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Potential uses of botanical extracts of *Larrea* and *Grindelia* in organic agriculture: Effects on soil properties and functional traits relative to growth

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ABSTRACT. Biostimulants—including botanical extracts—are natural preparations that have no negative effects on environmental health or populations and improve soil health and plant growth. Our objective was to evaluate the use of botanical extracts from two native species of the Patagonian Monte, an infusion of *Larrea nitida* (5%-L5 and 10%-L10) and a ferment of *Grindelia chiloensis* (5%-G5 and 10%-G10), on soil properties and the growth of a model plant (*Triticum aestivum*). While the 5% and 10% *Grindelia* ferments increased microbial respiration, soil N and C content, and biomass production, the 10% *Larrea* infusion inhibited microbial respiration and decreased total biomass. The *Grindelia* ferment has potential for use as a biostimulant in agroecological production, whereas the *Larrea* infusion does not show potential for such use. The inhibitory effect of phenolic compounds on microbial activity and plant growth should be addressed in future studies.

[Keywords: biostimulants, ferment, infusion, productivity, soil respiration]

RESUMEN. Usos potenciales de extractos botánicos de *Larrea* y *Grindelia* en agricultura orgánica: Efecto en propiedades del suelo y en rasgos funcionales relacionados con el crecimiento. Los bioestimulantes —dentro de los cuales encontramos a los extractos botánicos— son preparados naturales que no tienen efectos negativos sobre la salud del ambiente ni sobre las poblaciones, y mejoran la salud del suelo y el crecimiento de las plantas. Nuestro objetivo fue evaluar el uso de extractos botánicos de dos especies nativas del Monte Patagónico, infusión de *Larrea nitida* (5%-L5 y 10%-L10) y fermento de *Grindelia chiloensis* (5%-G5 y 10%-G10), sobre las propiedades del suelo y el crecimiento de una planta modelo (*Triticum aestivum*). Mientras que los fermentos de *Grindelia* al 5% y 10% incrementaron la respiración microbiana, el contenido de N y C del suelo, y la producción de biomasa, la infusión de *Larrea* al 10% inhibió la respiración microbiana y disminuyó la biomasa total. El fermento de *Grindelia* tendría potencial para ser utilizado como bioestimulante en producciones agroecológicas, mientras que la infusión de *Larrea* no presenta potencial para tal uso. El efecto inhibitorio de los compuestos fenólicos sobre la actividad microbiana y el crecimiento de la planta debería ser abordado en futuros estudios.

[Palabras clave: bioestimulante, fermento, infusión, productividad, respiración microbiana]

INTRODUCTION

The Green Revolution implemented in the 1960s was characterized by large increases in crop yields due to the extensive use of pesticides and fertilizers (Pereira et al. 2019; Rose et al. 2019). Long-term excess usage of these products has led to serious threats to human health and the environment worldwide (e.g., groundwater and air pollution, water eutrophication and soil health degradation; Ertani et al. 2014; Vejan et al. 2016; Campos et al. 2019; Costa et al. 2019; Ekin 2019; Pereira et al. 2019; Shang et al. 2019; Shukla et al. 2019; Zulfiqar et al. 2019). There is a growing interest in replacing these agrochemical products with cultivation methods based on natural products that allow to enhance plant growth and improve soil properties (Bulgari et al. 2014; Kocira et al. 2018). Furthermore, the demand from farmers and consumers for organic products that provide alternatives to synthetic inputs is also boosting the growth of the market. Biostimulants are among the natural preparations that are not harmful to the environment, improve the general health, vitality and growth of soil and plants and protect them against diseases, and are associated with the possibility of obtaining higher productivity (Kumar and Aloke 2020).

A plant biostimulant is defined as a product that stimulates plant nutrition processes

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independently of the product's nutrient content with the sole objective of improving one or more of the following characteristics of the plant or the rhizosphere of the plant: a) efficiency of the nutrient use; b) tolerance to abiotic stress; c) quality traits, and d) availability of nutrients in the soil or rhizosphere (du Jardin 2015). Natural stimulants often included under the term biostimulants encompass a diverse group of product technologies and may include bacterial or microbial inoculants, biochemical materials, amino acids, humic acids, fulvic acids, seaweed extracts, botanical extracts and more (Calvo et al. 2014; du Jardin 2015; De Pascale et al. 2018). Biostimulants have become more popular in sustainable agriculture in recent years because of their beneficial properties. They stimulate various physiological processes that promote the acquisition and use of nutrients by plants, improve root development (length and number of root hairs), shoot development, yield and nutritional quality of plants, counteract the effects of biotic and abiotic stresses, improve the activity of soil microbiota and fertilizer use and the content of undesirable compounds (e.g., nitrates and heavy metals) in cultivated plants (Ertani et al. 2014; Bulgari et al. 2015; Paradiković et al. 2018; Rouphael et al. 2018; Szparaga et al. 2018; Carillo et al. 2019a,b; Ekin 2019; Shukla et al. 2019; Cozzolino et al. 2020).

Botanical extracts are products generated from roots, barks, seeds, shoots, leaves and fruits, which have shown enormous potential as biostimulants in agricultural systems (Tudu et al. 2022). A variety of plants can be used to produce botanical extracts, biomass availability and wide abundance are the main selection criteria. Farmers or other growers choose plants that grow near their farms based on popular knowledge of traditional uses (traditional recipes passed on for generations; Godlewska et al. 2021a). Traditional, simple extraction methods with water as a solvent prevail, so that they can be used on a large scale and should not create difficulties for farmers. The extraction of plant biomass can also be carried out by boiling in water or at elevated temperatures and by fermentation (Oparaeke 2007; Desoky et al. 2019a,b; Jang and Kuk 2019; Findura et al. 2020).

The biochemical characterization is difficult, as botanical extracts are complex matrices containing many different biologically active molecules. Due to the complexity of the composition of botanical extracts, it is difficult to assign their beneficial effects on plants to a particular compound (Bulgari et al. 2015, 2019). Hence, their mechanism of action is still not well-understood, and these types of products should be categorized based on the physiological responses of plants (Bulgari et al. 2015). Generally, the stimulating properties of plant extracts are attributed to organic compounds such as secondary metabolites (i.e., terpenes, polyphenols, etc.), amino acids, plant hormones and vitamins, among others (Franzoni et al. 2022).

To develop affordable and effective biostimulants, we examined the effect from botanical extracts of two native shrubs of Monte-Patagonia, *Larrea nitida* Cav., and *Grindelia chiloensis* (Cornel.) Cabrera, on soil properties, resource use efficiency and growth of wheat plants under greenhouse conditions. These species were selected as candidates for new plants with potential for biostimulants due to their wide use in herbal medicine, cosmetology and industry. The botanical extracts of these species have been used in traditional medicine (Davicino et al. 2011; Amoroso et al. 2015; for *Larrea* uses) and with industrial uses (Ravetta et al. 1996; Wassner and Ravetta 2000; for *Grindelia* uses), but their potential use in organic agriculture was not tested.

We aimed to assess the potential use of botanical extracts of two widely distributed xerophytic shrubs in the Monte (Jarilla —*Larrea nitida*— and Melaza —*Grindelia chiloensis*—) as biostimulants. We evaluated their effects on the chemical (N and C content) and biological (microbial respiration) soil properties and plant growth (biomass production, allocation patterns and resource use efficient related traits). The botanical extract used was: 1) an infusion of leaves and a tender branch of *Larrea* and 2) a fermentation of leaves and reproductive structures of *Grindelia.*

We expected that both botanical extracts will enhance: 1) the biological activity and chemical properties of the soil: plants growing in soil with biostimulants will have higher microbial respiration, total N, and C content about the control soil, and 2) plant growth: plants growing in soil with biostimulants will increase the biomass production and plant height, and will change the morphological traits related with a higher resource use efficiency: thinner leaves with higher specific leaf area and fine and longer roots with higher specific root length, about control plants.

MATERIALS AND METHODS

Plant materials

This study assessed the potential of using *Larrea nitida* and *Grindelia chiloensis* plants for the production of plant-based extracts. Leaves and tender branches and reproductive structures (capitula only for *Grindelia*) were collected in a native population located in Trelew, corresponding to the biogeographic province of the Monte (43°18′5.52′′ S - 65°16′17.39′′ W).

Larrea nitida (Zygophyllaceae) is widely distributed in the northwest, center and southeast of Argentina (Discole et al. 1940). It is an evergreen shrub 0.9 to 1.8 m tall. The small, dark green, resinous leaves are borne mostly at the tips of twigs and the rest of the branches are bare. This species produces large amounts of phenolic resin on its leaves and stems (8-23% of leaf dry weight).

Grindelia chiloensis (Asteraceae) is a shrub native to Patagonia, Argentina, and is widely distributed in the center-west and south of Argentina and Chile. Trichomes on the surface of the leaves, capitula and stems of this species produce diterpene resin acids (Ravetta et al. 1996). It can accumulate as much as 25% resin by weight in its leaves, with net primary productivity between 90 and 170 $g.plant^{-1}.year^{-1}$ when growing in native stands (Ravetta et al. 1996).

Preparation of plant extracts

Aqueous extraction methods were chosen because they are the easiest method for farmers, are easily degradable, and do not leave toxic residues on the soil (Li and Zhihui 2009; Roy et al. 2010). The preparation methods (ferment for *Grindelia* and infusion for *Larrea*) were selected based on previous (unpublished) experiences of the authors; and evaluated concentrations (5% and 10%) were chosen taking into account the more effective concentration proposed by Godlewska et al. (2021b) (10%), and a 5% to evaluate whether the same results could be obtained using less plant material.

<u>Herbal infusion of Larrea</u>. 400 g of fresh biomass were introduced for 4 L of boil water and then filtered through a 0.2 mm fabric filter to obtain the 'extract L' (10% g/L). It has been reported that the infusion of *Larrea* contains phenolic compounds, lignans and flavonoids (Stege et al. 2006).

<u>Ferment of *Grindelia*</u>. 400 g of fresh aerial parts (leaves, tender branches and capitula) were macerated and fermented in 4 L of water for 20 days. After this time it was filtered through a 1 mm mesh to obtain the 'extract G' (10% g/L). Gastaldi (2019) found phenolic compounds such as flavonoids and phenolic acids (caffeic acid and chlorogenic acid) in *Grindelia*'s infusion. However, many compounds are transformed during fermentation into simpler molecules, including amino acids, free fatty acids, sugars and micro and macroelements (Rojas-Rojas et al. 2021; Prissa and Attanasio 2022).

Greenhouse growth bioassay

Bioassays, like greenhouse experiments, are small-scale tests that assess the biological activity of substances by evaluating their effect on living model organisms. We used small soil microcosms (3 L), containing 600 g of native soil, seeded with three wheat seeds (*Triticum aestivum* L.) as living model species, to investigate the effects of *Larrea* and *Grindelia* extracts (biostimulants) on soil microbial activity, soil N and C content and plant growth. The microcosms were arranged in a completely randomized design, with 6 repetitions for each botanical extract and concentration (5 treatments including control) and grown in a greenhouse.

Experimental treatments included: C: control, microcosms only received water during the growth cycle with any addition of plant extract; L: microcosms were treated with *Larrea* infusion with two concentrations, 5%-L5 and 10%-L10; and G: microcosms were treated with *Grindelia* ferment with two concentrations, 5%-G5 and 10%-G10. Plant extracts were added in one dose at the moment of sowing, simulating irrigation to soil field capacity (300 mL of plant extract for each microcosm).

Plants were maintained in a greenhouse, where they received 80% of outside light levels and a temperature range of 25/15 °C (average daytime/nighttime temperature) and were irrigated daily. After three months of growth, at the end of the growth period of the wheat plants, plants and soil of each treatment were harvested to soil properties and plant growth were analyzed.

Soil properties determination

Microbial respiration was measured based on alkali absorption of CO_2 at 25 °C for 24 hours, followed by titration of the residual OH^- with a standardized HCl solution and 20% barium chloride ($BaCl_2$) solution to precipitate the carbonates. In brief, a fresh soil sample of 60 g was taken from each microcosm and placed in a 500 mL glass jar. A 20 mL volume of 0.2 N NaOH solution was then injected into the internal trap to absorb the CO_2 released from the soil in the flask. Six blanks (controls) were also made, which consisted of flasks without soil, to which the same procedure was performed. The glass jars were incubated at 25 °C in the dark for 24 hours. Microbial respiration was calculated according to the following equation (Guerrero-Ortiz et al. 2012):

Respiration (mg de C mineralized by microbial respiration) = (B - M) x N x E
Equation 1

where B=mL of HCl needed to titrate the Na (OH) of the blanks (mL), M=mL of HCl needed to titrate Na (OH) of a glass jar with the soil of each treatment, N=acid normality (0.1 N) and E=equivalent weight of CO_2 (44 mg) (Guerrero-Ortiz et al. 2012).

At the end of the plant growth period, we measure the chemical properties of the soil with different botanical extract treatments. Soil total organic carbon and total nitrogen were measured using the dry combustion method with an elemental analyzer (Elementar vario MACRO CUBE, Elementar Co., Hanau, Germany) in soil samples corresponding to control (C) and G10 and L10 treatments.

Plant growth and resource use related traits measurements

A single wheat plant was used from each microcosm for measurement. Plant biomass was fractionated into roots, leaves and reproductive structures (spikes). For leaf traits measurements, fresh leaf thickness was measured using a Mitutoyo Digital Thickness Gage 547-301, when leaves were harvested. The total leaf area per plant was determined using UTHSCSA Image Tool for Windows, version 2.02. For root traits measurement, roots were suspended in 1 cm of water in a 30×25 cm black tray and then digitalized (8-bit grayscale image, resolution 300 dpi). GvSIG 1.11.0 software was used to calculate root diameter and total root length.

After scanning, plant samples were dried at 70 °C for 48 hours, and weighed. We estimated the proportional allocation to leaves (LMR; g leaf/g total biomass), to roots (RMR; g root/g total biomass) and reproductive biomass (reproductive effort; g reproductive structures/g total biomass). These data were used to calculate specific leaf area (SLA, cm^2 of total leaf area/g leaf) and specific root length (SRL, cm of total root length/g root).

Morphological traits were measured to evaluate the effect of the addition of botanical extracts on the resource use efficiency of plants. Plant resource use strategies can be quantified by measuring functional traits (Grime et al. 1997). One important set of functional traits describes differences in resource acquisition (Reich et al. 1997), known as the 'plant economics spectrum' (sensu Westoby et al. 2002), which runs from plants with quick returns on investment in nutrients and dry matter (i.e., acquisitive strategy) to plants with slower returns on their investments (i.e., conservative strategy). SLA and leaf thickness represent variation along the leaf economics spectrum and are indicative of a species' ability to respond to opportunities for rapid growth (Reich et al. 1999). SRL and root diameter have been suggested to be the belowground analog to SLA and thickness (Cornelissen et al. 2003). SRL is indicative of the potential rate of water and nutrient uptake and is considered to be a morphological index of belowground competitive ability (Lambers et al. 1998).

Statistical analysis

To compare differences in soil properties and plant growth-related traits between plants and soils under the effect of different biostimulants a one-factor ANOVA was performed for each extract type (*Grindelia*, with 2 levels-G5% and G10%; and *Larrea*, with 2 leves-L5% and L10%) compared with control (C) treatment. Differences between treatment means were analyzed using a Tukey test (P=0.05). The Shapiro-Wilk test and Levene's test for homogeneity of variance were used to check the normality of the data.

RESULTS

Effects of botanical extracts on soil properties

The botanical extracts evaluated did not have the same effect on the soil properties. On one hand, the application of *Grindelia* extract resulted in an increase in the biological activity of the soil, quantified by microbial soil respiration and enhance C and N content in the soil (Table 1). The increase in C and N content per pot (Δg C/pot and Δg N/pot, respectively) was statistically significant. On the other hand, *Larrea* extract application decreased the microbial activity in the soil and did not change their chemical properties (Table 1). The effects on the soil did not differ at different extract concentrations (5% vs. 10%).

Effects of botanical extracts on plant growth

All the seeds placed in the pots germinated (germination percentage 100%). The application of botanical extracts to the soil had significant effects on biomass production and the allocation pattern of wheat plants. Plants growing in soil treated with 5 and 10% of *Grindelia* ferment had a 67% higher biomass production than control plants (Figure 1), allocated preferentially to leaves (LMR) at the expense of roots (RMR; Figure 2). In 5% of *Grindelia*, the leaves biomass was higher than in the control, but no changes were observed in 10% of *Grindelia* (Figure 1). The proportional allocation to reproductive structures was not changed by *Grindelia* extracts, but the total biomass assigned to reproductive structures was higher than the control (Figure 1).

Larrea infusion at 10% applied to the soil resulted in a 36% of reduction in the biomass productivity of wheat plants (Figure 1) and also a change in the proportional allocation pattern: higher allocation to leaves (LMR) and expense of roots (RMR, Figure 2). The application of *Larrea* infusion at 5% did not affect plant growth or proportional allocation.

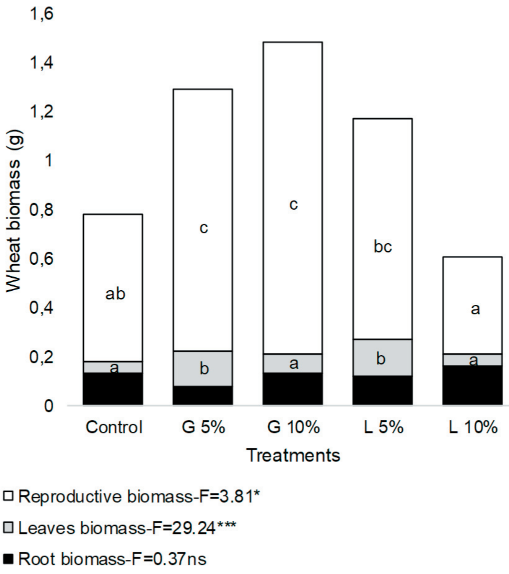

Figure 1. Biomass assigned to different organs (i.e., roots, leaves, and reproductive structures) and total biomass production (g) in wheat plants (living model organism) growing in soil with the application of *Grindelia* and *Larrea* extracts at 5% and 10% of concentration, compared with control plants (no treated soil). Different letters indicate significant differences between treatments and the control. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05. Control data are the same for the two extract treatments.

Figura 1. Producción de biomasa (g) diferenciada de acuerdo a la asignación a diferentes órganos (i.e., raíces, hojas y estructuras reproductivas) en de plantas de trigo (organismo modelo) creciendo en suelo con la aplicación de extractos de *Grindelia* y *Larrea* al 5% y 10% de concentración, comparada con plantas control (suelo no tratado). Letras diferentes indican diferencias significativas entre los tratamientos y el control. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05. Los datos del control son los mismos para los dos tratamientos.

Table 1. Chemical (C and N content per pot) and biological (microbial respiration) soil properties of soil treated with botanical extracts of *Grindelia* and *Larrea*, compared with control soil. Δg C/pot and Δg N/pot indicates the difference between C and N content in the treatment plot compared to control pots. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05.

Tabla 1. Propiedades químicas (contenido de C y N total en cada maceta) y biológicas (respiración microbiana) del suelo tratado con extractos botánicos de *Grindelia* y *Larrea*, comparadas con muestras de suelo control. Δg C/maceta y Δg N/maceta indican las diferencias entre el contenido de C y N del suelo tratado con respecto al suelo control. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05.

Treatments		Soil respiration (mg CO_2/day)	C		N	
			(g C/pot)	(Δg C/pot)	(g N/pot)	(Δg N/pot)
Control	*Grindelia*	13.54±2.54b*	12.3±0.57a		0.69±0.05a	
	Larrea	27.7±0.42a*				
Grindelia	5%	20.45±2.54a				
	10%	24.45±2.54a	21.0±0.47b	8.7±0.47 b	1.14±0.05b	0.45±0.05b
Larrea	5%	18.8±1.72b				
	10%	15.7±1.46b	14.1±0.67a	1.8±0.67 a	0.84±0.05a	0.15±0.05a
F-test	*Grindelia*	4.71*	168.2**	168.2*	45.0*	45.0*
	Larrea	21.4***	3.6^{ns}	3.66^{ns}	5.0^{ns}	5.0^{ns}

*For microbial respiration determination we used two blanks due to glass jars of each treatment were incubated separately, thus we used three blank samples for each incubation moment

However, in 5% of *Larrea*, the leaves biomass was higher than in the control. No changes were observed in 10% of *Larrea* (Figure 1). Allocation (in grams and proportional) to reproductive structures was not changed by *Larrea* extracts.

Both botanical extracts inhibited root growth of wheat plants: the total root length and SRL of plants growing at *Grindelia* 5% and 10%, and *Larrea* 10% were lower than that of control plants. However, the total root biomass did not change in any treatment compared to the control (Figure 1). The application of these botanical extracts also changed the leaf morphology of wheat plants: plants growing in soils treated with plant extracts had low SLA and thicker leaves, than control plants (Table 2). *Larrea* at 5% does not affect leaf and root traits.

Discussion

Our study shows that *Grindelia* increased soil biological activity (increased soil respiration, C and N content), while *Larrea* did not change soil biological activity compared to the control. Regarding effects on wheat plant growth: both treatments of *Grindelia* extract (5% and 10%) increased biomass production relative to the

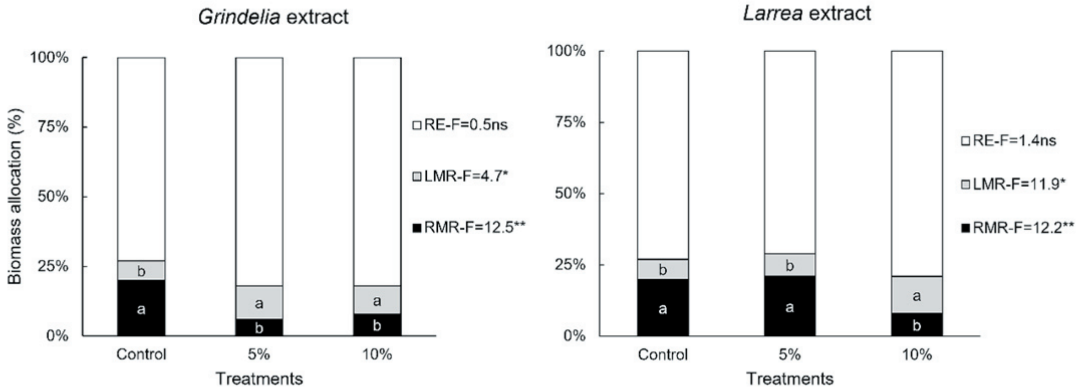

Figure 2. Proportional allocation pattern of wheat plants (living model organism) growing in soil with the application of *Grindelia* and *Larrea* extracts at 5% and 10% of concentration, compared with control plants (no treated soil). RMR: root mass ratio. LMR: leaf mass ratio. RE: reproductive effort. Different letters indicate significant differences between treatments and control within the same type of botanical extract. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05. The control data are the same for the two extract treatments, repeated in the panel of each extract.

Figura 2. Patrón de asignación proporcional de plantas de trigo (organismo modelo) creciendo en suelo con la aplicación de extracto de *Grindelia* y *Larrea* al 5% y 10% de concentración, comparado con plantas control (suelo no tratado). RMR: relación de masa de la raíz. LMR: relación de masa hacia las hojas. RE: esfuerzo reproductivo. Letras diferentes indican diferencias significativas entre los tratamientos y el control entre el mismo tipo de extracto botánico. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05. Los datos del control son los mismos para los dos tratamientos, repetidos en el panel de cada extracto.

Table 2. Morphological leaf and root traits of wheat plants (living model organism) growing in soil with the application of *Grindelia* and *Larrea* extracts at 5% and 10% of concentration, compared with control plants (no treated soil). SLA: specific leaf area. SRL: specific root length. Values are means±standard error. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05.

Tabla 2. Rasgos morfológicos de las hojas y raíces de plantas de trigo (organismo modelo) creciendo en suelo con la aplicación de extractos de *Grindelia* y *Larrea* al 5% y 10% de concentración, comparados con plantas control (suelo no tratado). SLA: área foliar específica. SRL: longitud específica de las raíces. Los valores son las medias±error estándar. *P<0.05; **P<0.01; ***P<0.001; ns P>0.05.

Treatments		Leaf traits		Root traits		
		SLA (cm^2/g)	Thickness (μm)	SRL (cm/g)	Diameter (cm)	Total length (cm)
Control		296.1±17.3a	190±12b	2304±43a	0.10±0.01	245.2±29.4a
Grindelia	5%	185.6±11.7b	230±10a	1678±48b	0.11±0.01	141.8±23.5b
	10%	175.4±22.3b	280±10a	1528±18b	0.10±0.01	167.6±21.7b
Larrea	5%	302.2±20.3a	200±20a	2401±52a	0.12±0.01	260.1±21.1a
	10%	175.3±28.9b	215±10a	1416±44b	0.13±0.01	126.1±19.3b
F-test	*Grindelia*	45.6***	7.1*	11.7*	0.4^{ns}	4.1*
	Larrea	48.7**	0.4^{ns}	12.1*	0.9^{ns}	9.3*

control. *Larrea* infusion only decreased biomass production by 10% compared to the control treatment. Both botanical extracts reduce the proportional allocation to root biomass in wheat plants. In the following, we discuss the possible causes or mechanisms involved in explaining the observed changes in 1) soil chemical properties, and 2) plant growth and nutrient use efficiency-related traits.

Effects on soil properties

Based on our results on the potential effects of extracts on soil properties, we conclude that *Grindelia* has a positive effect on soil health and has the potential to be used as a biostimulant, while *Larrea* extract would not be appropriate as it inhibited microbial activity. Differences in their effect could be related to the composition and solubility of secondary metabolites and proteins according to the extraction or transformation methods (i.e., infusion or fermentation). The release and respiration were promoted by *Grindelia* ferment. We related this finding to the fact that the ferment could have higher N content and soluble secondary metabolites extracted from leaves that could be considered as an additional source of labile carbon promoting higher metabolic activity and faster decomposition and N mineralization and release in the soil (Bradley et al. 1997; Castells et al. 2004; Gallo et al. 2009; Guerrero-Ortiz et al. 2012). The effect of *Grindelia* ferment on N-mineralization and microbial N release could not be evaluated because soil NH_4^+ and NO_3^- were not measured. Also, during the fermentation processes chemical compounds, such as resins of leaves and tender branches, are transformed (Hoffmann et al. 1984), losing their antifungal, insecticide and antimicrobial properties (Vilela et al. 2011; Gastaldi et al. 2019; Mensurado et al. 2021) or having a positive effect on soil microbial activity and plant growth (Prisa and Attanasio 2022). The enhanced microbial activity also could be explained by other two mechanisms. On one hand, the creation of a more favorable environment for microorganisms as a result of the improvement of physicochemical properties of the soil with the bioestimulant, such as aggregate stability, increased porosity and water-holding capacity (Fageria 2012; Halpern et al. 2015; Rouphael and Colla 2020). In particular for *Grindelia* extract, it could be possible that the resins incorporated into the soil form a hydrophobic chemical crust that prevents water loss (Rajnoch et al. 2022) and improve water retention. On the other hand, the incorporation of new beneficial microbes from the ferments into the soil, could enhance soil health and crop productivity (Sulok et al. 2021).

The infusions, on the other hand, have rapid extractive power due to higher temperatures that allow solubilizing of triterpenes, flavonoids and phenols (Jager et al. 2011). In the case of *Larrea* extracts, the high release of soluble phenolics presented in leaves could further reduce microbial activity and the availability of N in the soil by forming complexes with proteins decelerating the decomposition and mineralization of organic matter processes (Hattenschwiler and Vitousek 2000; Castells 2008; Adamczyk et al. 2011). In *Larrea*, we worked with aqueous extract or infusion which has very low amounts of nordihydroguaiaretic acid (NDGA) compared to phenolic extracts. NDGA is the main antibacterial compound of other species of *Larrea* (Mabry et al. 1977; Anesini and Perez 1993; Obermeyer et al. 1995; Amani et al. 1998; Quiroga et al. 2001; Zampini et al. 2007). Despite the potential lowest concentrations of Larrea aqueous extracts, it exhibited a marked inhibitory activity of soil microorganisms even at 5% of concentration. However, our results do not allow us to evaluate the negative effect in N mineralization, because we do not measure the availability of N.

In relation to the input of C and N into the soil, there are two potential sources: rhizodeposition of wheat root growing in the pots and de C and N added by biostimulants. Wheat rhizodeposits provide about 11.6 mg C/g of root dry matter (Hütsch et al. 2002) and 76 mg N/(3 plants) (Janzen 1990) to the soil. According to these data, the estimated C input for rhizodeposition in our experiment was 0.0049 g C and 0.0076 g N/plot on average for all treatments (Table 3). These values are lower compared with the increase in C and N obtained in the soil treated with biostimulants. Estimations of the soil C and N gain with the added biostimulants in this work were performed (Table 3) using published literature. For *Larrea* infusion (L10%), the estimates were based on the C and N content provided by the 2% infusion reported by Seggeso et al. (2019; 2.025 g C and 1.14 g N). The gain of C in the soil treated with L10% reported in Table 1 agrees with our estimates (Table 3). For *Grindelia* ferment (G10%), estimates were performed using data about C invested in nonstructural, C invested in defense compounds and total N content in dry biomass (1.09 g C 5.5 g C and 3.4 g N, respectively) (Zavala and Ravetta 2002). Our estimates do not take into account the structural C in the dry biomass or the transformation that can occur during fermentation in these compounds or the microorganisms added with the ferment. However, the estimated gain in C using G10% (Table 3) represents 75% of the C gain reported in Table 1, and can be considered a biostimulant effect. Estimates of soil N gain using biostimulants are higher than our data, possibly due to plant N uptake during the experiment.

Effects on plant growth and nutrient use efficiency-related traits

Neither of the two extracts evaluated had negative effects on seed germination or seedlings' survival, but they had different effects on plant growth. On the one hand, wheat plants growing in soil with *Grindelia* ferment was more productive and had a higher proportional allocation to the aboveground. This positive effect on growth could be related to the release of chemical compounds such as amino acids, sugars and different micro and macronutrients (e.g., calcium, potassium, magnesium, phosphorus and sulfur) during the fermentation process. Between those, amino acids are effective in increasing plant growth and improving plant health (Paleckiene et al. 2007; Popko et al. 2018; Singh et al. 2022; Kumari et al. 2023). Kocira et al. (2021) reported similar results using a flax seed macerate to improve the productivity of soybean crops. The lower root allocation of wheat plants growing with G5% and G10% can be explained using the optimal allocation theory: the effect of *Grindelia's* ferment increasing soil nutrient availability results in plants requiring lower root development (Bloom et al. 1985; Johnson and Thornley 1987). Against expected, at the leaf level plants growing in soils treated with ferment produce thicker and lower SLA leaves, traits related to a more conservative strategy (Wright et al. 2004). However, thicker leaves can be associated with higher productivity at the leaf level due to an increase in mesophyll width that enhances photosynthesis (González-Paleo and Ravetta 2018). Morphological changes in root, such as lower SRL, can be related to the physical properties of the soils with *Grindelia*´s ferment. *Grindelia* resins accumulate in soils and increase the soil hardness (Rajnoch et al. 2022), so in future studies these characteristics should also be measured to make a clearer interpretation of the response of the root system.

On the other hand, the effect of *Larrea* infusion was dependent on the concentration: 5% (L5) did not cause any changes in wheat plant growth or nutrient use efficiency-related traits compared to the control, while the addition of the infusion at 10% caused a decrease in productivity and a shift in the strategy to a more conservative root and leaves morphology (i.e., lower SLA, thicker leaves, lower SRL and lower root length, than control plants). Similar results using an aqueous extract of *Larrea divaricata* Cav. were reported to decrease the growth of native Patagonian grasses (*Poa ligularis* Nees ex Steud. and *Nassella tenuis* (Phil.) Barkworth; Segesso et al. 2019). The decrease in growth could be related to an allelopathic effect of the phenolic compounds present in these extracts. Phenols inhibit root growth and cell division and affect normal plant growth and development (Jacob and Sarada 2012). In native ecosystems, the growth of *L. tridentata* shrubs also affects root growth and establishment of other neighbor species (Elakovich and Stevens 1985; Mahall and Callaway 1992; Schafer et al. 2012).

General conclusion and potential use of Grindelia ferment

Based on our results on the potential effects of extracts on soil properties, we conclude that the 5% *Grindelia* ferment has a positive effect on soil health and plant growth and therefore the potential for use as a biostimulant. Organic carbon and labile substances (fermentation products) supplied to the soil using this extract enhance microbial activity and physicochemical properties of the soil. Improving soil health is critical to increase crop production, in a sustainable way. Furthermore, multiple application of *Grindelia* ferment could change the diversity and abundance of microbial (fungus and bacteria), as observed in other studies (Wang et al. 2016; Leite et al. 2017; Zhou et al. 2019). However, one of the main limitations of the application of this biostimulant is that it is required in large quantities for crop production. Based on our results, estimation of the amount of extract required to produce 1 ha, we would need one million L of biostimulant and 50 kg of dry *Grindelia* biomass. Therefore, it could only be applied to small-scale production, such as familiar or local agriculture and could be useful for the agroecological transition (i.e., less dependence on agrochemicals). An advantage of using *Grindelia* is that this species can be cultivated. The propagation (by seed and vegetative propagation), crop structure, irrigation requirements and harvesting time have been reported (Ravetta et al. 1996; Wassner and Ravetta 2000). The possibility of cultivation would reduce the extraction of plants from natural populations.

Due to the chemical composition of *Grindelia* ferment is not known, all the mechanisms proposed behind their effects on soil and plants, are only hypothetical until now. The input of C and N to the soil and the humification efficiency cannot be established. These topics should be addressed in future studies.

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