthropods were taken down to lower taxonomic levels. Teasing apart the lower taxa would be highly valuable in determining factors that make remnant vegetation attractive to beneficial arthropods. With this knowledge, remnant vegetation in pasture landscapes can be properly managed to foster robust populations of beneficial arthropods, which in turn can provide ES, such as pest suppression and pollination.

4.1 Conclusions

Arthropod diversity was found to be higher in remnant vegetation, which indicates its importance in conserving biodiversity in pasture landscapes. Abundances of individual arthropod groups displayed an array of different trends. Over two thirds of beneficial arthropod families had higher abundances in remnant vegetation. Pasture sampling sites farthest from remnant vegetation had in general the lowest abundances and diversity of beneficial arthropods. Nearly all ant genera had higher abundances in remnant vegetation, with many being found solely there. Remnant vegetation, having high vegetative heterogeneity, had many niches and food sources for ants to exploit. Based on these results, it appears remnant vegetation was acting as refuge for many beneficial taxa in this project. Although it was not clearly determined whether natural enemies were colonizing adjacent pasture from remnant vegetation, it appears remnant vegetation has an important role to play in maintaining and enhancing populations of beneficial arthropods in pasturelands.

The ANOSIM results showed that community changes occurred between the edge-pasture boundary over the seasons at the three taxonomic levels. It was unclear whether the changes in communities along the edge-pasture interface over time were being influenced by local populations or if recruitment was occurring from populations farther afield. The assemblage data also showed changes in arthropod communities between the core-edge of remnant vegetation over the seasons, which differed from a previous study where the core-edge of revegetated strips showed no difference in community structure over time (O'Donnell and Wright, 2021). These results indicate that non-crop habitat type impacts beneficial arthropod communities differently.

Non-crop habitat in agroecosystems provide refuge for beneficial invertebrates, but may also act as a sink where beneficial arthropods go but do not leave. To avoid the sink effect, detailed knowledge of arthropod assemblages, such as their life histories and essential resources, is required. This can be done by teasing apart the lower taxa of beneficial arthropod families. When analyzed from a species perspective, it will be possible to design refugia that fosters robust populations of ES-providing arthropods while also encouraging beneficial arthropods to colonize adjacent cultivated fields.

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