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Effects of *Bt* soybean on biodiversity are limited to target species and host-specific parasitoids in La Pampa province, Argentina

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Abstract

Bt crops have been incorporated in South America more than 20 years ago and their use has increased sharply since then. However, the potential negative environmental effects are still a concern, specifically the impacts on non-target arthropod species**.** The objective of this work was to determine if the effects of *Bt* soybean are limited to the removal of the larvae of target Lepidoptera species or if they indirectly affect the arthropod community. *Bt* soybean expressing *Cry1Ac* toxin and non-*Bt* soybean were planted under the same environmental and agricultural conditions on two planting dates (early and late planting dates). Neither cultivar received insecticide applications. The abundance of phytophagous insects and predatory arthropods was recorded in each treatment (cultivar × planting date). Moreover, lepidopteran larvae, stink bugs and eggs of both groups were collected and maintained under controlled rearing conditions to record parasitoids. Diversity of the arthropod assemblage, and of the phytophagous and entomophagous subassemblages of each treatment, and similarity between them were estimated with and without the presence of target species and their parasitoids. Range-abundance curves were plotted following the same criteria. Diversity was higher in the non-*Bt* cultivar than in the *Bt* one. No clear effect of planting date was detected. High similarity was found among treatments. Our results show that the removal of the target species larvae and their specific parasitoids is sufficient to explain the effect of the *Bt* soybean on richness and diversity of the arthropod assemblage as well as of the phytophagous and entomophagous subassemblages, with no evidence of additional indirect effects on biodiversity.

Keywords

Bt crops; arthropods; indirect effects.

Resumen

Los cultivos *Bt* se incorporaron ampliamente en los sistemas productivos de América del Sur siguiendo una pronunciada curva de adopción iniciada hace más de 20 años. Sin embargo, los posibles efectos ambientales negativos siguen siendo una preocupación, específicamente, los impactos sobre las especies de artrópodos no blanco**.** El objetivo de este trabajo fue determinar si los efectos de la soja *Bt* se limitan a la remoción de larvas de las especies lepidópteras blanco de la toxina o presentan efectos indirectos sobre la comunidad de artrópodos. Se sembraron Sojas *Bt* (que expresan la toxina Cry1Ac) y no *Bt* bajo las mismas condiciones ambientales y agrícolas en dos fechas de siembra (fecha de siembra temprana y fecha de siembra tardía). El ensayo se mantuvo sin aplicaciones de insecticidas. Se registró la abundancia de insectos fitófagos y de artrópodos predadores en cada tratamiento (variedad × fecha de siembra). Además, se colectaron larvas de lepidópteros, chinches fitófagas y huevos de ambos grupos. Los individuos y huevos recolectados fueron mantenidos bajo condiciones controladas con el fin de obtener y registrar parasitoides. Se estimó la diversidad y similaridad y se trazaron las curvas rango-abundancia para el ensamble de artrópodos y los subensambles de insectos fitófagos y artrópodos entomófagos con y sin presencia de especies blanco y sus parasitoides. La diversidad resultó alta en la variedad no *Bt*. No se detectaron efectos claros de la fecha de siembra. Una elevada similaridad fue encontrada entre los tratamientos para los ensambles y subensambles. Nuestros resultados muestran que los efectos de la variedad *Bt* sobre la comunidad de artrópodos de soja se debe a la eliminación de las larvas de especies blanco de la toxina y sus parasitoides específicos, sin evidencias de efectos indirectos adicionales sobre la biodiversidad.

Palabras claves

CULTIVOS Bt; ARTRÓPODOS; EFECTOS INDIRECTOS.

Introduction

Biodiversity is a key issue for sustainable development (Ammann, 2009), helps to maintain the structure of the communities and food chains (Carmona Galindo and Carmona, 2013) and provides a series of ecosystem services considered of environmental, economic and social importance (Mace et al., 2012). Therefore, biodiversity conservation is considered a priority for agriculture (Bàrberi, 2013). It has been argued that agricultural intensification can be the main driver of its deterioration (Andrade, 2017; Arpaia, 2021).

Arthropods are the animal group with the greatest presence and diversity in agroecosystems (Hernández-Aranda et al., 2022), contributing to important services such as pollination, pest control, decomposition and nutrient recycling (Haan et al., 2021). Less than 0.5 % of insect species are considered pests (Jankielsohn, 2018). *Bt* crops are genetically modified (GM) crops expressing genes derived from the soil bacterium *Bacillus thuringiensis* (Catarino et al., 2015; Manjunath, 2020). GM crops produce proteins toxic to specific insect pests (Abbas, 2018). *Bt* crops are considered important tools that contribute to the diversification of integrated pest management tactics (Anderson et al., 2019) and provide effective control of lepidopteran pests in soybean crops (Blanco et al., 2016). While part of the literature claims that *Bt* crops can benefit arthropod diversity through a lower use of insecticides (Hoy et al., 1998; Carpenter, 2011; Abbas, 2018; Anderson et al., 2019; Romeis et al., 2019), some concerns have been expressed regarding potential negative impacts on non-target species, which threaten biodiversity in agroecosystems (Garcia and Altieri, 2005; Arpaia, 2010; Naranjo et al., 2019). Possible negative effects could occur by direct and indirect mechanisms caused by *Bt* endotoxins on non-target species: 1) insects could come into direct contact with *Bt* proteins when they feed on plant tissues, or through their prey or hosts, which previously acquired the toxin by feeding on Bt plants (Romeis et al., 2009); 2) population abundances of non-target species could increase through non-competitive effects caused by the absence of the target species (Naranjo et al., 2008; Zeilinger et al., 2016) and 3) The presence or abundance of natural enemies may decrease as an indirect effect of reduced emission of attracting volatiles by plant tissues usually triggered by herbivore damage (Liu et al., 2015; Nascimento et al. 2021).

South America is one of the most important soybean producers globally, with Brazil and Argentina comprising 50% of the world production (Meira et al., 2019). *Bt* soybean cultivars expressing *Cry1Ac* toxin represent 25% and 80% of the whole area planted to soybeans in Argentina and Brazil, respectively (Horikoshi et al., 2021; REM, 2021). Soybean planting during the 2021/2022 season covered about 16.1 million hectares in Argentina and 74.5 million hectares in Brazil (Conab, 2022; MAGyP, 2022).

We pose the question whether the influence of *Bt* soybean on the arthropod community is limited to the removal of the lepidopteran species targeted by the toxin or the community composition is also influenced by indirect effects. Due to the high adoption level and regional importance of *Bt*-soybean crops in most of the soybean production area in South America, we proposed it as a model crop to explore this question. Under the working hypothesis of indirect effects reaching the entire community, we predicted that differences in the arthropod assemblages between *Bt* soybean and conventional soybeans would not lie only in the absence of target species.

Material and methods

Experimental design and data collection

During the 2018/2019, 2019/2020 and 2020/2021 growing seasons, two field experiments differing in planting date (early and late) were established in a split-block design at the Agricultural Experimental Station of INTA Guillermo Covas, Anguil, La Pampa province, Argentina (36°32’15”S; 63° 59’32”O). The environment in which the field experiments were located corresponded to the typical crops of the region (maize, sunflowers and alfalfa). In each of the experiments, *Bt* and non-*Bt* soybean cultivars belonging to the same maturity group were planted. Neither cultivar received insecticide applications. The treatments were determined by the planting date × soybean cultivar combination. Each treatment consisted of 4 replicates of 1750 m^2 each. The contrasting planting dates were used to estimate the abundance of phytophagous and entomophagous arthropods. To estimate arthropod abundance, each of the replicates were weekly monitored using a systematic design. Ten sampling units were taken following systematic patterns (“W”, “M”, “X”) on successive sampling dates to reduce the probability of overlapping between samples (Pedigo and Buntin, 1994). The vertical cloth technique (Gamundi, 1995) was used to sample arthropods present in the canopy. Each sample consisted of n=10 sampling units. A sweep net (36 cm hoop diameter, 60 cm deep sleeve and 70 cm handle) was used to collect jumping (Ves Losada and Baudino, 1998; Cuirolo, 2005) and flying (Chanthy et al*.,* 2015) insects. A sampling scheme of fixed sample size (n=10) was implemented. Each sample unit was represented by 5 180° angle-sweeps of the net (Vyavhare et al., 2015).

To determine the abundance of parasitoids, all the lepidopteran larvae, stink bugs, and the eggs of lepidoptera and pentatomid species were collected. Eggs were manually collected from the plants present in each 1-meter segment of the plant row (sampling unit), which was delimited using the vertical cloth method. All the collected specimens were transferred to the laboratory, where they were sorted out to species and labeled, including information of the treatment and date of collection. Each insect and each egg or egg mass was individually isolated in plastic containers of 7.5 × 10.5 Diam × Ht, covered with adherent film paper and kept under controlled rearing conditions (24 ± 1°C, 70 ± 10% RH) until its cycle was completed, or parasitoid emerged or egg hatched. The material was inspected weekly. Lepidoptera larvae were fed soybean leaves and pods extracted from the field and previously examined to avoid contamination with other insects. Stink bugs were fed pods of *Phaseolus vulgaris* L. The taxonomic determination of lepidopteran larvae (Barrionuevo, 2011), stink bugs (Saini, 1984; 1987; 1988), entomophagous arthropods and other groups of insects (Triplehorn and Johnson,

2005) was carried out based on specific literature and consultations with taxonomy experts. For all the analyses, each parasitoid obtained in the laboratory was incorporated and the date, treatment and replicate from which its host was collected were recorded. We assumed that each new parasitoid originated from a different female.

Diversity Analysis

The diversity of the assemblages of each treatment was calculated and compared through the Hill's numbers of order q = 0, 1 and 2. When q = 0, ^0D is species richness; If q = 1, ^1D is the Shannon entropy exponential; and when q = 2, ^2D is the inverse of Simpson's concentration index (Chao et al., 2014). The diversities were estimated in standardized samples with a common sample completeness value to compare assemblages and subassemblages with equal coverage (Chao et al., 2016). The iNEXT package (Hsieh et al., 2016; Hsieh et al., 2020) run in R software (R Core Team, 2021) was used to obtain the estimates of diversity and their 95 % confidence intervals (CI) for the minimum common coverage among all assemblages and subassemblages of each treatment. Additionally, in order to identify effects on the diversity of less frequent species, the Chao-1 index and its CI were estimated in the PAST software (Version 3.20) (Hammer et al., 2001). The CIs were used to compare the diversity estimates.

Similarity Analysis

The SpadeR software in its *Online* version was used to estimate similarity measures for orders q = 0 (Sørensen index), 1 (Horn) and 2 (Morisita - Horn index), based on abundance data (Jost, 2006; Chao et al., 2015). The CIs were used to compare the similarity measures.

Rank-Abundance curves

The rank-abundance curves for the assemblage of arthropods, as well as the subassemblages phytophagous insects and entomophagous arthropods, were elaborated in the PAST software (Version

3.20) (Hammer et al., 2001). Adult moths were removed from the records to avoid interference in the analysis, since they were recorded in both cultivars.

For the arthropod assemblages, rank-abundance curves were compared among *Bt* and non-*Bt* and early and late planting date combinations, with and without Lepidoptera larvae –the target insect of *Cry1Ac* protein– and their parasitoids. For the phytophagous and entomophagous subassemblages, rank-abundance curves were compared among the same combinations of soybean cultivars and planting dates, but with and without target Lepidoptera larvae of the *Cry1Ac* protein and with and without parasitoids of Lepidoptera larvae, respectively.

The plotted data were adjusted to a standard abundance model of the geometric type, which fits the abundances of species observed in very unequal communities with low diversity (He and Tang, 2008), as are the communities of agroecosystems, particularly those of extensive productive systems. The k parameter of the geometric series can be considered an indicator of the complexity of the species composition in the system (Ferreira and Petrere, 2008); therefore, it was used to compare the rank-abundance curves.

Results

The results of the diversity analysis show that, in general, the whole arthropod assemblage as well as entomophagous subassemblage were more diverse in the non-*Bt* cultivar, regardless of the diversity indices analyzed. More precisely, richness (q=0) was higher in non-*Bt* than *Bt* only in late plantings (Table 1 and 2). The subassemblage of phytophagous insects showed similar results, except for species richness, with no significant differences between treatments (Table 3). Planting date did not exhibit clear effects on the diversity of the assemblages and subassemblages studied. Sampling completeness was close to 100% (Tables 1, 2 and 3). The Chao-1 index also showed high similarity between the assemblages (Table 4).

Tables 1 - 4

The Sorenson index varied from 0.52 to 1 but showed no significant differences of similarity among treatment pairs, implying that similarities do not depend on cultivar or planting dates. Horn and Morisita-Horn indices yielded high similarity values, both for the arthropod assemblage (from 0.84 to 0.98; Table 5) and for the entomophagous (from 0.86 to 0.99; Table 6) and phytophagous subassemblages (from 0.82 to 0.99; Table 7) separately. When the Horn index was applied for arthropod assamblages (Table 5), the highest similarity occurred between early and late plantings of the non-*Bt* cultivar. However, this similarity did not differ from that between early and late plantings of the *Bt* cultivar. In other words, the similarity between early and late plantings is independent of the cultivar. In the case of phytophagous subassemblages (Table 7), the similarity between early and late planted plots with the non-*Bt* cultivar was significantly higher than the rest of the contrasts. This implies that the cultivar affects the similarity between the subassemblages present in plots of different planting dates. Regarding entomophagous (Table 6), no significant differences were found between any treatment combinations except the most contrasting one (early non-*Bt* - late *Bt*) in which the similarity was the lowest.

When the Morisita Horn index was used for arthropod assemblage (Table 5) and phytophagous subassemblage (Table 7) the similarity between early and late planted plots with the non-*Bt* cultivar was significantly higher than the rest of the contrasts. With respect to the entomophagous subassemblage (Table 6), the highest similarity was found for the early planted non-*Bt* and *Bt* treatments. But this was not different from the late planted *Bt* and non-*Bt* plots. In other words, planting date has no effect on the similarity between the assemblages of the different cultivars. The species composition and relative abundances within each assemblage (defined by planting date × soybean cultivar combination) are presented for each agricultural season in Tables A1 and A2.

Tables 5 - 7

Figures 1 – 3 show the rank-abundance curves for the arthropod assemblages and the subassemblages of entomophagous arthropods and phytophagous insects. The value of the parameter *k* is indicated in each curve to allow for comparisons between the different treatments of each assemblage and subassemblage. When data of target species larvae, their parasitoids, or both, were removed from the non-*Bt* cultivar data set, the values of *k* parameter increased considerably, equaling or exceeding those obtained in *Bt*-soybean. For the entomophagous arthropods subassemblage in late plantings, the non-Bt plots with parasitoids removed exhibited an intermediate k parameter value, lying between the non-Bt plots with parasitoids present and the Bt cultivars.

Figs. 1 – 3

Discussion

Bt crops have gained momentum in South America following a very fast adoption (Horikoshi et al., 2021; Manzur and Cárcamo, 2014). Nonetheless, the debate on their potential externalities is still present in the literature, with the direct or indirect influence on non-target species (Conner et al., 2003) as a special topic of discussion (Dang et al., 2021; Romeis, 2019). Among indirect effects, the most basic impact of *Bt* crops on natural enemies is thought to be the decrease in availability and quality of prey or hosts (Faria et al., 2007; Dhillon and Sharma, 2009; Lundgren et al., 2009). Natural enemies that tend to be more specialized (parasitoids) are the most affected by this mechanism

(Schuler et al., 1999; Fontes et al., 2002; Frizzas et al., 2017). The hypothesis of indirect effects predicts that arthropod communities differ between *Bt* and conventional crops. We tested this prediction focusing on classical properties of communities: richness, diversity and similarity.

To obtain robust results, the parameters selected to test the prediction require a high level of sample coverage. Therefore, when coverage is equalized, bias in diversity comparisons is reduced (Roswell et al., 2021). Low coverage levels provide inaccurate estimates (Chao and Jost, 2012). In this work, the coverages were 99% (Tables 1 – 3). Other studies on arthropod diversity in field crops show a range of coverage from 95 to 99% (García García et al., 2017; Sanchez et al., 2018). In studies of spider diversity in the tropical forests of Brazil, Azevedo et al. (2013) estimated that from 70% completeness, the sampling effort should be greatly increased to effectively increase the richness observed. Presumably it is easier to reach high levels of coverage in agroecosystems than in a pristine environment.

Studies on the potential effects of *Bt* crops on the arthropod communities present in various crops have yielded mixed results. In this work, the effect of the *Cry1Ac* protein, expressed in *Bt* soybean cultivars, is limited to the elimination of the larvae of the lepidopteran species targeted by the toxin and, consequently, to the absence of their parasitoids, due to the high host specificity of this group. Whitehouse et al. (2005) detected only subtle differences in the arthropod community between *Bt* and conventional cotton. All the diversity indices showed significant differences between treatments except for species richness index (D^0) of the phytophagous insects subassemblage. The Chao-1 index of the phytophagous and overall arthropod assemblages, showed no differences suggesting lack of effect of rare species while for entomophagous subassamblage in late plantings diversity in Non-*Bt* was significantly higher than in *Bt*. In general, we found a higher diversity in the non-*Bt* cultivar than in the *Bt* cultivar. *Rachiplusia nu* (Guenée) is the most abundant lepidopteran species in summer crops in the region where the trials were established; therefore, its parasitoids are also abundant (Sierra Fetter and Peralta, 2010; Cisnero and Virano, 2011; Lovera and Roggia 2021). The absence of parasitoids in the *Bt* cultivar may explain the results found in this work. Several authors found no differences in arthropods richness, diversity or dominance between *Bt* and non-*Bt* cultivars (Rose and Dively, 2007; Dhillon and Sharma, 2013; Sosa and Almada, 2014; Yu *et al.*, 2014; Chaves Resende *et al.*, 2016; García García *et al.,* 2017; Frizzas *et al.*, 2017 and Schutte, 2020). Regarding diversity of natural enemies' community, little or no effects of Bt cultivars of cotton and soybean compared to their non-Bt counterparts were detected (Whitehouse et al., 2005; Guo et al., 2016; Frizzas et al., 2017; Carbone, 2018; Kaur et al., 2021). Numerous fieldworks point out little to no differences in community structure or species diversity between *Bt* crops and their non-*Bt* counterparts (Ögür and Tuncer, 2012).

Our results agree with previous works reporting a decrease in species richness and arthropod diversity in *Bt* compared to non-*Bt* cotton (Sisterson et al*.,* 2004; Liu and Luo, 2019). However, for pest arthropods in the same crop, Men et al*.* (2003) reported an increase in diversity in *Bt* cultivars*.* These authors worked on agricultural field crops areas between 0.4 and 1.64 hectares. The similarity found between treatments was high for all assemblages and subassemblages and for all indicators that consider both only species composition and composition and abundance (Tables 5 – 7). This indicates that the assemblages differed by only a few species. Our results agree with those of Carbone (2018), who found that the *Bt* and non-*Bt* soybean cultivars shared the same predator species, evidencing similarity between cultivars. The same inference can be made by comparing the rank-abundance curves (Figures 1 – 3). For the arthropod assemblages as well as for the phytophagous insect or entomophagous arthropod subassemblages, differences were found between the *Bt* cultivar and its non-*Bt* counterpart, with the latter presenting a greater diversity and equitability. These differences diminished or were reversed when the larvae of the *Bt* target species and their parasitoids were removed from the analysis, suggesting that these species are the ones that differentiate one cultivar from the other in the field. No differences in the uniformity and composition of the insect community were found between *Bt* and conventional corn (Frizzas et al., 2017) and soybean (Schutte, 2020).

Studies on cotton and corn are relevant to this discussion. The cotton crop expresses the same protein as *Bt* soybean, the Cry1Ac protein. Therefore, it is possible to consider these studies as background information on the potential effects of this toxin on the arthropod community. On the other hand, although *Bt* toxins in corn are different from those in soybean (MRI, 2017; ArgenBio, 2021), direct or indirect influence on communities may be reflected in emergent properties of this level of organization, such as richness and diversity, beyond the effects on particular target species.

An important issue is the discussion about the scope of this study. Ecological processes have a scale of operation. A single process is regulated by different mechanisms and, therefore, can generate different patterns at different scales (García, 2008). In this work, each of the replicates covered an area equivalent to 0.18 hectare. The four replicates together did not occupy one hectare of cultivation (70 %), which suggests the occurrence of scale effects, especially for highly mobile species such as Hemiptera, Orthoptera and natural enemies. However, a process cannot be attributed to a particular scale, but specific space and time scales can be pointed out in which one process prevails over another (Schneider 1994, 2009). In studies about population dynamics, Bengtsson et al. (2004) suggest that analysis of small-scale movement patterns of animals can help to understand and predict movement on a larger scale. However, the results presented here should not be extrapolated to other environments or to larger scales. Studies at commercial plots or farm scales are necessary in order to verify the results found in this work. At the same time, the characteristics of the environmental matrix at the landscape level can also influence the size of the sources from which the colonization or dispersal of arthropods to crops occurs (Mitchell et al., 2014; González, 2015). Nonetheless, considering the homogeneity of the surroundings (typical crops of the region), we would not expect much influence of the spatial scale.

Conclusions

This work provides information on the arthropod assemblage of *Bt* soybean and adds to the background on the possible effects of crops expressing *Bt* proteins on the phytophagous insect and entomophagous arthropod subassemblages, as well as on the entire assemblage. Under the study conditions, the data support the conclusion that the effects of the *Cry1Ac* protein are limited to the suppression of larvae of the target species and their parasitoids as a consequence of the host specificity. Planting date had no influence on the diversity of arthropod assemblages present in soybean crops.

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Tables

Table 1. Arthropod assemblage: Species richness (^0D), Shannon entropy exponential (^1D), inverse of Simpson's concentration index (^2D), and their respective 95% confidence intervals (CI) for the minimum common cover among treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of 2018/2019, 2019/2020, and 2020/2021 growing seasons.

Soybean	**Planting date**	**Sample size** [a]	**Order *q***	**Estimated diversity (95% CI)** [b]
Bt	Early	8976	0	98.1 (70.3 – 125.9) ab
	Late	4205	0	71.3 (60.8 – 81.7) b
Non-*Bt*	Early	4875	0	89.7 (80.6 – 98.9) ab
	Late	8051	0	110.2 (85.1 – 135.3) a
Bt	Early	8976	1	7.6 (7.1 – 8.1) c
	Late	4205	1	7.9 (7.4 – 8.4) c
Non-*Bt*	Early	4875	1	14.1 (13.4 – 14.7) a
	Late	8051	1	12.1 (11.5 – 12.7) b
Bt	Early	8976	2	3.0 (2.8 – 3.1) c
	Late	4205	2	3.3 (3.2 – 3.5) b
Non-*Bt*	Early	4875	2	5.9 (5.7 – 6.2) a
	Late	8051	2	5.4 (5.2 – 5.7) a

[a] Sampling completeness: 0.99. [b] Different letters for the same order "*q*" indicate statistical differences between assemblages.

Estimation method: Extrapolation, because the sample size is greater than the reference sample size (Hsieh et al. 2016). Letters are arranged alphabetically from highest to lowest diversity value.

Table 2. Entomophagous arthropod subassemblage: Species richness (^{0}D), Shannon entropy exponential (^{1}D), inverse of Simpson's concentration index (^{2}D), and their respective 95% confidence intervals (CI) for the minimum common cover among treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of 2018/2019, 2019/2020 and 2020/2021 growing seasons.

Soybean	**Planting date**	**Sample size [a]**	**Estimation method [b]**	**Order *q***	**Estimated diversity (95% CI) [c]**
Bt	Early	1232	Extrapolation	0	27.8 (8.0 – 47.5) ab
	Late	643	Rarefaction	0	22.5 (17.0 – 27.9) b
Non-*Bt*	Early	1210	Rarefaction	0	37.0 (29.8 – 44.3) a
	Late	1964	Extrapolation	0	53.7 (31.4 – 76.0) a
Bt	Early	1232	Extrapolation	1	7.7 (7.1 – 8.3) b
	Late	643	Rarefaction	1	8.1 (7.4 – 8.7) b
Non-*Bt*	Early	1210	Rarefaction	1	9.8 (9.1 – 10.5) a
	Late	1964	Extrapolation	1	11.3 (10.4 – 12.2) a
Bt	Early	1232	Extrapolation	2	4.9 (4.6 – 5.3) b
	Late	643	Rarefaction	2	5.2 (4.7 – 5.6) b
Non-*Bt*	Early	1210	Rarefaction	2	6.1 (5.7 – 6.6) a
	Late	1964	Extrapolation	2	6.3 (5.7 – 6.9) a

[a] Sampling completeness: 0,99. [b] Estimation method: Rarefaction or Extrapolation, depending on whether the sample size is less or greater than the reference sample size (Hsieh et al. 2016). [c] Different letters for the same

order “*q*” indicate statistical differences between assemblages. Letters are arranged alphabetically from highest to lowest diversity value.

Table 3. Phytophagous insect subassemblage: Species richness (^{0}D), Shannon entropy exponential (^{1}D), inverse of Simpson's concentration index (^{2}D), and their respective 95% confidence intervals (CI) for the minimum common cover among treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of 2018/2019, 2019/2020 and 2020/2021 growing seasons.

Soybean	**Planting date**	**Sample size** [a]	**Estimation method** [b]	**Order *q***	**Estimated diversity (95% CI)** [c]
Bt	Early	6730	Extrapolation	0	64.8 (20.9 – 108.7) a
	Late	3744	Extrapolation	0	49.1 (25.0 – 73.2) a
Non-*Bt*	Early	3095	Rarefaction	0	50.4 (43.9 – 56.9) a
	Late	4677	Extrapolation	0	52.9 (35.4 – 70.3) a
Bt	Early	6730	Extrapolation	1	3.6 (3.3 – 3.8) c
	Late	3744	Extrapolation	1	3.8 (3.5 – 4.0) c
Non-*Bt*	Early	3095	Rarefaction	1	6.9 (6.5 – 7.3) a
	Late	4677	Extrapolation	1	5.9 (5.6 – 6.2) b
Bt	Early	6730	Extrapolation	2	1.7 (1.7 – 1.8) c
	Late	3744	Extrapolation	2	2.0 (1.9 – 2.1) b
Non-*Bt*	Early	3095	Rarefaction	2	3.2 (3.1 – 3.4) a
	Late	4677	Extrapolation	2	3.3 (3.1 – 3.4) a

[a] Sampling completeness: 0,99. [b] Estimation method: Rarefaction or Extrapolation, depending on whether the sample size is less or greater than the reference sample size (Hsieh et al. 2016). [c] Different letters for the same

order “*q*” indicate statistical differences between assemblages. Letters are arranged alphabetically from highest to lowest diversity value.

Table 4. Arthropod assemblage, Entomophagous arthropod subassemblage and Phytophagous insect subassemblage: Chao-1 index and their respective 95% confidence interval (CI) for the treatments determined by the planting date (early and late) and soybean cultivar (Bt and non-Bt) combination for data of 2018/2019, 2019/2020 and 2020/2021 growing seasons.

Group	**Soybean**	**Planting date**	**Chao-1 (95% CI)***
Arthropod assemblage	*Bt*	Early	118.6 (70.6 - 119.0) a
		Late	78.0 (59.0 - 92.6) a
	Non-*Bt*	Early	98.1 (81.7 - 114.9) a
		Late	123.8 (82.4 - 130.2) a
Entomophagous arthropod	*Bt*	Early	36.3 (21.5 - 45.0) ab
		Late	24.2 (19.0 - 32.5) b
	Non-*Bt*	Early	42.7 (32.5 - 54.0) ab
		Late	58.0 (36.0 - 75.2) a
Phytophagous insect	*Bt*	Early	76.5 (53.3 - 81.0) a
		Late	54.0 (44.1 - 65.0) a
	Non-*Bt*	Early	54.6 (51.8 - 69.3) a
		Late	52.6 (48.2 - 69.0) a

* Different letters for the same group indicate statistical differences between assemblages. Letters are arranged alphabetically from highest to lowest diversity value.

Table 5. Arthropod assemblage: Similarity index between treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of three growing seasons.

Index	**Planting date - Soybean**	Early - non-*Bt*	Early - *Bt*	Late - non-*Bt*
Sørensen*	Late - *Bt*	0.59 (0.46 – 0.72)	0.78 (0.53 – 1.00)	0.58 (0.45 – 0.70)
	Late - non-*Bt*	0.74 (0.55 – 0.93)	0.52 (0.34 – 0.71)	
	Early - *Bt*	0.62 (0.45 – 0.80)		
Horn	Late - *Bt*	0.87(c) (0.86 – 0.89)	0.94 (a) (0.93 – 0.95)	0.88 (c) (0.86 – 0.89)
	Late - non-*Bt*	0.96 (a) (0.95 – 0.97)	0.85 (c) (0.84 – 0.87)	
	Early - *Bt*	0.90 (b) (0.89 – 0.92)		
Morisita – Horn	Late - *Bt*	0.94 (b) (0.93 – 0.95)	0.89 (c) (0.87 – 0.91)	0.92 (bc) (0.91 – 0.93)
	Late - non-*Bt*	0.98 (a) (0.97 – 0.99)	0.84 (d) (0.82 – 0.86)	
	Early - *Bt*	0.90 (c) (0.89 – 0.92)		

* No significant differences were found for this similarity index.

Table 6. Entomophagous arthropod subassemblage: Similarity index between treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of three growing seasons.

Index	**Planting date - Soybean**	Early - non-*Bt*	Early - *Bt*	Late - non-*Bt*
Sørensen*	Late - *Bt*	0.70 (0.51 – 0.89)	0.91 (0.66 – 1.00)	0.56 (0.36 – 0.76)
	Late - non-*Bt*	1.00 (0.69 –1.00)	0.70 (0.53 – 0.87)	
	Early - *Bt*	0.83 (0.53 – 1.00)		
Horn	Late - *Bt*	0.92 (b) (0.91 – 0.93)	0.96 (a) (0.95 – 0.97)	0.96 (a) (0.95 – 0.98)
	Late - non-*Bt*	0.97 (a) (0.96 – 0.98)	0.98 (a) (0.96 – 0.99)	
	Early - *Bt*	0.98 (a) (0.97 – 0.99)		
Morisita – Horn	Late - *Bt*	0.86 (c) (0.84 – 0.89)	0.90 (bc) (0.88 – 0.93)	0.98 (ab) (0.95 – 1.00)
	Late - non-*Bt*	0.93 (b) (0.90 – 0.95)	0.95 (b) (0.93 – 0.98)	
	Early - *Bt*	0.99 (a) (0.98 – 1.00)		

* No significant differences were found for this similarity index.

Table 7. Phytophagous subassemblage: Similarity indices between treatments determined by the planting date (early and late) and soybean cultivar (*Bt* and non-*Bt*) combination for data of three growing seasons.

Index	**Planting date - Soybean**	Early - non-*Bt*	Early - *Bt*	Late - non-*Bt*
Sørensen*	Late - *Bt*	0.56 (0.40 – 0.72)	0.70 (0.41 – 0.98)	0.68 (0.42 – 0.93)
	Late - non-*Bt*	0.71 (0.50 – 0.91)	0.54 (0.33 – 0.75)	
	Early - *Bt*	0.59 (0.41 – 0.77)		
Horn	Late - *Bt*	0.86 (c) (0.85 – 0.88)	0.94 (b) (0.93 – 0.95)	0.86 (c) (0.84 – 0.87)
	Late - non-*Bt*	0.96 (a) (0.95 – 0.97)	0.82 (d) (0.81 – 0.84)	
	Early - *Bt*	0.88 (c) (0.87 – 0.89)		
Morisita – Horn	Late - *Bt*	0.94 (b) (0.93 – 0.95)	0.89 (cd) (0.87 – 0.90)	0.92 (c) (0.90 – 0.93)
	Late - non-*Bt*	0.99 (a) (0.98 – 0.99)	0.83 (d) (0.81 – 0.85)	
	Early - *Bt*	0.90 (c) (0.89 – 0.91)		

* No significant differences were found for this similarity index.

Figures

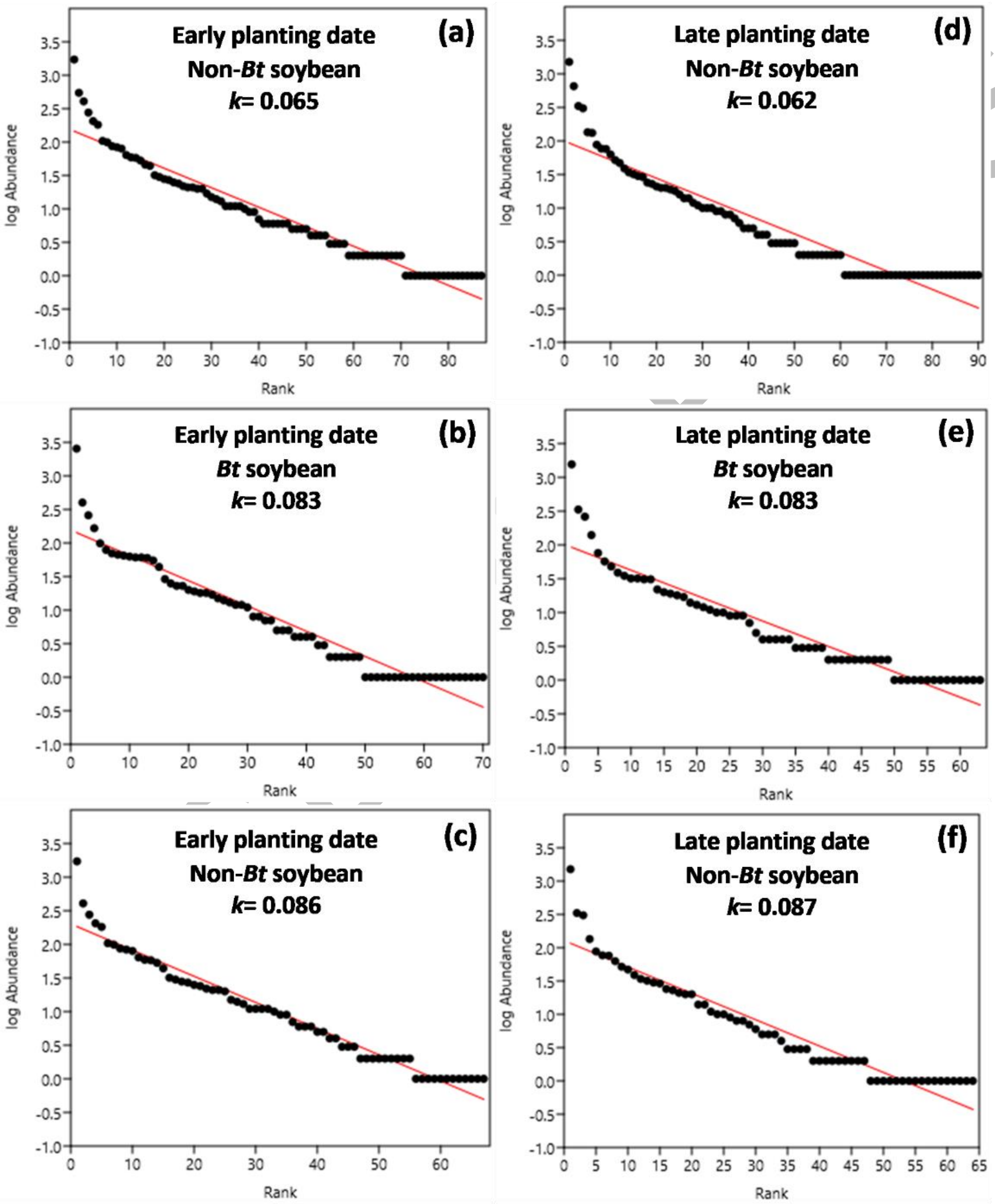

Figure 1. Arthropod assemblage: Rank–Abundance curves and estimates of the parameter *k*. Panels a and d: non-*Bt* soybean; Panels b and e: *Bt* soybean; Panels c and f: Non-*Bt* soybean without the presence of lepidopteran larvae target of Cry1Ac toxin and their parasitoids. Ensamble de artrópodos: Curvas de rango - abundancia y

estimación del parámetro *k*. Figures a y d: Soja no-*Bt*; Figures b y e: Soja *Bt*; Figures c y f: Soja no-*Bt* sin la presencia de larvas de lepidópteros blanco de la toxina Cry1Ac y sus parasitoides.

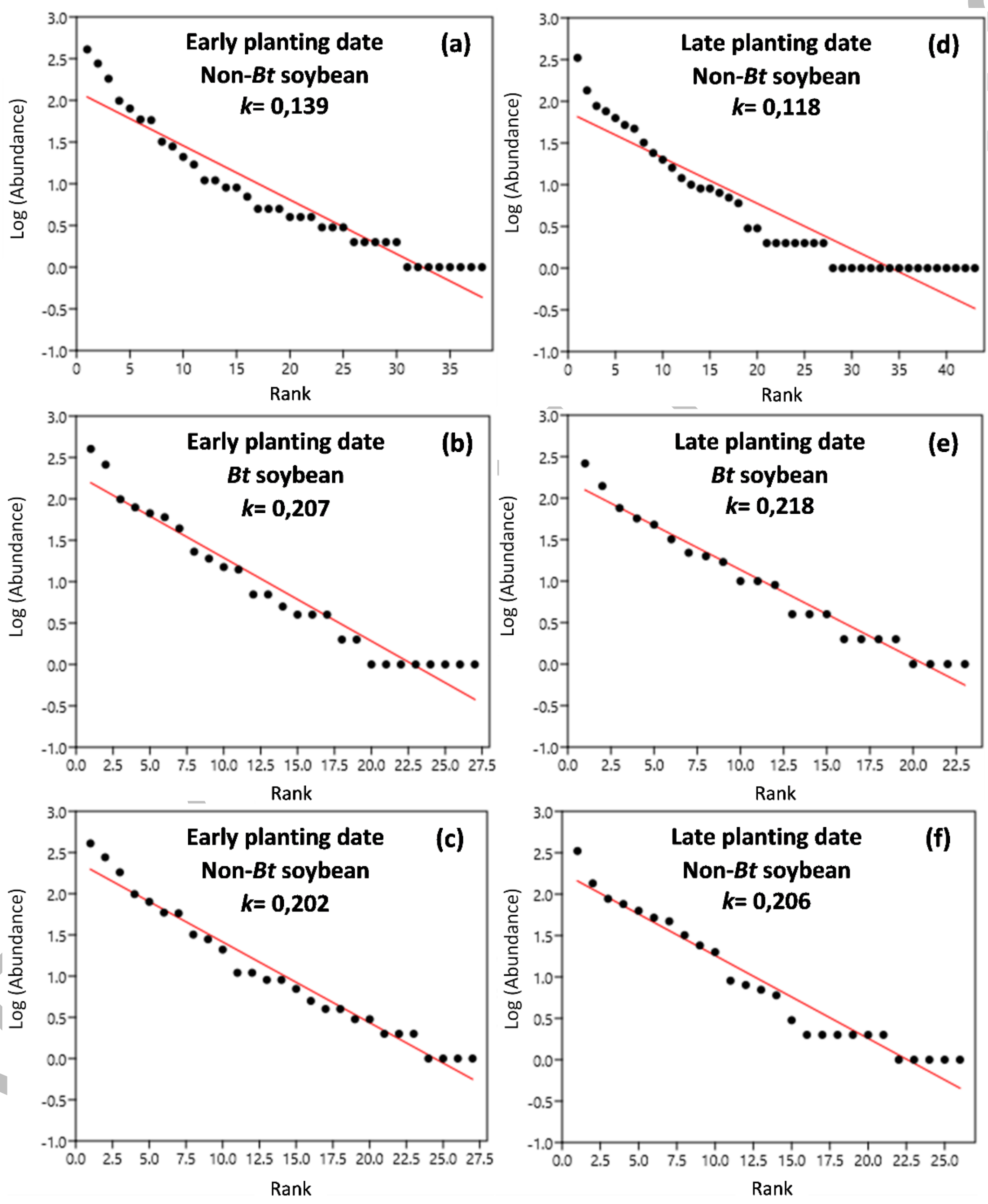

Figure 2. Entomophagous arthropod subassemblage: Rank–Abundance curves and estimates of the parameter *k*. Panels a and d: Non-*Bt* soybean; Panels b and e: *Bt* soybean; Panels c and f: Non-*Bt* soybean without the presence of parasitoids of lepidopteran larvae target of Cry1Ac toxin. Subensamble

Entomófagos: Curvas de rango - abundancia y estimación del parámetro *k*. Figures a y d: Soja no *Bt*; Figures b y e: Soja *Bt*; Figures c y f: Soja no-*Bt* sin la presencia de parasitoides de larvas de lepidópteros blanco de la toxina Cry1Ac.

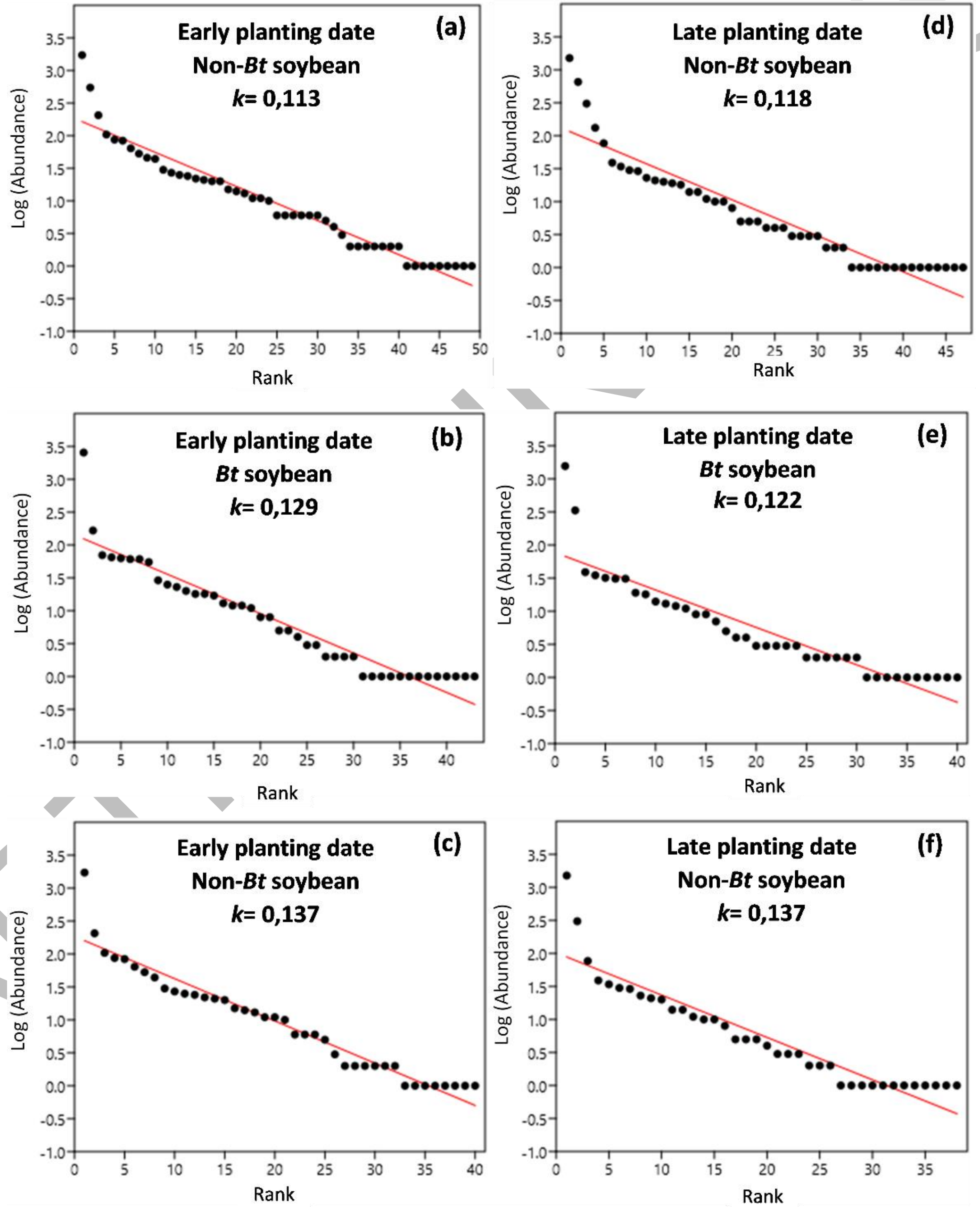

Figure 3. Phytophagous insect subassemblage: Rank–Abundance curves and estimates of the parameter *k*. Panels a and d: Non-*Bt* soybean; Panels b and e: *Bt* soybean; Panels c and f: No-*Bt* soybean without the presence of lepidopteran larvae target of Cry1Ac toxin. Subensamble Fitófagos: Curvas de rango - abundancia y estimación del parámetro *k*. Figures a y d: Soja no-*Bt*; Figures b y e: Soja *Bt*; Figures c y f: Soja no-*Bt* sin la presencia de larvas de lepidópteros blanco de la toxina Cry1Ac.

APPENDIX

Table A1. Abundance of phytophagous species recorded across different treatment combinations involving planting date (early vs late) and soybean cultivar (Bt vs non-Bt) for the 2018/2019, 2019/2020, and 2020/2021 growing seasons in Anguil, La Pampa (Semiarid Pampean Region).

Seasons	**2018/19**				**2019/20**				**2020/21**			
Planting date	**Early**		**Late**		**Early**		**Late**		**Early**		**Late**	
Soybean	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***
Acanaloniidae sp1.	0	1	0	0	0	0	0	0	0	0	0	0
Achyra sp.	0	0	0	0	0	6	8	7	6	2	18	8
Acledra modesta	1	1	0	0	0	0	0	0	0	0	0	0
Acrididae sp1.	0	0	0	0	0	1	0	0	0	0	0	0
Acyrthosiphon pisum	1	2	3	3	0	0	1	0	0	0	1	1
Agalliana ensigera	1	2	5	6	6	0	0	1	18	10	3	4
Anasa sp.	0	0	0	0	0	0	0	0	1	0	0	0
Anticarsia gemmatalis	11	0	10	0	7	0	4	0	2	0	4	0
Aphididae sp1.	0	1	1	0	0	0	0	0	0	0	0	1
Aramigus tessellatus	9	7	2	2	3	2	1	1	10	9	0	1
Aricoris sp.	0	0	0	0	0	0	0	0	2	0	0	0
Astylus atromaculatus	0	1	0	0	0	0	0	0	0	0	0	0
Astylus vittaticollis	0	3	0	0	0	0	0	0	0	0	0	0
Athaumastus haematicus	1	0	0	1	0	0	1	0	5	0	0	1
Atrichonotus taeniatulus	0	0	0	0	0	0	0	1	2	2	0	2
Baeacris sp.	18	13	11	15	60	49	13	14	6	3	6	2
Byllis subgranulata	1	0	1	0	1	1	0	0	0	0	0	0
Calopteron sp.	0	0	0	0	0	0	0	1	3	0	2	1
Ceresa brunnicornis	1	0	0	0	2	0	5	1	8	4	0	2

Chauliognathus scriptus	45	28	12	15	14	24	12	9	28	9	5	7
Chelymorpha varians	0	0	0	0	1	0	0	0	0	0	0	0
Chinavia musiva	0	0	0	1	0	0	0	0	0	0	0	0
Chrysolina sp.	1	0	1	0	0	0	0	0	1	0	0	0
Cicadellidae sp1.	0	0	0	0	0	0	0	0	1	0	0	0
Colaspis sp.	52	34	36	25	40	13	40	8	12	8	1	6
Colias lesbia	2	0	3	0	1	1	1	0	4	0	0	0
Conoderus sp.	1	0	0	0	0	0	0	0	0	0	0	0
Crocidosema aporema	0	0	0	0	0	1	0	1	0	0	0	0
Curculionidae sp1.	4	3	0	0	7	4	0	1	2	1	0	2
Dargida albilinea	0	1	0	0	0	0	0	0	0	0	0	0
Diabrotica speciosa	15	8	18	8	0	1	2	1	0	2	0	0
Diceraeus furcatus	24	19	13	15	16	22	6	10	24	20	15	10
Dichroplus conspersus	0	0	0	0	2	0	0	0	0	0	0	3
Dichroplus elongatus	3	5	3	2	20	16	6	7	1	2	5	4
Dichroplus maculipennis	0	0	0	0	0	0	0	0	0	0	1	1
Dichroplus pratensis	2	4	4	2	11	12	4	5	1	1	3	2
Edessa meditabunda	5	7	7	5	5	4	3	0	1	1	0	2
Epicauta adspersa	1	0	0	1	3	1	3	2	2	0	0	0
Epicauta leopardina	0	0	0	0	0	0	0	1	0	0	0	0
Euplectrotettix sp.	0	0	0	0	1	1	0	0	0	0	0	0
Feltia deprivata	6	0	2	0	0	0	0	0	0	0	0	0
Fulgoridae sp1.	0	0	1	0	0	0	0	0	0	0	0	0
Geometridae sp1.	0	0	0	0	0	0	0	0	2	0	0	0
Harmostes procerus	15	18	2	3	0	0	0	1	5	0	1	1
Helicoverpa gelotopoeon	2	0	0	0	30	2	43	1	14	0	90	0
Largus fasciatus	0	0	0	0	0	0	1	2	2	0	0	0
Lygus albonatus	0	0	0	0	0	2	0	0	0	1	0	0
Manduca sexta	0	0	0	0	0	1	0	0	0	0	0	0
Megascelis sp.	0	0	0	0	0	0	0	1	0	0	0	0
Mythimna adultera	0	1	0	0	0	0	0	0	0	0	4	0
Mythimna unipuncta	0	1	0	0	0	0	0	0	0	0	0	0
Naupactus argentatus	0	0	0	0	0	0	1	0	0	0	0	0
Naupactus leucoloma	19	29	4	6	32	33	34	26	2	8	1	0
Naupactus verecundus	0	4	1	0	0	8	1	1	0	1	0	1

Nezara viridula	102	57	135	133	71	84	147	186	33	25	25	15
Nysius simulans	39	58	21	17	0	1	0	0	5	4	0	1
Pachybrachis sp.	0	2	0	1	0	1	0	0	0	2	1	1
Pantomorus auripes	8	8	1	1	13	12	12	12	6	0	10	6
Pantomorus ruizi	9	8	0	0	1	0	0	0	0	0	1	0
Pentatomidea sp1.	0	0	0	0	0	0	0	0	0	2	1	1
Phaedon sp.	12	22	13	13	0	6	0	0	9	1	1	1
Piezodorus guildinii	360	256	735	409	797	1536	596	931	565	758	178	223
Priocyphus bosqui	0	1	0	0	0	0	0	0	0	0	0	1
Rachiplusia nu	104	1	124	4	381	3	457	9	64	0	86	0
Sciaridae sp1.	6	5	0	0	0	0	0	0	0	0	0	0
Scotussa lemniscata	0	1	0	0	0	0	0	0	0	0	0	1
Sitona discoideus	0	0	0	0	0	0	0	0	0	0	1	0
Spilosoma virginica	0	0	0	0	0	0	0	0	6	0	0	0
Spodoptera cosmioides	2	0	2	1	0	1	0	0	0	0	0	0
Spodoptera frugiperda	0	0	0	0	0	0	0	0	1	0	1	0
Staleochlora viradicata	0	0	0	0	0	0	0	0	0	0	0	1
Strymon eurytulus	1	0	1	0	1	0	1	0	0	0	2	0
Thyanta sp.	0	0	0	0	0	0	0	0	0	0	5	0
Trimerotropis pallidipennis	0	0	0	0	4	1	4	0	1	1	0	0
Tropidacris collaris	0	1	0	0	0	0	0	0	0	0	0	0
Xerophloea viridis	13	8	4	2	3	4	0	1	14	13	6	9
Zoniopoda tarsata	0	0	0	0	1	0	0	0	0	0	0	0

Table A2. Abundance of entomophagous species recorded across different treatment combinations involving planting date (early vs late) and soybean cultivar (Bt vs non-Bt) for the 2018/2019, 2019/2020, and 2020/2021 growing seasons in Anguil, La Pampa (Semiarid Pampean Region).

Season	**2018/19**				**2019/20**				**2020/21**			
Planting date	**Early**		**Late**		**Early**		**Late**		**Early**		**Late**	
Soybean	**Non -*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non -*Bt***	***Bt***	**Non-*Bt***	***Bt***	**Non-*Bt***	***Bt***
Alabagrus sp.	0	0	0	0	0	0	0	0	0	0	1	0
Aleiodes brethesi	4	0	7	0	0	0	1	0	0	0	2	0
Araneidae	6	10	4	5	11	4	8	6	63	65	64	46
Archytas sp.	0	0	0	0	1	0	0	0	0	0	0	0

Archytas marmoratus	0	0	0	0	0	0	0	0	0	0	1	0
Asilidae	2	0	0	1	2	0	0	1	0	1	0	0
Atrachelus cinereus	1	0	1	0	0	0	1	2	1	1	0	0
Carabidae sp1.	0	0	0	0	6	1	8	9	15	18	1	1
Carabidae sp2.	0	0	0	0	0	0	0	0	1	0	0	0
Calosoma argentinense	1	0	0	0	0	0	0	0	0	0	0	0
Casinaria plusiae	0	0	3	0	0	0	0	0	0	0	0	0
Chysoperla sp.	3	8	16	8	2	0	7	9	6	7	1	3
Coccinella ancorallis	1	2	1	1	3	1	0	1	3	1	1	0
Conura sp.	0	0	1	0	1	0	0	0	0	0	0	0
Copidosoma floridanum	2	0	9	0	0	0	0	0	3	0	3	0
Cotesia ayerza	0	0	0	0	1	0	1	0	0	0	0	0
Cotesia marginiventris	0	0	1	0	0	0	0	0	0	0	0	0
Cotesia lesbiae	0	0	0	0	1	0	1	0	1	0	0	0
Cotesia sp.	0	0	0	0	0	0	0	0	1	0	1	0
Crematogaster sp.	18	16	10	5	46	54	34	25	118	29	8	18
Eriopis connexa	100	114	50	41	201	199	166	138	107	88	116	83
Geocoris callosus	11	11	7	10	12	9	18	11	36	40	7	11
Gonia sp.	0	0	0	0	0	0	0	0	0	0	1	0
Harmonia axyridis	0	0	1	1	3	1	0	1	2	3	0	0
Hippodamia convergens	0	1	0	0	1	0	1	0	0	0	1	0
Ichneumonidae sp1.	0	0	0	0	0	0	0	0	0	0	1	0
Incamyia nuda	0	0	0	0	0	0	0	0	3	0	2	0
Mantodea	4	5	3	2	3	2	1	2	2	0	3	0
Microplitis sp.	0	0	1	0	0	0	1	0	5	0	7	0
Nabis argentinus	15	11	25	10	6	4	36	7	11	29	2	0
Nabis capsiformis	1	3	5	2	6	1	3	2	4	1	0	0
Odonata sp1.	0	0	0	0	0	0	0	0	0	0	1	0
Orius sp.	0	0	0	1	0	0	0	0	9	4	6	8
Oxyopidae	38	37	42	33	28	9	18	18	33	21	28	25
Patelloa sp.	0	0	0	0	0	0	0	0	0	0	1	0
Podisus maculiventris	0	0	0	0	1	0	0	0	0	0	0	0
Podisus nigrispus	0	0	0	0	2	0	1	0	1	0	0	0
Reduviidae sp1	0	0	0	0	0	0	0	0	0	1	0	1

Salticidae	7	4	7	2	5	8	5	6	16	11	8	2
Syrphidae	0	2	2	1	0	0	0	2	4	0	1	1
Solenopsis sp.	0	0	0	0	10	8	37	16	48	6	10	6
Tachinidae sp.	1	0	0	0	1	0	0	0	0	0	1	0
Telenomus aff. *podisi*	1	1	0	0	0	0	1	0	1	1	0	0
Tettigoniidae	2	1	0	0	0	0	0	0	0	0	2	1
Thomisidae	123	117	55	60	94	69	45	37	60	73	35	43
Trichopoda giacomelli	2	5	1	1	0	0	1	0	1	2	0	0
Trissolcus aff. *leviventris*	0	0	0	0	0	0	0	0	0	0	1	0
Trissolcus aff. *teretis*	0	0	0	0	0	0	0	0	0	1	0	0
Trissolcus basalis	0	0	0	0	0	0	0	0	0	1	0	0
Tylospilus nigrobinotatus	0	0	0	0	0	0	2	0	0	0	0	0
Voria sp.	5	0	5	0	12	0	10	0	0	1	1	0
Zelurus sp.	0	0	0	0	0	0	0	0	0	0	0	1