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## Population analysis of white grubs (Coleoptera: Melolonthidae) throughout the Brazilian Pampa biome



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### ABSTRACT

The replacement of natural grassland by cultivated areas might favor the increase in abundance of some root-feeding species such as the white grubs, which may become a constraint for field crop production. This research aimed to assay the population density and geographical distribution of white grubs pest and other species in natural grassland and cultivated areas throughout the Brazilian Pampa biome. White grubs were sampled in 18 locations in both landscape use types and identified. Population density (number of larvae  $m^{-2}$ ) was calculated for each recorded species and sorted within two groups (pest species and other species), compared between natural grasslands and cultivated areas, as well as among locations. A dendrogram to evaluate species similarity among locations was built based on combined data obtained from both landscape use types throughout the region. In total, 31 species were found in the Brazilian Pampa, and four of them are considered as crop pests: *Diloboderus abderus* (Sturm, 1826), *Euethola humilis* (Burmeister, 1847), *Lyogenys fusca* (Blanchard, 1830), and *Phyllophaga triticophaga* Morón & Salvadori, 1998. The average population density of pest species in cultivated areas was less than five larvae  $m^{-2}$ , at most of locations. Some species had a wide geographical distribution (e.g. *D. abderus* and *Cyclocephala modesta* Burmeister), while other melolonthids occurred at only one location. The knowledge of which white grub species are present in a field and its population densities assist farmers to take proper management decisions.

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### Introduction

The Brazilian Pampa biome is located in the of Rio Grande do Sul State, where it occupies an area of 176.5 mil  $km^2$  (IBGE, 2004). Its vegetation is composed primarily of grass species (*Paspalum* spp., *Axonopus jesuiticus*, *Aristida* spp., and *Bouteloua megapotamica*) (Boldrini et al., 2010). The climate of this region is classified as “Cfa” and “Cfb” according to the Köppen climatic classification (Alvares et al., 2017).

The natural grasslands areas of the Brazilian Pampa have been replaced by croplands (Boldrini, 2009; Silveira et al., 2017), with a large increase in the area sowed with summer crops (over a million hectares) in the period from 2000–2015 due to a rise in commodity prices (Silveira et al., 2017). The increase in crop acreage has been from soybean, wheat, and rice (Gressler, 2008; Silva, 2012).

The areas with increase soybean and corn acreage during the warm season, and oats (*Avena* spp.), wheat (*Triticum aestivum*), and ryegrass (*Lolium* spp.) in the cool season have been the uplands, while the areas with increase rice acreage are in the lowlands (Gressler, 2008).

The conversion of natural grassland into cultivated areas and the adoption of some agricultural practices might favor some root feeding insects such as the white grubs (Morón, 1996). White grubs are larvae of the beetles belonging to the family Melolonthidae. Some species are rhizophagous and considered serious pests of many cultivated plants, despite its ability to improve soil quality (Oliveira and Salvadori, 2012). Thus, an increase of population densities of pest species might become a predicament for crop production (Salvadori and Pereira, 2006).

In Brazil, less than one percent of all Melolonthidae species recorded have been reported damaging crops and about five percent are associated to croplands (Morón, 2004). In most cases, control measures have been taken without knowing the accurate population density and species of white grubs present in a field,

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leading to incorrect management strategies. The use of inappropriate management strategies may affect the abundance of white grubs and reduce beneficial species, while triggering the increase of population density and dispersion of pest species (Solís and Morón, 1998).

In the 1980s, some species of Melolonthidae became pest on crops in the state of Rio Grande do Sul (southern Brazil) (Salvadori and Oliveira, 2001; Silva and Costa, 2002). The pasture white grub, *Diloboderus abderus* (Sturm) and the wheat white grub, *Phyllophaga triticophaga* Morón & Salvadori are the most important white grub pests of wheat, corn, and soybean in the Planalto region of Rio Grande do Sul state (Salvadori and Pereira, 2006). *Cyclocephala flavipennis* Arrow has also been found abundantly in cultivated areas (Salvadori and Pereira, 2006; Cherman et al., 2014a). This species has been considered as harmless even at high population density at farming conditions in southern Brazil, despite of feeding on wheat roots in greenhouse bioassays (potted wheat plants) (Salvadori and Pereira, 2006). However, *C. flavipennis* was reported damaging blueberry roots in southern Rio Grande do Sul (Diez-Rodríguez et al., 2015) and perennial winter pastures in Santa Catarina State (Duchini et al., 2017). In addition, *Plectris* (= *Demodema*) *brevitarsis* (Blanchard) has been found feeding on soybean roots (Morón and Salvadori, 2006) and *Liogenys fusca* Blanchard associated to winter crops (Cherman et al., 2011). Distribution and population density of non-pest species are well documented for the north region of Rio Grande do Sul (e.g. Cherman et al., 2013, 2014b).

For the south region of Rio Grande do Sul, the area that comprises the Brazilian Pampa, *Euethleola humilis* (Burmeister) was reported

damaging rice (Ferreira and Barrigossi, 2006). In the same region, *E. humilis* and *D. abderus* were reported damaging *Eucalyptus* spp. (Bernardi et al., 2008; Garlet et al., 2009). Despite these records, knowledge on population density and distribution of melolonthids “as a whole”, is lacking for the Brazilian Pampa. This is necessary to develop and implement successful and site-specific management strategies.

Here, we report the population density and distribution of white grub pest and other species in the Brazilian Pampa biome. White grubs were collected in cultivated and natural grassland areas in 18 locations to test the following hypotheses: (1) cultivated areas increase the population density of pest species; (2) population density of other species is greater in natural grassland areas than in cultivated areas; (3) the white grub species composition varies according to the phytophysionomic units of the Brazilian Pampa.

## Materials and methods

### Study area and locations

Within the Brazilian Pampa 18 locations (Table 1; Online resource 1) were chosen to sample white grubs, localized at different phytophysionomic units (Hasenack et al., 2010). Representative areas in terms of crop production or that have been converted from natural grassland to cultivated areas were sampled. Moreover, the locations and areas were selected based on information gathered by extension field agronomists and farmers, regarding the occurrence of white grubs, land accessibility, and the presence of a natural grassland area nearby a cultivated

**Table 1**  
Locations, previous and current crop, collection date, sampled area, and number of trenches (n) for cultivated and natural grassland areas in the Brazilian Pampa.

Location	Latitude (S)	Longitude (W)	Previous crop	Current crop	Data	Area (hectares)	n
<i>Cultivated areas</i>							
Alegrete	29°45'S	55°40'W	Corn	Oat + Italian ryegrass	10/25/2012	9.5	25
Caçapava do Sul	30°28'S	53°28'W	Corn	Oat + Italian ryegrass	10/19/2012	3	25
Cacequi	29°54'S	54°47'W	Soybean	Oat	10/26/2012	9.2	25
Dom Pedrito	31°03'S	54°29'W	Corn	Oat + Italian ryegrass	11/15/2012	5	25
Itacurubi	28°55'S	55°11'W	Soybean	Oat + Italian ryegrass	10/10/2012	5	25
Itaqui	29°13'S	56°28'W	Rice	Italian ryegrass	10/9/2012	6	25
Rosário do Sul	30°17'S	54°58'W	Corn	Oat + Italian ryegrass	10/26/2012	6.2	25
São Borja	28°38'S	55°59'W	Soybean	Wheat	10/9/2012	9	25
Uruguaiana	29°48'S	57°54'W	Millet	Italian ryegrass	10/25/2012	12.5	25
Vila Nova do Sul	30°21'S	53°57'W	Corn	Italian ryegrass	10/19/2012	6.5	25
Cachoeira do Sul	29°51'S	52°41'W	Soybean	Oat + Italian ryegrass	5/25/2013	5.67	34
Restinga Seca	29°49'S	53°17'W	Soybean	Italian ryegrass	5/31/2013	3.79	31
São Gabriel	30°22'S	54°23'W	Corn	Italian ryegrass	8/20/2013	4.5	25
São Sepé	30°00'S	53°41'W	Soybean	Oat	8/20/2013	5	25
Bagé	31°19'S	53°58'W	Soybean	Italian ryegrass	8/27/2013	4	25
Pinheiro Machado	31°35'S	53°15'W	Corn	Italian ryegrass	8/27/2013	1.5	25
Arroio Grande	32°13'S	53°14'W	Soybean	Italian ryegrass	8/28/2013	10	25
Herval	32°03'S	53°23'W	Corn	Italian ryegrass	8/28/2013	6	25
<i>Natural grassland areas</i>							
Alegrete	29°44'S	55°40'W	Natural grassland		10/25/2012	7.5	25
Caçapava do Sul	30°27'S	53°28'W	Natural grassland		10/19/2012	9.5	25
Cacequi	29°54'S	54°47'W	Natural grassland		10/26/2012	1.5	25
Dom Pedrito	31°04'S	54°29'W	Natural grassland		11/15/2012	6	25
Itacurubi	28°54'S	55°11'W	Natural grassland		10/10/2012	6	25
Itaqui	29°11'S	56°30'W	Natural grassland		10/9/2012	6	25
Rosário do Sul	30°17'S	54°59'W	Natural grassland		10/26/2012	5.3	25
São Borja	28°38'S	55°59'W	Natural grassland		10/9/2012	1	25
Uruguaiana	29°48'S	57°54'W	Natural grassland		10/25/2012	6	25
Vila Nova do Sul	30°20'S	53°57'W	Natural grassland		10/19/2012	5	25
Cachoeira do Sul	29°52'S	52°42'W	Natural grassland		5/25/2013	5.56	34
Restinga Seca	29°49'S	53°16'W	Natural grassland		5/31/2013	5.1	32
São Gabriel	30°22'S	54°24'W	Natural grassland		8/20/2013	10.5	25
São Sepé	30°00'S	53°40'W	Natural grassland		8/20/2013	7	25
Bagé	31°18'S	53°58'W	Natural grassland		8/27/2013	8	25
Pinheiro Machado	31°36'S	53°16'W	Natural grassland		8/27/2013	3.5	25
Arroio Grande	32°13'S	53°15'W	Natural grassland		8/28/2013	3	25
Herval	32°03'S	53°23'W	Natural grassland		8/28/2013	8	25

one. Natural grassland areas were considered those with natural vegetation (grasslands) used to raise cattle, and cultivated areas were those that have been used as cropland for at least ten years.

#### Sample procedure

Samples were taken during 2012 and 2013 in 18 locations throughout the Brazilian Pampa biome. We performed soil samples from May to November because species with known bioecology are at larval stage during this period in Rio Grande do Sul State (e.g. *D. abderus* and *P. triticophaga*; Pereira and Salvadori, 2006; Salvadori and Pereira, 2006). At least 25 trenches (50 × 25 × 30 cm deep) were randomly opened in both cultivated and natural grassland areas at each location. The soil of each trench was carefully handled to verify the presence of larvae and adults of Melolonthidae. The specimens were individually placed into 70 mL plastic pots containing trench's soil and transported to the Laboratório de Manejo Integrado de Pragas of the Universidade Federal de Santa Maria.

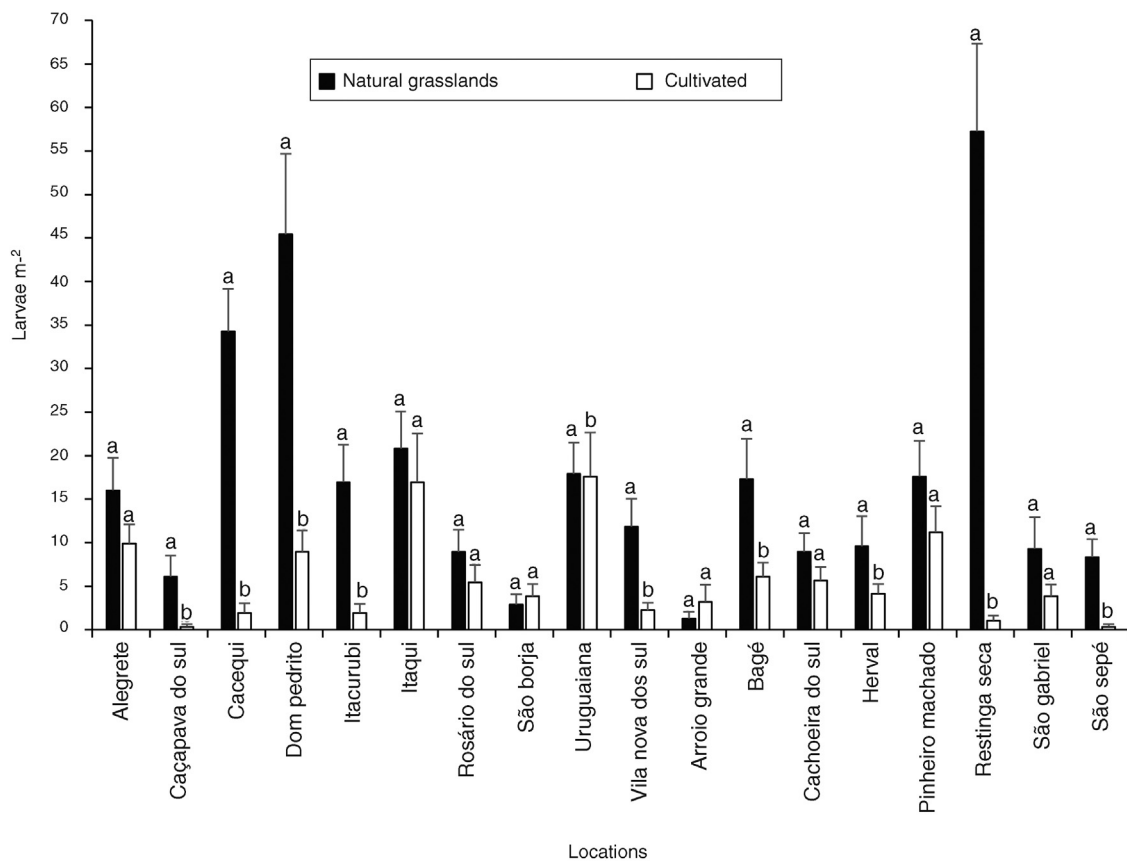
#### Specimen identification

Melolonthidae larvae were identified using a stereoscopic microscope (5x magnification), taxonomic keys and morphological descriptions of Frana (2003), Pereira and Salvadori (2006), and Cherman et al. (2013). For those specimens at larval stage that was not possible to identify, we reared them to adult stage. For this, the larvae were kept individually into 70 mL pots containing trench's soil and a carrot dice in growth chambers (25 ± 1 °C and 12:12 h light:dark cycle) (Aragón et al., 2005). The pots were verified weekly for adult emergence, soil moisture, and to replace the carrot dices. Vouchers of larvae and adults were deposited at

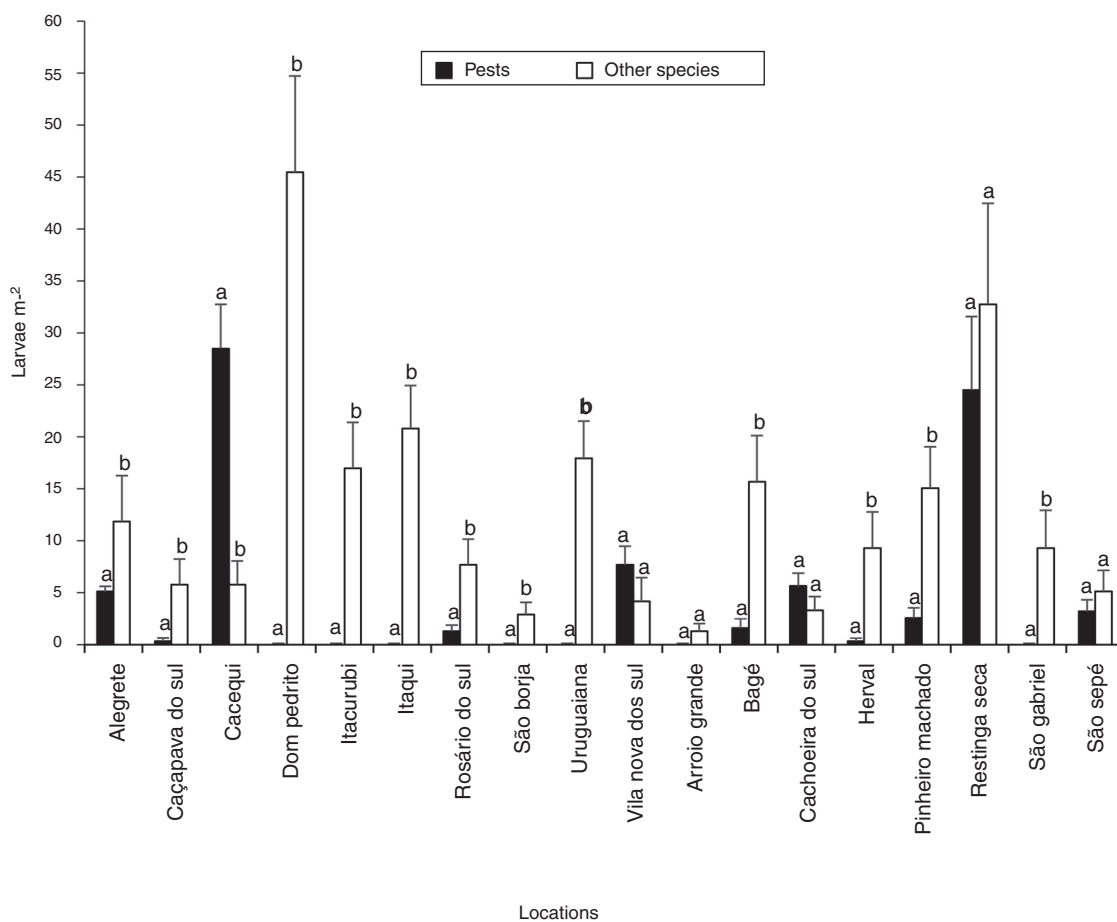
the following collections: Coleção Entomológica do Departamento de Defesa Fitossanitária (Universidade Federal de Santa Maria, Rio Grande do Sul); DZUP (Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba), and CERPE (Coleção Entomológica da Universidade Federal de Pernambuco, Recife).

#### Statistical analysis

The absolute abundance data (number of specimens of each species) of each sampled area was used to calculate the relative abundance for each species (larvae m<sup>-2</sup>). Then, the species were sorted within two groups based on available literature: pest and other species. The “other species” category is not considered as simply “non-pests” because it may have both beneficial and/or potential pest species (facultative rhizophagous habits, e.g. *C. flavipennis*). In the category of “pests” are only those that have already been recorded in the literature causing injuries to cultivated plants. The total average of white grubs density (larvae m<sup>-2</sup>) was compared between cultivated and natural grassland areas and within groups (pest species vs other species), using bootstrap *t*-test analysis (10,000 repetitions) at 95% confidence interval, performed in the software BIOPSTAT 5.0 (Ayres et al., 2007). Data from cultivated and natural grassland areas of each location and all sampled sites was combined into a unique data set to compare similarities of species composition among locations. Cluster analysis based on Jaccard's similarity relationship (as it counts presence/absence) was plotted using the software PAST 3.0 (Hammer et al., 2001).



**Fig. 1.** Population density of white grubs (mean ± SE) in natural grassland and cultivated areas in the Brazilian Pampa. Pairs of columns with the same letters do not differ significantly by bootstrap *t*-test ( $p \leq 0.05$ ).



**Fig. 2.** Population density of white grubs pest and other species (mean  $\pm$  SE) in natural grassland areas in the Brazilian Pampa. Pairs of columns with the same letters do not differ significantly by bootstrap *t*-test ( $p \leq 0.05$ ).

## Results

### Population density of white grubs in cultivated and natural grassland areas

In total, 1365 specimens belonging to 31 species were collected in cultivated and natural grasslands areas of the Brazilian Pampa. Population density of white grubs in natural grassland areas was significantly greater than in cultivated areas in nine out of 18 sampled sites (Fig. 1). On average, larval density of all natural grassland areas was significantly greater than in cultivated areas ( $p < 0.05$ , 17.25 and 5.8 larvae  $m^{-2}$ , respectively), ranging from 57.25 to 1.28 (natural grassland) and 17.6 to 0.32 larvae  $m^{-2}$  (cultivated areas). The greatest number of white grubs in natural grasslands were observed in Restinga Seca (57.25 larvae  $m^{-2}$ ), Dom Pedrito (45.44 larvae  $m^{-2}$ ), and Cacequi (34.24 larvae  $m^{-2}$ ). The species composition was mainly by *Plectris griseovestita* Moser, *Plectris* sp.5, and *L. fusca*, respectively. São Borja and Arroio Grande were the only two localities where the mean white grub density was greater in cultivated than in natural grassland areas; however, it did not differ significantly ( $p > 0.05$ ). Uruguaiiana (17.6 larvae  $m^{-2}$ ), Itaquí (16.9 larvae  $m^{-2}$ ), and Pinheiro Machado (11.2 larvae  $m^{-2}$ ) had the greatest density of white grubs in cultivated areas.

### Population density of white grubs pest and other species

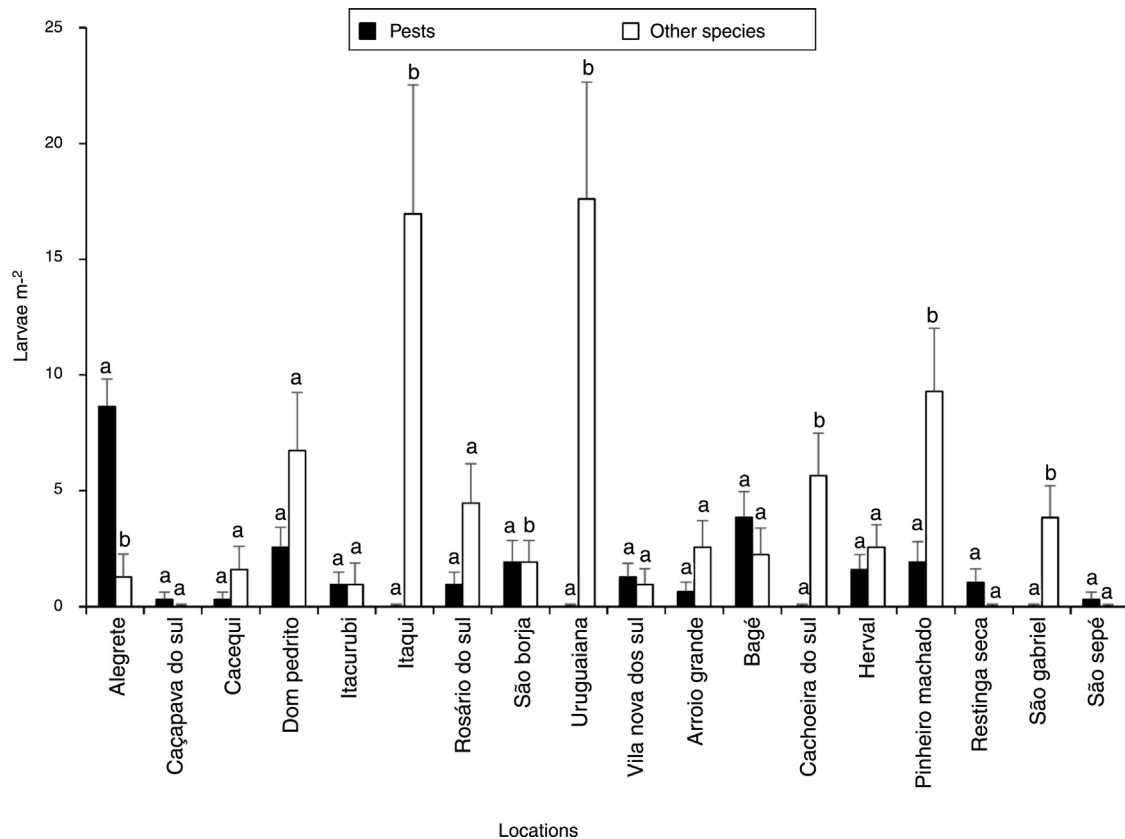
*D. abderus*, *E. humilis*, *L. fusca*, and *P. triticophaga* are among the species collected that have been reported damaging plants. In natural grasslands areas, the mean density of white grubs pest was

4.48 larvae  $m^{-2}$  (28.48 to zero), while in cultivated areas it was 1.46 larvae  $m^{-2}$  (8.64 to zero) (Figs. 2 and 3). Cacequi and Restinga Seca were the two locations with population of pest species over 10 larvae  $m^{-2}$  in natural grassland areas. The mean population density for other species (non-pest species) was 12.82 in natural grassland areas (45.44 to 0.32) and 4.36 larvae  $m^{-2}$  in cultivated areas (17.6 to zero). The population density of white grubs pest was significantly greater in Cacequi (natural grasslands) and in Alegrete under cultivated areas (Figs. 2 and 3). Great densities of *D. abderus* and *L. fusca* were observed in Cacequi (natural grassland area) and *D. abderus* in Restinga Seca (natural grassland area) and Alegrete (cultivated area) (Online resources 2 and 3).

### Occurrence and distribution of white grub pest and other species

*D. abderus* was found in 15 out of 18 locations sampled (five only in cultivated areas, three in natural grassland areas, and seven locations in both land use types). This species was followed by *E. humilis* that was found in four locations in natural grasslands and one in both land use types. *L. fusca* was found in one natural grasslands, one cultivated area, and two locations in both landscapes. For those considered as other species, *Cyclocephala modesta* Burmeister was found in 13, followed by *Dicrania* sp. (six locations), and *Leucothyreus* sp.1 and *P. griseovestita* (five locations). Eight species occurred in two locations and a group of eleven species was found only in one locality each (Fig. 4).

The clustering analysis using Jaccard index (Fig. 5) demonstrated a pattern of species distribution throughout Brazilian Pampa. The pattern of species distribution formed groups comprising habitats



**Fig. 3.** Population density of white grubs pest and other species (mean  $\pm$  SE) in cultivated areas of the Brazilian Pampa. Pairs of columns with the same letters do not differ significantly by bootstrap  $t$ -test ( $p \leq 0.05$ ).

according to the phytophysionomic units of the Brazilian Pampa. The phytophysionomic “Vegetação Parque” included the locations of Uruguaiana and Itaquí. The “Campos do Centro do Estado” included Rosário do Sul and Cacequi, and “Campos Barbas-de-bode” comprised the locations of Itacurubi, São Borja, and Alegrete. In addition, Vila Nova do Sul, Caçapava do Sul, São Sepé, and Restinga Seca were included in another group. Two groups were formed comprising habitats between the phytophysionomic units of “Campos Savanóides” and “Campos Solos Profundos” (Arroio Grande, Pinheiro Machado, Bagé, and Herval). Moreover, the location of São Gabriel that belongs to a transition zone and Dom Pedrito that belongs to “Campos Solos Profundos” did not cluster with any other group.

## Discussion

The overall mean density of pest species in cultivated areas was lower than the known economic threshold established for pest control. For *D. abderus* and *P. triticophaga* in winter cereals, the economic threshold is five white grubs m<sup>-2</sup> (Salvadori and Pereira, 2006). Density of pest species above this in cultivated areas was observed only in Alegrete, where *D. abderus* was predominant. The occurrence of high densities of white grubs in a cultivated area does not mean all larvae are of pest species, and when misidentified, it might lead to management mistakes (Cherman et al., 2014b). For this, site-specific monitoring of white grubs should be taken each growing season as a basis of an Integrated Pest Management (IPM). Moreover, the economic thresholds may change due to economic conditions (e.g. treatment cost, production value) and pest density (Pedigo et al., 1986), and it is prudent to consider before taking any management decisions.

We assessed population density and distribution of white grub pest and other species in the Brazilian Pampa. Four out of 31 species found in this study are considered as pest species; three of them (*D. abderus*, *E. humilis*, and *P. triticophaga*) had already been reported damaging crops in southern Brazil (Ferreira and Barrigossi, 2006; Salvadori and Pereira, 2006). *L. fusca* was included as a pest species due to its crop damage in the Central-West Region of Brazil (Santos et al., 2008a; Costa et al., 2009). *D. abderus*, *L. fusca*, and *P. triticophaga* also were reported in cultivated areas of the Planalto region of Rio Grande do Sul (Cherman et al., 2014b), and can be considered the most common pest species of cultivated areas in the state of Rio Grande do Sul.

Among the pest species, *D. abderus* was widely distributed throughout the Brazilian Pampa (15 out of 18 sampled locations) suggesting this species is well adapted to the environmental and land management uses in southern Brazil. Indeed, *D. abderus* is known to be present in cultivated and natural areas throughout Rio Grande do Sul State (Silva and Salvadori, 2004; Cherman et al., 2014a). Its presence and dispersion into cultivated areas has increased after implementation of no-tillage system because females fly to find places with crop residues to build nests and lay their eggs (Silva et al., 1994).

This study also reports the occurrence of *P. triticophaga* in the Brazilian Pampa. *P. triticophaga* was found only in Dom Pedrito (cultivated area) and Pinheiro Machado (natural grassland area). This species has a two-year life cycle and is found in both tillage and no-tillage fields (Salvadori and Pereira, 2006), and has a wide distribution in the north of Rio Grande do Sul (Salvadori and Pereira, 2006; Cherman et al., 2013). *P. triticophaga* and *D. abderus* are considered the most important white grubs pest of rainfed crop fields in South Brazil due to their damage potential to winter cereals, corn, and soybean (Salvadori and Pereira, 2006).

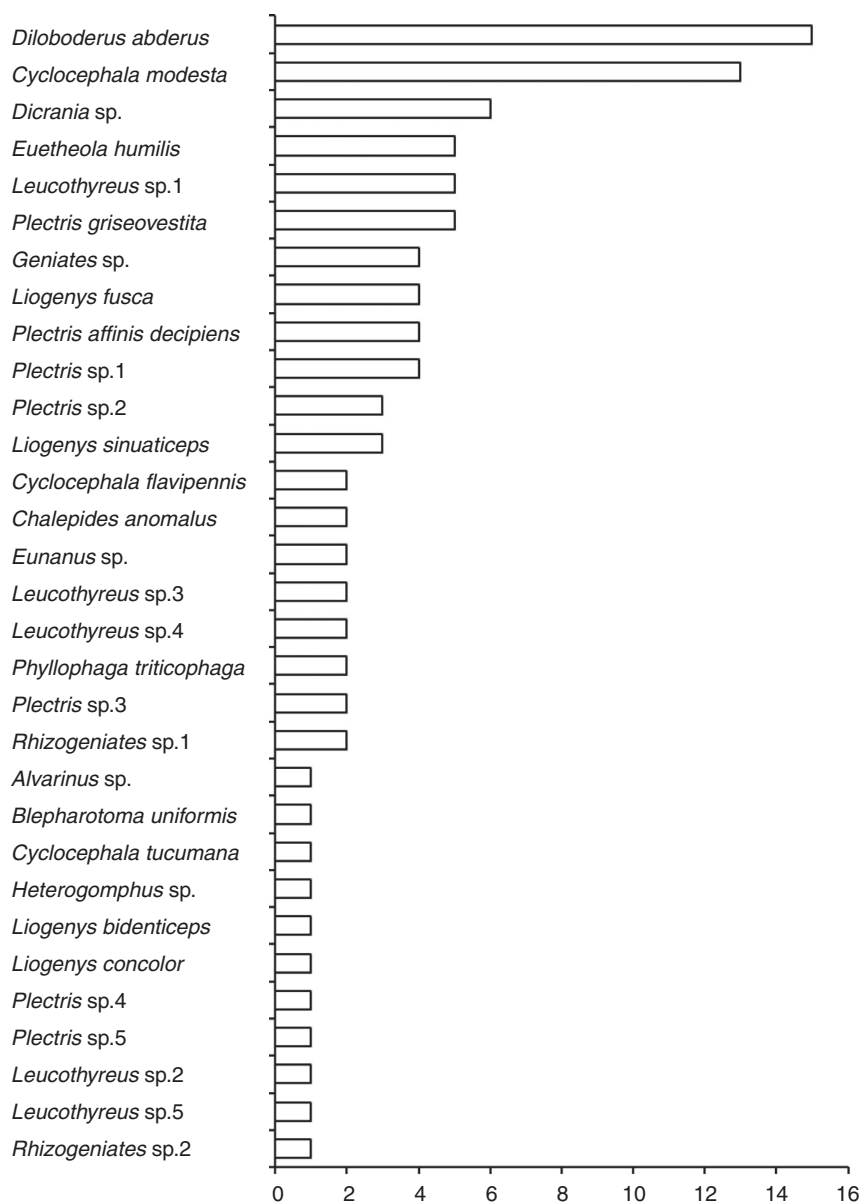


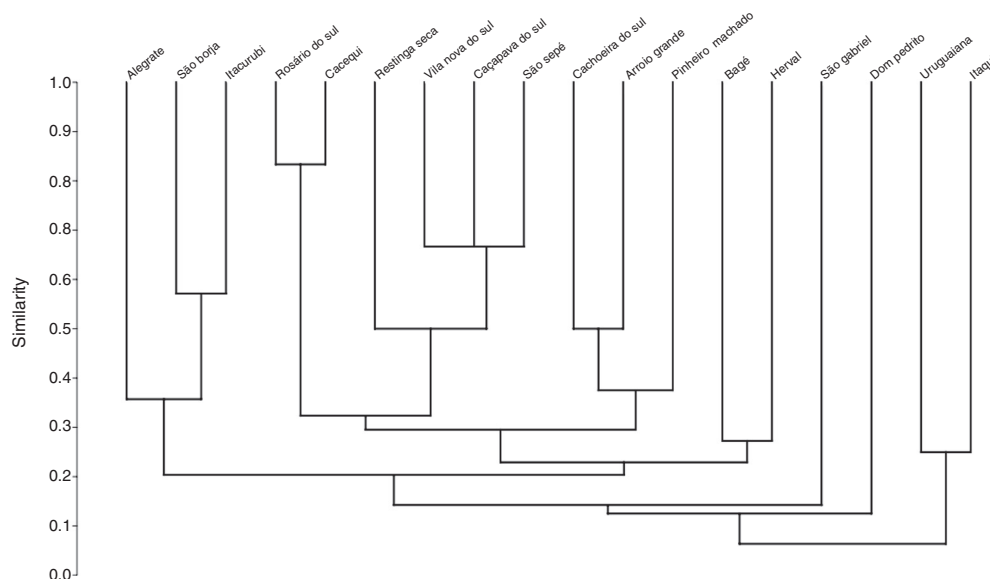
Fig. 4. Melolonthidae species and number of locations that each species was found.

In the group of species that are not considered pests, *C. modesta* was reported in 13 out of 18 locations sampled (nine in cultivated areas). This species is widely distributed and very abundant in both cultivated and non-cultivated areas throughout north of Rio Grande Sul (Cherman et al., 2014a). The occurrence of *C. modesta* in cultivated areas might be linked to the insertion of plants of the family Fabaceae (e.g. soybean) in crop rotation systems (Zerbino, 2002). To date, no injury on field crops was reported in Brazil; however, *C. modesta* was reported damaging improved natural pasture fields in Uruguay (Morelli and Alzugaray, 1990) and is associated to the white grubs complex of wheat in Argentina (Massaro, 2010). The Monitoring of *C. modesta* and other species that have not yet been reported as field crop pest, but belong to genus that contain pest species in Brazil (e.g. *Plectris* and *Liogenys*) is important to earlier identification of problems and to properly apply strategies of pest management.

Due to difficulties to sample and identify Melolonthidae at larval stage, farmers have adopted preventive control approaches to reduce population densities of white grubs in cultivated areas. Current management strategies to control white grubs in Brazil rely

on the use of synthetic insecticides delivered by seed treatment (Santos et al., 2008b), yet without knowing the accurate population density and white grubs species. The use of insecticides every growing season over large areas increase the chance of resistance development and disrupting of natural enemies (predators and parasitoids), which may increase the dependency on chemical control (Koppenhöfer and Fuzy, 2008).

The cluster analysis allowed us to detect patterns of species distribution linked to the phytophysionomic units of the Brazilian Pampa based on vegetation and soil composition proposed by Hasenack et al. (2010). For example, *Eunanus* sp. only occurred in the physiognomy “Vegetação Parque” that comprises the locations of Itaqui and Uruguiana, and *Chalepides anomalus* Martínez was present only in the “Campos do Centro do Estado” (Cacequi and Rosário do Sul). The assemblage of white grubs species differs in structure and abundance from one region to another, depending on the landscape, vegetation, and climate of different localities (Pardo-Locarno et al., 2003). Although these findings demonstrate that species occurrence might be associated to the phytophysionomic units of the Brazilian Pampa, sampling white grubs throughout the



**Fig. 5.** Dendrogram representing results of a cluster analysis of Melolonthidae species showing Jaccard index among the 18 locations sampled throughout the Brazilian Pampa.

year and using other sampling methods (e.g. light trap) is important to confirm the patterns observed.

Our results demonstrated that at most of cultivated areas, the population density of pest species did not exceed economic threshold for winter cereals. Furthermore, the relationship between the occurrence of white grubs in one year and its presence in the same or next year is tenuous because larval occurrence might be influenced by many biotic and abiotic factors. Due to many particularities regarding the white grubs biology, we highly encourage farmers and extension agronomists to scout fields before planting to take proper management decisions, follow economic thresholds for each pest species, and combine control tactics to have better outcomes. In conclusion, control decisions for white grub pests must follow the basis for IPM and threshold levels for each pest species, taking into consideration the relation between white grub density, crop, damage, and control costs.

### Conflicts of interest

The authors declare no conflicts of interest.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.rbe.2018.08.002](https://doi.org/10.1016/j.rbe.2018.08.002).

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