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Exploring the use of residues from the invasive *Acacia* sp. for weed control

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Abstract

A sustainable practice for weed control and crop protection is the incorporation of green manures with phytotoxic potential. It is gaining attention as a way to reduce the use of synthetic herbicides in agriculture and so pot experiments and field trials were conducted to explore the possible use of residues of *Acacia* species to alleviate weed emergence. We assessed, under greenhouse conditions, the herbicidal effect of phytotoxic manures from *Acacia dealbata* and *Acacia longifolia* applied to soil at different doses (1.5 and 3% w/w) on maize growth, some accompanying weeds, and the physiological profile of soil microbes. Applied at a higher dose, *A. dealbata* residues reduced the emergence of dicotyledons in the short-term ($P < 0.05$) and, after 30 days, there was a decrease in total weed emergence ($P < 0.005$) and a mild effect on weed composition, while total weed biomass remained unaffected. Regardless of the inclusion of *Acacia* residues, the physiological profile of the soil bacterial community did not show significant alterations. Additionally, we tested *A. dealbata* residues as a mulch or a green manure at the field scale. Although the effects of manures were site-dependent and affected monocot and dicot weeds differentially, dicots were more sensitive. The herbicide potential of acacia residues was only evident for dicots at sites with low-weed density in the seed bank. Nevertheless, due to the absence of phytotoxic effects on maize and minor modifications in the functional profile of bacterial communities, residues of acacia could be used as a complementary tool used together with other practices to reduce the reliance on synthetic herbicides in maize-based cropping systems.

Introduction

Organic farming is considered as one of the most promising and sustainable options for assuring the long-term stability of the four pillars of sustainability in agriculture: production, environment, economy and wellness (Reganold and Wachter, 2016). Intensive agriculture is currently driven by yield maximization and satisfaction of enormous demand from the increasing human population and is based on the use of large amounts of synthetic pesticides and fertilizers (Bhadoria, 2011; Rótolo et al., 2015). The continued use of synthetic herbicides in agriculture results in the emergence of herbicide-resistant weeds and leads to environmental pollution with impacts on human health and ecosystems (Dayan et al., 2009; Bhadoria, 2011). For these reasons, many agrochemicals used for decades have been banned (EC, 834/2007), and the use of synthetic herbicides is being reduced, i.e., it is one of the main objectives in the European Union agenda for agriculture (Integrated Pest Management). Nowadays, there is a demand for the development of alternative, integrated and sustainable farming practices based on new, easily degradable natural products and environmentally friendly methodologies that are, at the same time, inoffensive to agroecosystems and human populations (FAO, 1995; Swaminathan, 2006).

To adequately manage weeds, there is a growing need for new herbicides with safer toxicological and environmental profiles and new modes of action (Dayan and Duke, 2014). Thus, natural plant extracts, mulches and green manures that possess phytotoxic compounds can be directly used as eco-friendly tools for pest management (Xuan et al., 2005; Narwal, 2010; de Albuquerque et al., 2011; Tabaglio et al., 2013) and are appropriate to use in organic agriculture where synthetic pesticides are not allowed (EC, 834/2007). Allelopathic cover crops or mulches are also being increasingly used in conventional agriculture to reduce the inputs of synthetic herbicides (Caamal-Maldonado et al., 2001; Dhima et al., 2009). For instance, cultivable legumes such as *Vicia faba* were found to control several broadleaved and grass weeds with a notable fertilizing effect on crops (Álvarez-Iglesias 2016; Álvarez-Iglesias et al., under review). Similarly, Tabaglio et al. (2013) incorporated rye (*Secale cereale*) as a cover crop for integrated weed management with significant results, reducing the germination and seedling growth of some broadleaf weeds, mainly *Amaranthus retroflexus* and *Portulaca oleracea*. The structural diversity and evolved biological activities of natural compounds from plants offer new complementary opportunities to control resistant weeds (Dayan et al., 2009; Dayan and Duke, 2014).

Another environmental concern related to sustainable agroecosystems is the increasing pressure of invasive species (Vilá et al., 2004, 2011). Exotic plants cultivated out of their native ranges can further become invaders producing damage on various levels (Weidenhamer and Callaway, 2010; Vilá et al., 2011), e.g. affecting ecosystem services (Le Maitre et al., 2011; EC, 2014), colonizing agricultural lands and reducing crop yield (Vilá et al., 2004; Early et al., 2016), and provoking biodiversity loss (Simberloff et al., 2013; Parker et al., 2013). Within the strategies responsible for the invasiveness of exotic plant species, an important role is played by allelopathy (i.e., the release of allelopathic compounds that interfere with the normal performance of surrounding species) (Wolfe et al., 2008; Thorpe et al., 2009; Lorenzo et al., 2011, 2013). Furthermore, invasive plants usually produce a great amount of biomass, such as *Ailanthus altissima*, so that some of them are increasingly viewed as biomass sources (Annighöfer et al., 2012; Kurokochi and Toyama, 2015). The management of invasive plants has become a worldwide priority in recent years and costs billions of dollars every year (van Wilgen et al., 2016), which means the assessment of economic investment in invasive alien plant management is currently gaining importance (Sims et al., 2016).

In this study, we bring together both concerns (i.e. the reduction in synthetic herbicide use and the need for sustainable management of invasive plants) by evaluating the bioherbicidal potential of manures from two invasive allelopathic species: *Acacia dealbata* Link and *Acacia longifolia* (Andrews) Willd, with the aim of appraising their use in sustainable maize cropping systems. Novel uses for phytotoxic residues from the management of invasive plants are proposed to reduce agriculturally derived problems as a cost-effective alternative to controlling their spread, as a part of operationally integrated agroecosystems and biomass recycling. Previous works reported the feasibility of the use of *Eucalyptus globulus* plant material as a phytotoxic green manure for weed control (Puig et al., 2013; Puig et al., under review). Here, we selected exotic species from the *Acacia* genus because it is widely considered a highly invasive group that transforms forest and agricultural systems worldwide (Le Maitre et al., 2011; Richardson and Rejmanek, 2011; Lorenzo and Rodríguez-Echeverría, 2015). Within this genus, *A. dealbata* and *A. longifolia* produce severe impacts on invaded ecosystems (Marchante et al., 2008; Rodríguez-Echeverría et al., 2009; Fuentes-Ramírez et al., 2010; Lorenzo et al., 2010b; Lazzaro et al., 2014; Souza-Alonso et al., 2015). Moreover, there is evidence for allelopathic effects from both species. *Acacia dealbata* releases phytotoxic compounds into the surrounding environment that affect the growth of neighbouring plants (Lorenzo et al., 2011; Aguilera et al., 2015), whereas *A. longifolia* has the potential to inhibit chemically the establishment of co-evolved plants (Ens et al., 2009) and its volatile compounds released from flowers and leaves have shown phytotoxic character (Souza-Alonso et al., 2018). Consequently, the phytotoxic activity exhibited justifies the inclusion of *A. dealbata* and *A. longifolia* in our assay, in the search for potential uses as natural herbicides.

Soil quality and properties greatly depend on established soil microbial communities given that microbes play a key role in nutrient dynamics (Berg and McClaugherty, 2008). Because of its rapid response compared with other soil parameters, the physiological activity of the microbial community can be considered a good early indicator of soil biological changes (Masciandaro et al., 2004). In this sense, external inputs based on green manures can produce substantial changes in soil microbial activity (Masciandaro et al., 2004, Tejada et al., 2008). This fact is particularly relevant when dealing with allelopathic plants as they can negatively affect soil microbes (Inderjit et al., 2011). In the *Acacia* case, soil enzymatic activities are altered in invaded areas (Marchante et al., 2008; Souza-Alonso et al., 2015) and functional diversity of soil bacteria was modified after being watered for 1 month with chemicals naturally released by *A. dealbata* (Lorenzo et al., 2013). Nevertheless, the phytotoxic effect of *Acacia* residues on microbial communities from agricultural soils has so far not been properly addressed.

With these premises, the hypothesis here is that the phytotoxic activity of plant material obtained from *A. dealbata* and *A. longifolia* can be used to prevent the germination and the establishment of weeds in maize crops. A further hypothesis is that these phytotoxic green manures may alter the functional diversity of soil bacteria. To test the hypotheses, we aimed to (i) evaluate *under greenhouse conditions* the phytotoxic activity of *A. dealbata* and *A. longifolia* manures incorporated into the soil as green manure on the germination and early growth of maize and several highly problematic weeds, and also on the physiological profile of soil microbial communities and, (ii) assess the temporal herbicidal activity of *A. dealbata* as a mulch and green manure to reduce the establishment of spontaneous weeds in maize fields *at field scale*.

Material and methods

Greenhouse experiment: Evaluation of the phytotoxic activity of Acacia manures on the early growth of weeds and maize and functional diversity of soil microbes

Soil and plant material collection

During May 2015, agricultural soil (A_p horizon) was collected from an agricultural field located in Tui, NW Spain (42° 06′21.05″N, 8°39′10.84W). After sieving (2 mm), 4 aliquots of fresh soil (10 g each) were submitted to the physiological characterization of soil microbial community. Plant material (leaves and fine branches) from *A. dealbata* was collected in Tui (Pontevedra, Spain; 42°06′06.3″N, 8°39′29.8″W) whereas material from *A. longifolia* was collected in *Mata do Camarido* (Moledo, NW Portugal; 41°51′11.7″N, 8°51′55.9″W).

Experimental setup

According to the method used by Puig et al. (2013), *A. dealbata* and *A. longifolia* fresh leaves and small branches were collected in the above-mentioned populations, slashed in 1–2 cm pieces and tested at two different doses in soil: 3 and 1.5% (w/w) on a dry soil mass basis, which correspond to 40 and 80 g of fresh plant material per four-liter pot, respectively. Each pot was supplemented with Patent PK (K + S KALI GmbH, Kassel, Germany; P_2O_5 12%, K_2O 15%, MgO 5%) at a dose of 800 kg ha^{-1}, and Lithothamne 400 (Timac Agro, Orcoyen, Spain; MgO 2.5%, CaO 36%) at 3000 kg ha^{-1}, as a basal dressing for maize. We assayed a total of four treatments, named AD3 and AD1.5 (*A. dealbata* at 3 and 1.5%, respectively), in addition to AL3 and AL1.5 (*A. longifolia* at 3 and 1.5%, respectively). There were also adequate controls without acacia plant material. In this case, drinking straw pieces (1 cm) were added to control pots to mimic the padding effect of the same volume of acacia leaves incorporated into the soil (Wuest et al., 2000). Each treatment was replicated four times. Pots were watered to maximum water retention capacity and then weighed. Then, five seeds of maize (*Zea mays* L. cv. Anjou) per pot were sown equidistant at 2 cm depth, and a mixture of 4 monocot and dicot weeds (a total of 24 mg) composed of redroot pigweed (*Amaranthus retroflexus* L.), common purslane (*Portulaca oleracea* L.), black nightshade (*Solanum nigrum* L.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] were seeded on the surface and covered with a thin soil layer. Proportions were based on Dhima et al. (2009), simulating seed bank densities of small seeded weeds in infested corn fields. Additionally, five seeds of field bindweed (*Convolvulus arvensis* L.) were sown at 2 cm depth. Seeds of weeds were purchased from Herbiseed© (Twyford, England, UK RG10 0NJ). Pots were maintained during 30 days under greenhouse conditions (daylight regime, $T \leq 26$°C). Every 2 days, pots were weighed and water loss by evapotranspiration (ET) was replaced.

Harvest and plant measurements

Pots were examined for maize and weed emergence every day during the first 10 days until the identification of seedlings was unfeasible in control pots. During this time, weed seedlings were classified in monocot and dicot weeds. After 30 days of growth, the final number of emerged weeds was counted and then harvested at soil level, identified and separated into different species. After that, plants belonging to different species were placed in paper bags and dried at 50°C for 3 consecutive days to measure aerial dry biomass (g dry weight). Maize plants were carefully removed from the soil and separated into shoots and roots. Shoots were measured and specific leaf area (SLA, $m^2\ kg^{-1}$) of V3 was assessed. Roots were gently washed and dried to remove adhered soil particles and then measured. Both roots and shoots were dried and weighed as described for weeds. Soil pH (1:2.5 H_2O), humidity and total ET were determined at the end of the experiment according to Puig et al. (2013).

Soil community level physiological profile (CLPP)

After harvest, 5 g of fresh soil from each pot was diluted with 25 mL of NaCl (0.85%) in Falcon tubes (50 mL) and vortexed for 2 min. Suspensions were then settled for 3 min and further diluted to 10^{-3} to achieve a readable concentration of soil microorganisms (Souza-Alonso and Guisande-Collazo, unpublished data). The assessment of the physiological profile of the bacterial community was carried out using BIOLOG Ecoplate™ (Biolog Inc., Hayward, CA) 96-well plates, containing 31 C-sources. Each well of the Biolog Ecoplates was inoculated with 150 μL of soil suspension. In each microplate, microbial response based on C-substrate consumption that expressed average well-color development (AWCD) was calculated following the equation $AWCD = \sum(C-R)/n_i$, where C is the color production of each well, R is the absorbance value of the control well to correct for background color and n_i is the number of substrates (Garland and Mills, 1991). The evolution of the AWCD value was recorded daily for 8 days. Relative rates of color production among samples were compared based on similar AWCD values following Garland (1996). Following these criteria, comparisons should preferably be made on the basis of reference points between 0.7 and 1 units of absorbance. In this case, to allow comparisons the selected AWCD values were between 0.85 and 0.90 abs units for each treatment. Wells that had negative values were set to zero for the analyses. Substrate richness, diversity and evenness were calculated as described in Zak et al. (1994).

Field experiment: assessment of temporal phytotoxicity of A. dealbata mulch and green manure for weed establishment

The field trial was designed after Álvarez-Iglesias (2016) to evaluate whether green manures of *A. dealbata* have herbicidal activity immediately after application (hereafter called short-term herbicidal activity) or during the decomposition of residues (hereafter called long-term herbicidal activity) on the emergence of spontaneous weeds in agricultural fields. The experiment was conducted at three agricultural sites named *Pesegueiro*, *Centieira* and *Xesta* in Tui, NW Spain (42°06′21.05″N, 8°39′10.84W) from late April to early October 2015. In this area, *A. dealbata* patches usually surround agricultural fields. Fresh leaves and thin young branches (up to 2 cm diameter) of *A. dealbata* were collected in April 2015 in Tui, NW Spain (40°06′25.33″N 8° 39′35.71″W), characterized by European Atlantic climate with an average annual temperature and total precipitation of 14°C and 1930 mm, respectively (Carballeira et al., 1983). Experimental sites were located at least 200 m apart from each other. Soil properties for each site are shown in Table 1. These fields were traditionally dedicated to maize production and had not been treated with herbicides over the previous 3 years. During the experiment, agricultural sites were left fallow, but they had a seed bank enriched by accompanying weeds of maize crops.

Before setting up the experiment, soils at each site were ploughed and then earth milled. Treatments consisted of chopped residues of fresh *A. dealbata* assayed at a similar dose to the greenhouse experiment, i.e., at 28 Mg fresh weight ha^{-1} in two different forms: directly placed on the soil surface as mulch (AM) or slashed residues incorporated into the first 10 cm-top soil layer as green manure (AGM). Additional controls without mulch or green manure were also established (C). Treatments were randomly established in 1.5 × 1.5 m plots separated 1 m from each other and replicated five times at each experimental site. The dose of 28 Mg ha^{-1} was defined by considering the future practical feasibility of the approach. Allelopathic mulches are generally applied using dry residues. However, *A. dealbata* is a woody leguminous-evergreen tree, the leaves being the most phytotoxic part (Lorenzo et al., 2016). To facilitate further collection and use of acacia residues by farmers, we decided to use fresh material directly including leaves and small woody branches (up to 2 cm diameter), which helped achieve the dose of 28 Mg ha^{-1}. However, this dose is the equivalent to 8.9 Mg dry weight ha^{-1} (Lorenzo et al., unpublished data), which is similar to doses of allelopathic mulches or manures (8–15 Mg ha^{-1}) commonly used for weed control (Kandhro et al., 2015; Abbas et al., 2017; Farooq et al., 2017). The dose of 28 Mg ha^{-1} represented the minimum quantity of plant material needed to cover the soil without it being a thick layer (2–3 cm thick) that physically prevented the emergence of seedlings. In addition, fresh acacia residues also release chemical volatiles with phytotoxic potential (Souza-Alonso et al., 2014a), that could be lost during the drying process.

To evaluate the short-term herbicidal activity of *A. dealbata* manures, spontaneous weeds from the soil seed bank were left to grow for 6 weeks. After this time, the aerial biomass of weeds was harvested at soil level from three random 20 × 20 cm^2 squares within each plot. Once in the laboratory, weeds were identified at species level when possible, otherwise classified at the family level. Weeds were classified into monocot and dicot weeds and biomass of each group was determined after drying at 60°C until constant weight. The long-term phytotoxic activity of *A. dealbata* treatments was similarly assessed after 20 weeks.

Table 1. Chemical characteristics of soils from the three agricultural sites and limiting factors for plant production

Soil properties		Pesegueiro	Centieira	Xesta
pH (1:2.5, H_2O)		5.0	4.8	4.7
Organic matter (%)		10.8	7.5	8.3
C total (%)		4.82	4.10	3.60
N total (%)		0.41	0.32	0.29
P Available (ppm)		36	12	20
K^+ Assimilable (ppm)		362	114	86
Ca^{2+} exc. ($cmol_{(+)}\ kg^{-1}$)		2.40	1.30	1.00
Mg^{2+} exc. ($cmol_{(+)}\ kg^{-1}$)		0.66	0.35	0.22
Na^+ exc. ($cmol_{(+)}\ kg^{-1}$)		0.10	0.14	0.14
Limiting factor	Limitation			
OM	Organic matter content <1.5%	N.L.	N.L.	N.L.
N	Total N content <0.1%	N.L.	N.L.	N.L.
P	$P < 18$ mg kg^{-1}	N.L.	L.	N.L.
mg1	<0.4cmol$_{(+)}$ kg^{-1} in CEC	N.L.	L.	L.
ca1	<1.5 cmol$_{(+)}$ kg^{-1} in CEC	N.L.	L	L.
ca2	Ca/Mg<0.5	N.L.	N.L.	N.L.

Exc., exchangeable; L, limiting factor; N.L., not limiting factor.

Statistical analyses

Data were tested for normality using the Kolmogorov–Smirnov test and Levene's test for homogeneity of variances. The mean values of each soil parameter after harvest, besides maize growth, weed emergence and composition in the different treatments, were statistically compared using one-way ANOVA with Dunnet T3 test or Tukey ($P \leq 0.05$) for post hoc multiple comparisons. To analyze the soil physiological profile, correspondence analysis (CA) was performed on normalized data for each well. Data from the field experiment were analyzed by using linear mixed models (LMMs) via restricted maximum likelihood (REML) to test whether *A. dealbata* treatments (AM, AGM, C) had an effect on weed variables for each short- and long-term assay at each experimental site. Data were analyzed considering sampling quadrats nested within plots as variance components (random factors) and *A. dealbata* treatments as an explanatory variable (fixed factor). Differences between least-squares means were tested pairwise with the Tukey method when the effect of treatment was significant. LMMs and LSMEANS were conducted using the 'nlme' and 'lsmeans' packages, respectively, in R. The level of significance was set at $P \leq 0.05$ for all of the analyses. All statistical analyses were carried out using R version 3.2.2 (The R Foundation for Statistical Computing Platform).

Results

Greenhouse experiment

Weed emergence

Only the treatment with *A. dealbata* at 3% tended to inhibit or delay the emergence of dicot weeds during the first 10 days (Fig. 1). The other treatments did not produce any effect or slightly promoted the germination of weeds. In the case of monocot weeds, the increase in germination was statistically significant for AD3 at day 10 ($P < 0.05$), if compared with the control. For dicot weeds, AL3 significantly enhanced their presence in comparison with *A. dealbata* treatments at day 6 ($P < 0.05$); however, no differences were found with respect to control pots.

Weeds and maize growth, and soil parameters

Thirty days after green manure incorporation, AD3 significantly reduced the quantity of total emerged weeds, notably *P. oleracea,* whereas AL1.5 improved the establishment of *A. retroflexus* (Table 2). In terms of total weed biomass (kg of dry matter ha^{-1}) there was no significant effect from *Acacia* treatments; nevertheless, AD3 significantly modified the percentage of each species' contribution to the total biomass (Fig. 2). AD3 increased the relative contribution of *D. sanguinalis* but reduced the proportion of *C. arvensis, P. oleracea* and *A. retroflexus* (Fig. 2). Maize emergence was not affected by any treatment; however, leaf length measured at the end of the experiment was significantly reduced by the AL1.5 treatment. The remaining morphological parameters (root length, SLA, R/S ratio, leaf and root biomass, and total biomass) were not affected (Table 2). Soil pH was significantly increased ($P < 0.001$) in all cases by *Acacia* manures (Table 2), whereas no effect was observed on humidity and total ET.

Soil functional profile

The ordination plot of the CA showed a trend of separation between different treatments (Fig. 3), indicating a different C-substrate consumption. On the one hand, fresh soils and potted soils appeared distantly situated along dimension 1, which explained more than 40% of the variance. Some C-compounds seemed to be highly related to fresh soils (glycogen, cellobiose, erythritol, N-acetyl-D-glucosamine, L-asparagineand L-threonine) but separated from control soils and those with *Acacia* manures. Treatment position in this first dimension is homogeneously related to several components, with a spatial distribution mainly explained by the amino acid L-arginine (11.37%) the polyamine

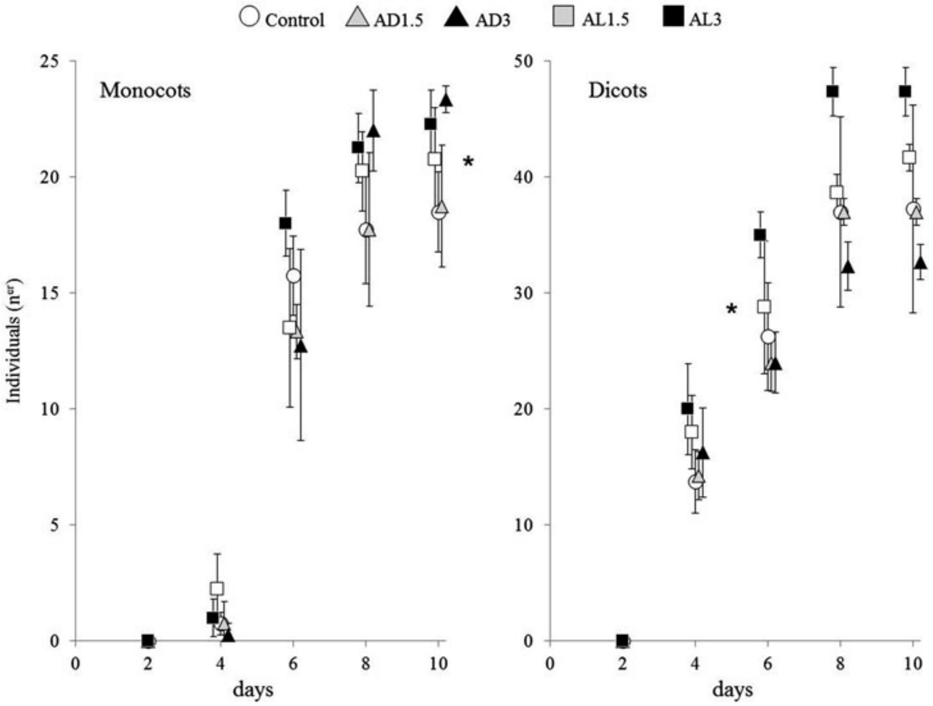

Fig. 1. Number of individuals of monocot (*left chart*) and dicot weeds (*right chart*) observed at 2, 4, 6, 8 and 10 days after sowing. Asterisks indicate significance at a significant level ($P \leq 0.05$) in ANOVA. Bars indicate standard error (SE).

Table 2. Effects of different proportions of *A. dealbata* and *A. longifolia* on maize growth and weed emergence after harvest in the greenhouse experiment

Parameters	Control	AD1.5	AD3	AL1.5	AL3	$F_{(4,15)}$	P
Soil pH	4.37 ± 0.05^{a}	4.51 ± 0.02^{b}	4.54 ± 0.05^{b}	4.51 ± 0.02^{b}	4.58 ± 0.03^{b}	21.27	***
Soil humidity (%)	26.14 ± 0.47	26.57 ± 1.44	26.93 ± 0.77	26.29 ± 1.78	26.06 ± 1.06	0.353	n.s.
Total ET (mL)	3649.9 ± 319.46	3864.45 ± 139.59	3888.5 ± 161.63	3719.87 ± 144.86	3661.22 ± 172.47	1.282	n.s.
Maize							
Leaf length (cm)	78.22 ± 4.83^{a}	77.51 ± 3.71^{a}	75.56 ± 2.33^{a}	67.05 ± 3.21^{b}	71.04 ± 2.32^{ab}	7.701	**
Root length (cm)	85.42 ± 15.85	74.9 ± 12.70	73.48 ± 12.63	69.46 ± 1.77	70.19 ± 3.08	1.405	n.s.
R/S ratio	1.09 ± 0.18	0.96 ± 0.13	0.97 ± 0.16	1.04 ± 0.07	0.99 ± 0.05	0.679	n.s.
SLA (cm^2 g^{-1})	395.43 ± 25.38	445.05 ± 24.6	409.42 ± 22.54	420.95 ± 18.31	406.49 ± 17.66	0.740	n.s
Biomass (g)	1.44 ± 0.62	1.69 ± 0.24	1.6 ± 0.27	1.17 ± 0.13	1.37 ± 0.27	1.337	n.s.
Leaf biomass (g)	1.02 ± 0.45	1.17 ± 0.17	1.02 ± 0.17	0.77 ± 0.11	0.91 ± 0.19	1.443	n.s.
Root biomass (g)	0.42 ± 0.18	0.89 ± 0.73	0.92 ± 0.75	0.40 ± 0.03	0.46 ± 0.10	1.224	n.s.
Weeds per pot							
Total weeds (*n*)	85.33 ± 10.21^{b}	78.0 ± 5.22^{ab}	63.25 ± 9.36^{a}	96.25 ± 11.58^{b}	69.66 ± 7.50^{ab}	3.484	**
Digitaria sanguinalis (*n*)	17.25 ± 7.41	20.5 ± 2.38	23 ± 1.15	22.25 ± 4.86	21.75 ± 4.27	0.346	n.s.
Solanum nigrum (*n*)	0	0.5 ± 0.58	0.25 ± 0.5	0	0.25 ± 0.5	1.050	n.s.
Amaranthus retroflexus (*n*)	20.67 ± 6.03^{ab}	23 ± 4.55^{ab}	17.75 ± 4.92^{b}	29.25 ± 1.71^{a}	24 ± 4.16^{ab}	3.743	*
Portulaca oleracea (*n*)	18.67 ± 5.51^{a}	9 ± 2.58^{ab}	2.33 ± 1.53^{b}	7.33 ± 3.51^{ab}	9.25 ± 7.80^{ab}	4.312	*
Convolvulus arvensis (*n*)	3.33 ± 0.58	2.75 ± 0.96	3.25 ± 0.5	2.5 ± 0.58	3.5 ± 0.55	1.578	n.s.
Other monocots (*n*)	5 ± 4.24	7.25 ± 5.68	2.67 ± 0.58	8 ± 1.01	6.5 ± 2.38	1.078	n.s.
Other dicots (*n*)	16.33 ± 5.03^{ab}	18 ± 4.36^{ab}	9 ± 1.73^{b}	21.25 ± 3.86^{a}	12.67 ± 1.15^{ab}	5.852	*
Total biomass (g)	3.20 ± 1.48	3.20 ± 0.46	4.20 ± 1.11	3.22 ± 0.55	3.33 ± 0.91	0.790	n.s.

Within each column, mean values of different parameters (n = 4, ± SD) measured after harvest. For each variable, different letters indicate significant differences between treatments according to ANOVA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) and using Tukeys´s HSD as the post hoc test for multiple comparisons. AD1.5 = *A. dealbata* 1.5%, AD3 = *A. dealbata* 3%, AL1.5 = *A. longifolia* 1.5%, AL3 = *A. longifolia* 3%, R/S ratio = root/shoot ratio, SLA = specific leaf area, Total ET = total Evapotranspiration, n = number.

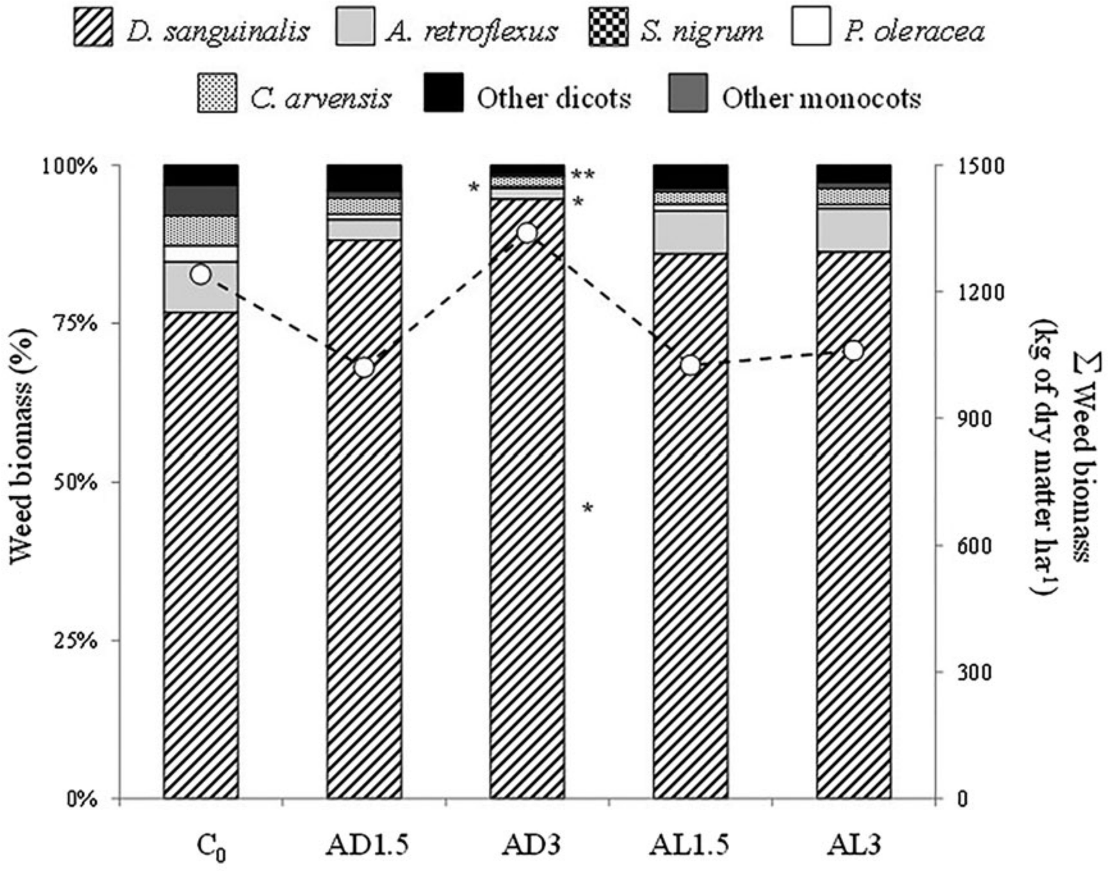

Fig. 2. Biomass proportion of each weed group with respect to the total content found in pots after the addition of *Acacia* residues (100%, bars, left axis) and total weed biomass expressed by hectare (white circles, right axis). Asterisks indicate significant differences with respect to the control for the biomass proportion (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). C, control, AD1.5 = *A. dealbata* 40 g, AD3 = *A. dealbata* 80 g, AL1.5 = *A. longifolia* 40 g, AL3 = *A. longifolia* 80 g.

Putrescine (8.25%) but mostly by carbohydrates (Lactose, 9.95%; Glycerol-P, 9.84%; N-acetyl-D-glucosamine, 9.24%; β-methyl-D-glucoside, 8.07%). When differences across dimension 2 (25% of the variance) are explored, AD treatments (both AD1.5 and AD3) appear distanced from the non-treated soils (control and fresh). Dimension 2 is relatively well explained by three compounds of different origin: polymer (Glycogen, 12.16%), carbohydrates (Erythritol, 22.41%) and carboxylic acids (Itaconic acid, 26.34%). Additionally, control, fresh and AL3 soils appear positioned together and separated from other treatments. Complementary to this, the presence of *A. dealbata* and *A. longifolia* material did not significantly affect the richness, diversity or evenness of C-substrate consumption (data not shown).

Field experiment

From the soil characterization at the beginning of the experiment (Table 2), field site Centieira evidenced the soil limiting factors *P*, *mg1* and *ca1*, and Xesta *mg1* and *ca1*.

Linear mixed models indicate that monocot, dicot and total weeds showed a different trend in biomass depending on field site. The effects of *A. dealbata* treatments on weed biomass also had contrasting magnitudes over time. Furthermore, spontaneous weeds were not differentially distributed among treatments but the composition and abundance differed among experimental fields (Table 3). Pesegueiro presented noteworthy high infestation levels (from 2500 to 4000 kg dw ha^{-1}, in the short- and long-term, respectively) if compared with Centieira and Xesta, which showed manageable weed densities from an agronomical point of view (from 150 to 1000 kg dw ha^{-1}, in the short- and long-term, respectively).

At Pesegueiro site, AM significantly reduced the biomass of monocot and total weeds in the short-term ($P \leq 0.05$), but increased the biomass of dicot weeds in the long-term ($P < 0.001$), thus diluting the weeding effects on the total biomass (Fig. 4a,b). This field site suffered a shift in weed population, being dominated by dicotyledons in the short-term (*Chenopodium album*, *Polygonum persicaria*, *Raphanus raphanistrum* and *Stellaria media*, Table 3) and then by monocots in the long-term, mainly the aggressive *D. sanguinalis* and *E. crus-galli* (Table 3).

At Centieira, biomass of monocot weeds was enhanced by the AM treatment in the short-term ($P \leq 0.05$, Fig. 4c) but it is worth noting that dicots were significantly reduced by both AGM and AM ($P < 0.001$, Fig. 4c). In the long-term, the biomass of the dominant dicot weeds (*Coleostephus myconis, Medicago arabiga, Mentha suaveolens, Plantago lanceolata, R. raphanistrum* and an unidentified *Asteraceae*; Table 3) and consequently total weed biomass suffered very significant inhibition by AM ($P \leq 0.01$, Fig. 4d). The abundance of monocot weed species also declined in the long-term.

Finally, at Xesta the AM treatment promoted monocot and total weed biomass in the short-term ($P \leq 0.05$, Fig. 4e), with no significant effects in the long-term (Fig. 4f). *Corrigiola litoralis, Polygonum persicaria, Medicago arabiga, Mentha suaveolens, Polygonum* sp., *Calistegia* sp. and an unidentified *Asteraceae*

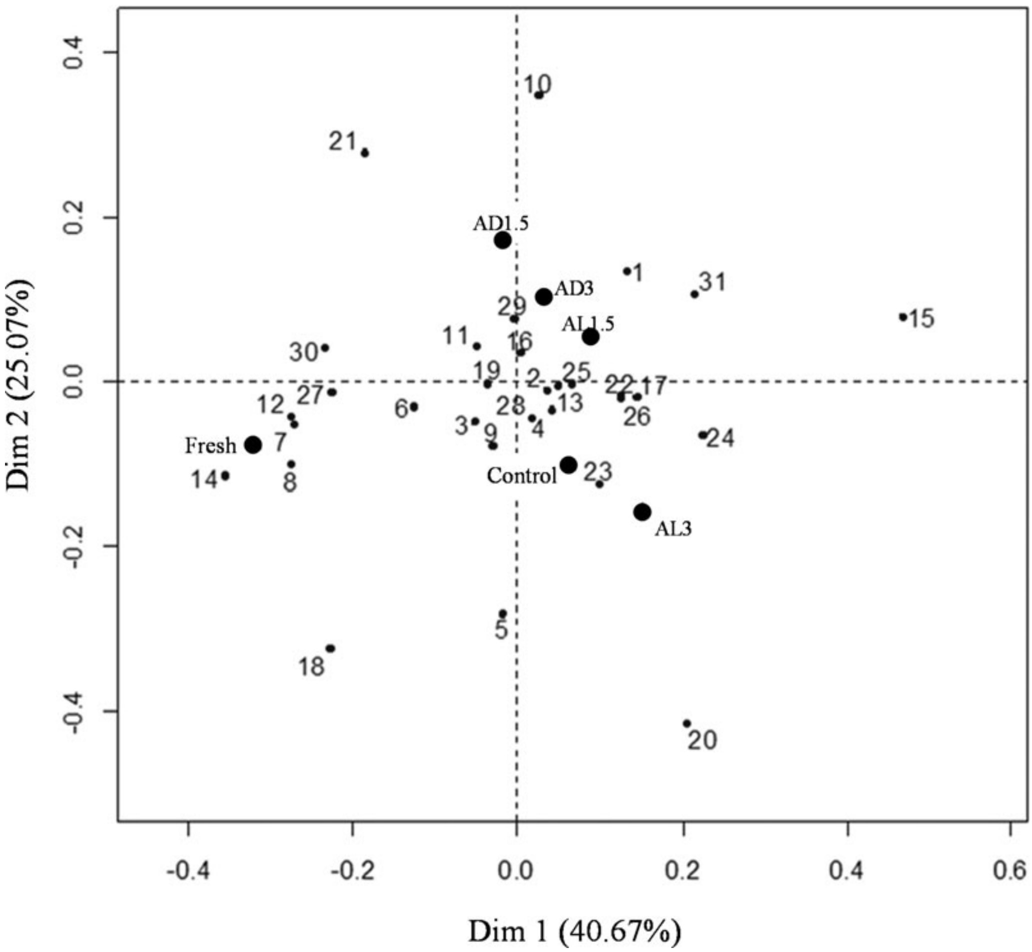

Fig. 3. Two-dimensional plot obtained from correspondence analysis (CA) of C substrate utilization patterns. Carbon-substrates included in Biolog Ecoplates are divided in six classes: carbohydrates(a), carboxylic acids(b), amino acids(c), polymers(d), amines/amides(e) and phenolic compounds(f). From 1 to 31 are: 1.Pyruvic acid(b), 2.Tween 40(d), 3.Tween 80(d), 4.α-cyclodextrin(d), 5.Glycogen(d), 6.Cellobiose(a), 7.Lactose(a), 8.β-methyl-D-glucoside(a), 9.Xylose(a), 10.Erythritol(a), 11.Manitol(a), 12.N-acetyl-D-glucosamine(a), 13.D-glucosaminic acid(b), 14.Glucose(a), 15.D, L-α-Glicerol-P(a), 16.D-Galactonic-γ-Lactone(b), 17.D-Galacturonic acid(b), 18.2-Hydroxybenzoic acid(f), 19.4-Hydroxybenzoic acid(f), 20.α-hydroxybutyric acid(b), 21.Itaconic acid(b), 22.α-ketobutyric acid(b), 23.L-malic acid(b), 24.L-arginine(c), 25.L-asparagine(c), 26.L-phenylalanine(c), 27.L-serine(c), 28.L-threonine(c), 29.L-glutamic acid(c), 30.Phenyletilamine(e), 31.Putrescine(e).

dominated in the short-term, also accompanied by *Plantago lanceolata, Fumaria* sp. and *Geranium* sp. in the long-term. Monocot abundance also declined with treatments in the long-term, towards a less diverse community (Table 3) and the presence of *Cynodon dactylon* and *Dactylis glomerata* dramatically decreased when associated with acacia treatments.

Discussion

To the best of our knowledge, this is the first time that fresh residues from *Acacia* have been tested for agronomic purposes, specifically for weed control. Results from our pot experiment evidenced that green manures from *A. dealbata* applied at 3% in soil were able to reduce the emergence of some dicotyledon weeds significantly. Significant weed reduction at field scale was only conspicuous for the dicot fraction at Centieira, where the seed bank was dominated by dicots. Additionally, AD3 treatment applied in pots tended to change the weed composition, enhancing the dominance of the monocot *D. sanguinalis*. The same trend was observed at those experimental sites where weed amounts were discrete (Centieira and Xesta) but not in the infested field (Pesegueiro), indicating that the weeding effects of acacia were site-dependent. In fact, the most drastic shifts in the monocot-dicot balances were observed in Pesegueiro, where the competence of the aggressive monocots is prone to be more intense due to resource limitation under carrying capacity (Taylor et al., 1990). Attending to soil fertility, Pesegueiro presented no limiting factors for plant productivity, producing the highest levels of infestation and weed biomass and, thus, hindering weed control.

The apparently higher phytotoxic effects observed on dicot weeds could be related to their prompt germination, as observed in pots and at Centieira site (with manageable weed densities), probably due to an early exposure to the chemical compounds released by acacia residues. Kobayashi (2004) and Xuan et al. (2005) stated that the highest phytotoxic activities are usually observed during the first days immediately after green manures incorporation into the soil, and then they progressively decrease their effectiveness for weed control. Hence, the later germination of monocot weeds possibly allowed them to escape from this early effect and become highly aggressive and abundant. In addition, weeds usually show differential sensitivity to applied compounds from allelopathic plants conditioning plant response (Xuan et al., 2005).

Table 3. Distribution and abundance of spontaneous weeds found in all treatment in the three agricultural sites

	Short-term									Long-term								
	Pesegueiro			Centieira			Xesta			Pesegueiro			Centieira			Xesta		
	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM
Dicotyledons																		
Amaranthus retroflexus L.										+					+			
Anthemis arvensis L.								+										
Calystegia sp.							+	+	+							+	+	
Chenopodium album L.	++	+	++		+	+				+	+	+	+	+				
Coleostephus myconis (L.) Cass.	++	++	+	+	+	+		+		+	+		++	+	+++	+	+	
Corrigiola litoralis L.	+	+		+			+++	++	+		+		+	++	++	+++	+++	+++
Fumaria sp.	+	+	+					+	+						+	+	+	+
Geranium sp.				+				+						+		+	+	+
Medicago arabica (L.) Huds.	++	+	+	+++	++	++	+++	+++	+				+	++	+++	+++	++	+
Mentha suaveolens Ehrh.				++	++	+++	++	++	+++				++	+	++	++	++	++
Plantago lanceolata L.				++	++	++								+	+	+	+	+
Polygonum persicaria L.	+++	+++	+++				+	+	+		+	+					+	+
Polygonum sp.							+	+	+									
Portulaca oleracea L.													+			+		
Prunella vulgaris L.								+										
Ranunculus sp.				+		+												
Raphanus raphanistrum L.	+++	+++	+++	+++	+	+	+			++	+++	++	+	++	+	+	+	+
Rubus sp.					+													
Rumex obtusifolius L.		+																
Rumex sp.				+		+		+										
Solanum nigrum L.	+	+	+															
Spergula arvensis L.															+	+		
Stachys arvensis L.																+	+	+
Stellaria media (L.) Vill.	++	++	++							+	++	++						+
Trifolium repens L.					+		+	+								+		
Asteraceae (Other species)	+	+	+	+	++	+	+	++	+		+		+	++	++	+++	+++	+++
Other dicotyledons	+	+		+	+	+		+	+	++	++	++	++	+++	+	+	+	+

(*Continued*)

Table 3. (*Continued.*)

	Short-term									Long-term								
	Pesegueiro			Centieira			Xesta			Pesegueiro			Centieira			Xesta		
	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM	C	AM	AGM
Monocotyledons																		
Cynodon dactylon (L.) Pers.	+		+	+	+++	+++	++	+	++							+		
Cyperus sp.					+		+	+	+					+		+	+	
Dactylis glomerata L.		+		+	+	++	+++	+++	++							+		
Digitaria sanguinalis (L.) Scop.	++	+++	++			+		+		+++	+++	+++	+			+	+	+
Echinochloa crus-galliI (L.) *Echinochloa crus-galliI* (L.) *Echinochloa crus-galliI* (L.)										+++	+++	+++						
P.Beauv.																		
Setaria viridis (L.) P.Beauv.										+								
Other monocotyledons	+++	+++	+++	+++	++	++	++	++	++		+		+	+++	+	++	++	+++
Ferns																		
Pteridium aquilinum (L.) Kuhn					+								+	++	+			

C = control, AM = *A. dealbata* mulch, AGM = *A. dealbata* green manure. ‘ + ’ indicates presence in 1–5 sampling quadrates. ‘ + +’indicates presence in 6–10 sampling quadrates. ‘ + + + ’ indicates presence in 11–15 sampling quadrates.

Contrary to expectations, our results are not consistent with previous studies reporting strong allelopathy or phytotoxicity of *A. dealbata* and *A. longifolia* (Ens et al., 2009; Lorenzo et al., 2010c, 2011, 2013, 2016; Aguilera et al., 2015). The mild weed control observed in our greenhouse and field trials may be related to the fact that former studies testing *Acacia* phytotoxicity did not consider the soil function (Lorenzo et al., 2010c; 2011; Souza-Alonso et al., 2014a; Aguilera et al., 2015). In nature, allelopathy and phytotoxicity are processes that depend highly on soil chemical concentrations. Similarly, weed suppression based on allelopathic materials is usually proportional to the applied dose in agriculture (Xuan et al., 2005). In fact, some chemical components from plant residues can be toxic at higher doses, but also innocuous or even stimulating at low doses (Viator et al., 2006), an effect that has also been recognized for certain commercial herbicides (see Belz and Duke 2014, and references therein). In the NW of the Iberian Peninsula, *A. dealbata* and *A. longifolia* usually conform massive and dense stands (Marchante et al., 2003; Lorenzo et al., 2010a) assuring high soil concentrations of chemical compounds that, probably, exceed those achieved in our assay and consequently produce more evident allelopathic effects.

With a key role in the soil function, soil microbial function can help explain the weak phytotoxic effect observed. Plant chemistry influences soil physicochemical and microbial community changes that take place in their surroundings (Wolfe et al., 2008; Thorpe et al., 2009; Weidenhamer and Callaway, 2010), remarkably so in the case of acacias (Marchante et al., 2008; Ens et al., 2009; Lorenzo and Rodríguez-Echeverría, 2015; Souza-Alonso et al., 2015). Based on the CLPP obtained in the pot experiment, fresh soils appeared distantly positioned from control and treatments along the main axis suggesting that the growth of maize and weeds was the most influencing factor conditioning the soil microbial community function. In this sense, the continuous release of C-organic exudates from roots rather than the release of phytochemical compounds from *Acacia* manure seems to dominate the provision of C sources for heterotrophic soil microorganisms. Nevertheless, *A. dealbata* manure had some influence on the soil physiological profile attending to the second dimension of the CA. Here, treated soils were slightly separated from the control, indicating that functional activity of soil bacterial communities was partially modified by the *A. dealbata* manure.

Using natural leachates from *A. dealbata*, Lorenzo et al. (2013) indicated that chemical compounds that reach the soil through the canopy are able to induce changes in the catabolic profile of bacterial communities. However, it is hard to identify the role/s of one specific chemical compound due to interactive factors affecting behavior and phytotoxicity of allelochemicals in the soil (Kobayashi, 2004). Moreover, many of the compounds claimed to be allelochemicals have little or no biological activity in nature, due to their instability, interactions with soil particles or rapid degradation by microbes (Dayan and Duke, 2014), which employ, degrade or transform phytotoxic chemical compounds released into the environment (Inderjit and van der Putten, 2010; Inderjit et al., 2011). Therefore, differences in C-consumption could be attributed, to a certain degree, to the chemical composition of green manure from *A. dealbata*. The observed specific inhibition of certain botanical groups, the shifts in weed dominance and the slight changes in the functional structure of the bacterial community might also be partly driven by nutrients released by *A. dealbata* leaves. In fact, both *A. dealbata* and *A. longifolia* are woody N-fixing legumes that enrich soil nutrients (mainly C, N and P) through the incorporation of

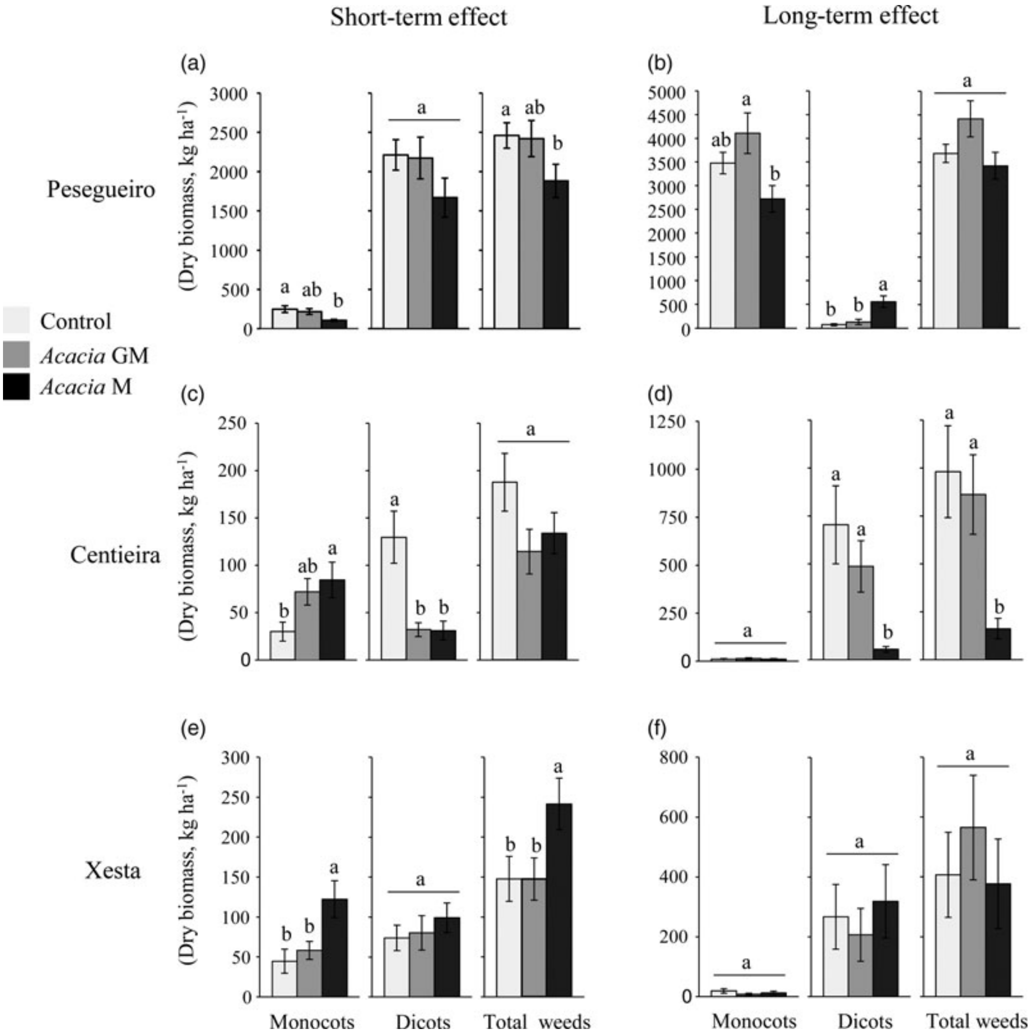

Fig. 4. Short- and long-term phytotoxic effects of *A. dealbata* treatments (mulch, green manure or control) on the biomass of monocot, dicot, or total weeds at three agricultural fields (Pesegueiro, Centieira, Xesta). Model-adjusted least square means values ± SE are shown, *n* = 5. Bars labeled with different letters are statistically different ($P \leq 0.05$), and groups of bars labeled with distinct letters are statistically different ($P \leq 0.01$) based on linear mixed models. Note the different scale for experimental sites. GM, green manure, M, mulch.

high amounts of litter to the system (Marchante et al., 2008; Lorenzo et al., 2010b; Souza-Alonso et al., 2014b). Mulches of leguminous species are known to stimulate the growth of crops by nutrient inputs (Båth et al., 2006; Narwal, 2010), reducing erosion and favoring conservation and infiltration of water into the soil. In fact, composted residues of *A. longifolia* recently showed positive effects on the production of horticultural species (Brito et al., 2015a). However, the nutritional supply of green manures with high N could mask the expected phytotoxic effects or vice-versa (Hanifi and El Hadrami, 2008), and thus facilitate the rapid growth of highly competitive species, such as some monocot weeds. Interestingly, our results also indicated that the incorporation of *Acacia* manures significantly increases soil pH (up to 0.21 units after the incorporation of *Acacia* manure), a limiting factor for plant growth, suggesting the use of acacia residues as an inexpensive amendment to ameliorate poor acid soils.

Nowadays, weed control and herbicide toxicity and accumulation, in addition to herbicide resistance, lead us to a crossroads that requires up-to-date approaches. Although we cannot infer large assumptions due to different results obtained from different agricultural fields, we suggest that *Acacia* mulches could be applied in low infested crop fields dominated by dicot weeds to reduce herbicide dependence, at least as a complementary tool in combination with other control strategies. Furthermore, acacia manures are a source of available soil nutrients, help buffer pH, and did not evidence negative effects on maize, which indicates that a complementary use as organic amendments for *Acacia* residues at field scale needs further elucidation.

Within a global context of economic recession, it is fundamental to ensure that funds invested in invasive plant management are adequately employed, by minimizing ecological and economic risks. Although we did not evaluate the costs of the present study, the applicability of *Acacia* green manures for agricultural purposes either to reduce the abundance of dicot weeds, as a source of nutrients or to correct pH in acid soils may be feasible. In the area of the study, both *A. dealbata* and *A. longifolia* are highly competitive species that form dense and widely distributed populations providing large quantities of fresh material throughout the year, mainly through resprouting after cutting (Lorenzo et al., 2010a; Souza-Alonso et al., 2013). Indeed, a recent inventory evaluating the spread of *A. dealbata* from 1998 to 2008 in NW Spain showed that the occupied area by this species has expanded by 0.8% (i.e. 25,400 ha), increasing its stock (Hernández et al., 2014). In addition, the distribution and productivity of *A. dealbata* are expected to continue increasing in this area (Hernández et al., 2014), especially in pine forests, with 900 new individuals per hectare and year (Rodríguez et al., 2017), which can provide abundant plant material. In Portugal, the expansion and high productivity of some invasive *Acacia* species (*A. dealbata, A. melanoxylon* and *A. pycnantha*) have led to consider them as potential biomass sources (Carneiro et al., 2014). Although specific productivity data for *A. longifolia* is not available, Brito et al. (2015b) indicated that this species could be used as a renewable source due to the high availability of its biomass in NW of the Iberian Peninsula. The collection of high quantities of *Acacia* material and the subsequent work on these residues by using specific machinery has already been carried out (Brito et al., 2013, 2015b), and is therefore possible. The application of these green manures or mulches by farmers should also be feasible since this practice is largely implemented at field scale with different residues (Kandhro et al., 2015; Abbas et al., 2017; Farooq et al., 2017). As a final remark, innovative actions included in this work are focused to contribute to sustainable practices and directed to address several agroecological concerns: reduction in synthetic herbicide application, amelioration of soil and sustainable management of invasive plants.

Conclusions

As far as we know, this is the first time that *Acacia* residues have been added to agricultural soils as green manure to test their potential for weed control and other agroecosystems services. Summarizing, our results suggest that *A. dealbata* green manure was effective at diminishing dicot weed density in pots. Effects at field scale were site-dependent and affected monocot and dicot weeds differentially, dicots being more sensitive to *A. dealbata* manure. Based on former evidence of phytotoxicity reported in the literature, weed control was not as effective as expected. However, due to the abundance and high availability of plant material, the incorporation of *A. dealbata* residues to complement other control strategies might be a viable option within an integrative weed management approach if economically feasible. Furthermore, due to the apparent absence of phytotoxic effects on maize and the slight modifications in the functional profile of bacterial communities, the feasibility of using *Acacia* green manures as soil amendments should be further explored.

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