



## RESEARCH ARTICLE

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# Seed density is more effective than multi-trait limiting similarity in controlling grassland resistance against plant invasions in mesocosms

Florencia A. Yannelli<sup>1,2</sup> | Gerhard Karrer<sup>3</sup> | Rea Hall<sup>3</sup> | Johannes Kollmann<sup>1,4</sup> | Tina Heger<sup>1,5</sup>

<sup>1</sup>Chair of Restoration Ecology, School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

<sup>2</sup>Department of Botany and Zoology, Centre for Invasion Biology, Stellenbosch University, Matieland, South Africa

<sup>3</sup>Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life Sciences Vienna, Wien, Austria

<sup>4</sup>Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway

<sup>5</sup>University of Potsdam, Biodiversity Research and Systematic Botany, Potsdam, Germany

## Correspondence

Florencia A. Yannelli, Chair of Restoration Ecology, School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany.  
Email: florencia.yannelli@tum.de

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## Abstract

**Question:** Disturbed areas offer great opportunities for restoring native biodiversity, but they are also prone to invasion by alien plants. Following the limiting similarity hypothesis, we address the question of whether or not similarity of plant functional traits helps developing seed mixtures of native communities with high resistance to invasive species at an early stage of restoration.

**Location:** Centre of Greenhouses and Laboratories Dürnast, Technische Universität München, Freising, Germany.

**Methods:** Using a system of linear equations, we designed native communities maximizing the similarity between the native and two invasive species according to ten functional traits. We used native grassland plants, two invasive alien species that are often problematic in disturbed areas (i.e., *Ambrosia artemisiifolia* and *Solidago gigantea*) and trait information obtained from databases. The two communities were then tested for resistance against establishment of the two invaders separately in a greenhouse experiment. We measured height of the invasive species and above-ground biomass, along with leaf area index, 4 and 8 months after sowing respectively.

**Results:** Both invasive species were successfully reduced by the native community designed to suppress *S. gigantea* dominated by small-seeded species. These results could be considered as partial support for the limiting similarity hypothesis. However, given the success of this mixture against both invasive species, suppression was better explained by a seed density effect resulting from the smaller seed mass of the native species included in this mixture. Further, the dominance of a fast-developing competitive species could also contribute to its success.

**Conclusions:** There was no unequivocal support for the limiting similarity hypothesis in terms of the traits selected. Instead we found that increasing seeding density of native species and selecting species with a fast vegetative development is an effective way to suppress invasive plants during early stages of restoration. If limiting similarity is used to design communities for restoration, early life-history traits should be taken into account.

## KEYWORDS

*Achillea millefolium*, *Ambrosia artemisiifolia*, biotic resistance, competition, density-driven suppression, disturbed areas, restoration, seed mixtures, *Solidago gigantea*

## 1 | INTRODUCTION

Human activities often result in land-use change and ecosystem degradation, with negative effects on biodiversity and ecosystem services worldwide (Millennium Ecosystem Assessment 2005). The intensification of land use and the transformation of near-natural areas to agricultural or urban developments call for ecological restoration in many regions (Mitsch, 2012). Examples of degraded ecosystems offering opportunities for restoration include roadsides (Bochet, Tormo, & García-Fayos, 2010; Tormo, García-Fayos, & Bochet, 2008), abandoned mines (Baasch, Kirmer, & Tischew, 2012) and set-aside fields (Munro, Fischer, Wood, & Lindenmayer, 2009; Török et al., 2010). Given the great loss of species-rich grasslands (Poschlod & Wallis De Vries, 2002), other disturbed sites such as urban habitats also offer an opportunity for restoring biodiversity and ecosystem services of grassland-like systems (Fischer, von der Lippe, & Kowarik, 2013; Klaus, 2013). However, these habitats are prone to invasion by alien plants, which effectively colonize restoration sites with insufficient vegetation cover. The susceptibility of these areas to invasion highlights the importance of increasing the early biotic resistance of restored grassland communities (Yannelli, Hughes, & Kollmann, 2017; Yannelli, Koch, Jeschke, & Kollmann, 2017).

Introduction of native species is an essential step during grassland restoration because of dispersal limitation and missing seed banks of these species (Hedberg & Kotowski, 2010). A promising approach is to design native species mixtures to accelerate vegetation establishment and at the same time prevent the establishment of undesired plants. Instead of site-specific trial-and-error attempts, Funk, Cleland, Suding, and Zavaleta (2008) proposed assembling communities for ecological restoration based on the limiting similarity hypothesis. According to this hypothesis, invaders will be unlikely to establish if native species with similar traits are present in the resident community (Abrams, 1983; MacArthur & Levins, 1967). Commonly used traits are linked to resource acquisition, and it is assumed that niche overlap under limiting resources would lead to competitive exclusion (Hooper & Dukes, 2010). Therefore, this approach provides the opportunity to design seed mixtures by choosing species that might affect not only biotic resistance, but also other ecosystem properties relevant to the respective restoration project, i.e., reduced erosion, improved water purification and carbon storage (Funk et al., 2008; Ostertag, Warman, Cordell, & Vitousek, 2015).

Results of experiments using a trait-based approach to design resistant communities have been mixed, as reviewed by Price and Pärtel (2013), underlining the challenge of selecting the most relevant traits (Funk et al., 2016). The lack of consistency could also be explained as a consequence of attempting to test limiting similarity using only broad functional traits such as phenology, life history and life form (Emery, 2007; Hooper & Dukes, 2010; Sheley & James, 2010; Young, Barney, Kyser, Jones, & DiTomaso, 2009). Indeed, other studies have suggested portraying plant niches using a multi-trait approach based on several traits related to competitive ability or resource use, for instance using cluster analysis (Byun, de Blois, &

Brisson, 2013; Yannelli, Koch et al., 2017). Although this method allows more specific trait selection, significant patterns explained only by a few traits might be masked by non-significant ones included in the analysis (Yannelli, Koch et al., 2017). Further, the method does not allow for different native species abundances, which would make the communities more realistic. To overcome these challenges, Laughlin (2014) proposed a framework where trait-based targets can be translated into relative abundance of native species by means of undetermined systems of linear equations. This method allows calculation of native species proportions in a seed mixture designed for use in restoration, in a way that maximizes functional trait similarity of the native with the invasive alien plant species (Laughlin, 2014). Although this framework offers exciting opportunities for examining limiting similarity in this context, to our knowledge no study has tested it so far.

In this contribution we aimed to investigate the potential of using the limiting similarity hypothesis to design seed mixtures resulting in native grassland communities with increased biotic resistance. Specifically, in a greenhouse experiment we studied whether this resistance could be accomplished by maximizing the similarity in functional traits between the native community and a specific invasive alien species. We used calcareous grassland species commonly utilized for restoration, given their marked decline due to land-use changes in Central Europe (Poschlod & Wallis De Vries, 2002), and two common invasive plants as test species. Based on ten functional traits, we hypothesized that an invasive plant will be more effectively suppressed through the respective community designed to prevent its establishment based on trait similarity.

## 2 | METHODS

### 2.1 | Plant and trait selection

We tested our hypothesis using two invasive species known to occur in disturbed urban areas in Central Europe (Essl et al., 2015; Weber & Jakobs, 2005), namely *Ambrosia artemisiifolia* and *Solidago gigantea*. Seeds of *A. artemisiifolia* were collected nearby the River Danube in Vienna, eastern Austria (48°16'01" N, 16°22'10" E), and *S. gigantea* along River Isar in Freising, southern Germany (48°23'57" N, 11°45'16" E). We selected the native competitors for the experiment from a pool of 28 grassland species occurring with a frequency  $\geq 10\%$  in >100 surveys of calcareous grasslands in the agricultural landscapes north of Munich, Germany (Conradi & Kollmann, 2016). Calcareous grassland species were used as target native species since, from their decline starting in the 19th century, due to land-use change and fragmentation (Poschlod & Wallis De Vries, 2002), they are commonly used for restoration projects. The seed material for the native species was acquired from the local seed producer Johann Krimmer (Pulling, Germany).

Ten functional traits were selected in order to test the limiting similarity hypothesis, i.e., seed mass, plant height at maturity, specific leaf area (SLA), dry leaf mass, flowering time, growth form, plant longevity, life form, reproduction type and functional type (following

**TABLE 1** Native species composition of the two experimental communities and the proportion of each species in the seed mixture (% of total seed mix weight) based on similarity in ten functional traits with the respective invasive species

Species	Proportion AA	Proportion SG
<i>Achillea millefolium</i>	-	0.43
<i>Agrostis capillaris</i>	-	0.001
<i>Betonica officinalis</i>	0.002	-
<i>Buphthalmum salicifolium</i>	-	0.001
<i>Campanula rotundifolia</i>	0.001	0.001
<i>Centaurea scabiosa</i>	0.4	-
<i>Dactylis glomerata</i>	0.001	0.001
<i>Festuca rubra</i>	-	0.002
<i>Filipendula vulgaris</i>	-	0.001
<i>Galium album</i>	0.05	0.44
<i>Linum perenne</i>	0.004	-
<i>Medicago lupulina</i>	0.29	-
<i>Peucedanum oreoselinum</i>	0.001	-
<i>Poa angustifolia</i>	-	0.12
<i>Teucrium montanum</i>	0.25	-
<i>Veronica chamaedrys</i>	0.001	0.003

AA: community designed to prevent establishment of *Ambrosia artemisiifolia*; SG: community to prevent establishment of *Solidago gigantea*. All species had reproduction by seeds but some species also had clonal growth.

-, not included.

the legume–forb–grass scheme). These traits have been described as related to species dispersal ability, establishment success, persistence and competitive ability (Funk et al., 2008; Westoby, Falster, Moles, Vesk, & Wright, 2002). Temporal niche overlaps were partly represented by including traits such as plant longevity and flowering time (Byun et al., 2013; Wilsey, Daneshgar, & Polley, 2011). Trait information was obtained from BioFlor (Klotz, Kühn, & Durka, 2002) and LEDA (Kleyer et al., 2008). Our nomenclature follows Wißkirchen and Haeupler (1998).

## 2.2 | Community design

The system of linear equations proposed by Laughlin (2014) was used to design the communities, since it allows transformation of multi-trait targets into communities with ranges of native species abundances. Systems of linear equations can be used to estimate unknown probabilities, given a set of equality and inequality constraints. Specifically in this context, using systems of linear equations it is possible to determine which relative abundances are necessary to obtain communities of native species whose traits are maximally similar to the traits of a focal invasive species (Laughlin, 2014). For this, the following equations were used:

$$\sum_{i=1}^S t_{ik} p_i = \bar{T}_k, \quad (1)$$

$$\sum_{i=1}^S p_i = 1, \quad (2)$$

$$p_i > 0, \quad (3)$$

where  $S$  is the size of the species pool and  $k$  the total number of traits. Therefore, Equation 1 constrains the linear combination of the  $k^{\text{th}}$  trait of the  $i^{\text{th}}$  native species ( $t_{ik}$ ) and the unknown relative abundances of native species  $p_i$  to the  $k^{\text{th}}$  trait value of an invasive species ( $\bar{T}_k$ ). Given that  $p_i$  represents relative abundances, Equation 2 constrains the sum of all  $p$ -values to 1, and Equation 3 forces it to be non-negative. In order to find the unknown values using linear algebra, we can translate Equations 1 and 2 to a matrix form  $Ax = B$ , where  $A$  is a matrix containing the native species mean traits,  $x$  is the vector of unknown native species abundances and  $b$  is the vector of constraints, e.g., invasive species traits for Equation 1 (Laughlin, 2014).

Based on these equations we designed the seed mixtures for two sets of communities with the aim of preventing the establishment of our two focal invasive species, *S. gigantea* and *A. artemisiifolia*. For this, we converted non-numerical traits of all species into dummy variables with binary values. Then the matrix  $A$  was compiled using the ten mean trait values for each native species and the vector  $b$ , with the ten mean trait values of the targeted invasive species. To obtain the native communities that were most similar in traits to the invasive species, we used the ‘maxent’ function within the FD library of R (Laliberté & Shipley 2010; R Foundation for Statistical Computing, Vienna, Austria), resulting in a proportion for all native species in the pool. To control for an effect of the species richness, and given that there are some species that would have extremely low proportions, we sorted them from high to low proportions, and included only the first ten species in each seed mixture (Table 1).

As a result, the seed mixture for community AA designed to prevent establishment of *A. artemisiifolia* was dominated by *Centaurea scabiosa* (40% of the seed mixture weight), a species with large seeds, and mostly contained forbs but also some legume and semi-rosette species (Table 2). Conversely, the seed mixture for community SG designed for *S. gigantea* was dominated by *Galium album* and *Achillea millefolium* (44% and 43%, respectively). SG consisted of smaller-seeded species and had approximately a ten-fold density compared to the AA community. Further, the proportion of grasses was higher than in AA but still low compared to the proportion of forbs.

## 2.3 | Experimental design

In a greenhouse experiment, we tested whether the design of resistant native communities could be accomplished by maximizing the similarity in functional traits between the native community and a specific invasive alien species based on Laughlin (2014). The experiment had a fully randomized factorial combination of two treatments, namely the invasive species to be tested (‘invasive species’,

Characteristics	AA	SG	<i>A. artemisiifolia</i>	<i>S. gigantea</i>
Seed mass (mg)	0.32	0.03	3.18	0.06
Height (m)	0.50	0.41	0.80	1.38
Flowering start (months)	6.36	5.88	7	8
SLA (mm <sup>2</sup> /mg)	21.80	21.10	32.10	20.30
Proportion clonal growth	0.75	0.99	-	1
Proportion forbs	0.71	0.88	1	1
Proportion grasses	0.00	0.12	-	-
Proportion legumes	0.29	0.00	-	-
Proportion erosulate	0.31	0.44	1	1
Proportion semi-rosette	0.69	0.56	-	-

**TABLE 2** Characteristics of the study communities and the two invasive species using information on ten functional traits obtained from databases

Quantitative traits are provided as weighted community means according to species proportion in the respective mixture and mean values for the invasive species. In the case of qualitative traits, the proportions of species with that trait included in the mixture are given in different rows. AA: community designed based on limiting similarity for *Ambrosia artemisiifolia*; SG: community based on limiting similarity for *Solidago gigantea*.

i.e., *Ambrosia artemisiifolia* or *Solidago gigantea*) and the native communities ('treatments', i.e., AA, SG or control). The control treatment was a monoculture of each invasive species and all treatment combinations were replicated six times. In other words, each treatment combination consisted in the sowing of a community containing native plants whereby the suppression of the invasive species was tested by comparing it with the control.

Native communities were sown at a total density of 3 g/m<sup>2</sup>, which corresponds to regular practice for grassland restoration in Central Europe (Kiehl, Kirmer, Donath, Rasran, & Hölzel, 2010). The seed mixtures were prepared by dividing this total density according to the respective proportion in seed weight for each native species in each mixture (Table 1). The invasive species were sown 1 week later at a density of 1 g/m<sup>2</sup> by scattering the seeds on top of the soil across the experiment to mimic invasion at an early stage of grassland restoration by seed rain. The density of the invasive species was selected according to average values of viable seeds found in soil seed banks for *A. artemisiifolia* (Fumanal, Gaudot, & Bretagnolle, 2008; Vitalos & Karrer, 2008) and extrapolated to *S. gigantea* to make it comparable.

All treatment combinations were grown in 40 cm × 30 cm × 6 cm plastic trays with a peat-based substrate (Einheits Erde®, Einheitserdewerke Werkverband e.V., Altengronau, Germany; N, 180 g/m<sup>3</sup>; P, 240 g/m<sup>3</sup>; K, 240 g/m<sup>3</sup>; pH 5.8). Pots were initially watered every day from above until plant establishment, and then changed to watering from beneath on demand. The greenhouse experiment was carried out at the Greenhouse Laboratory Centre Dürnast, Technical University of Munich (48°24' N, 11°41' E). Starting in late May 2015, the experiment ran for 8 weeks in an unheated closed greenhouse with an average temperature of 21 ± 5°C.

## 2.4 | Measurements and data analysis

The height of up to ten randomly selected individuals of each invasive species was recorded 4 weeks after sowing to assess the

initial effects of the treatments on invasive species emergence. Before collecting biomass, the leaf area index (LAI), defined as the green leaf area per unit ground surface area, was calculated for each treatment combination. For this, we used a ceptometer and measured three times light interception above the canopy and below the canopy. Subsequently, 8 weeks after sowing, total above-ground biomass was collected through harvesting the plants at 1 cm above ground, sorting the invasive plant material from the natives, and placing them in different paper bags. All samples were then dried at 65°C for 3 days and weighted immediately after this period.

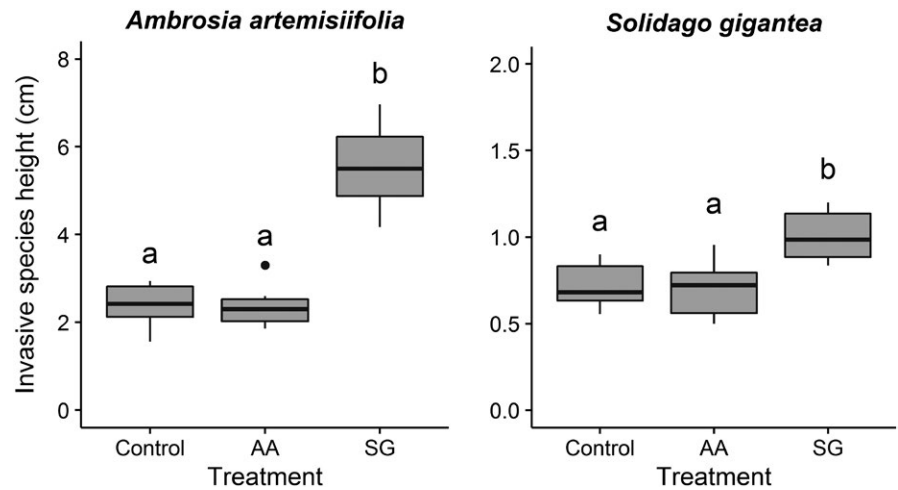
Using ANOVA we tested whether height, LAI and above-ground biomass of both invasive species were affected by community type (AA, SG, control), with subsequent multiple pair-wise comparisons using Tukey test. A *t*-test was used to identify differences in above-ground biomass between the two types of native communities. We used R v 3.1.0 for all statistical analysis.

## 3 | RESULTS

Four weeks after sowing, the height of the invasive species was significantly affected from the competing community (*A. artemisiifolia*:  $F = 36.2$ ,  $p < .0001$ ; *S. gigantea*:  $F = 7.0$ ,  $p < .0001$ ). The tallest *A. artemisiifolia* and *S. gigantea* plants occurred under competition with the SG community, while there were no significant differences between the AA community and the control consisting of invasive plants growing in a monoculture (Figure 1).

Eight weeks after sowing, the LAI was significantly affected by the community type ( $F = 125.0$ ,  $p < .0001$ ; Figure 2). The higher LAI values were found for the SG community, with no differences between the AA and control treatments. Community biomass also differed significantly, with SG having 49% more biomass than AA ( $t = 235.7$ ,  $p < .05$ ). In terms of the invasive species biomass, we found a significant effect of native community identity for both

**FIGURE 1** Four weeks after sowing height of the invasive species was different between monocultures of invasive species (control) and communities designed to specifically suppress *Ambrosia artemisiifolia* (AA) or *Solidago gigantea* (SG). Different letters indicate significant differences among treatments ( $p < .05$ )

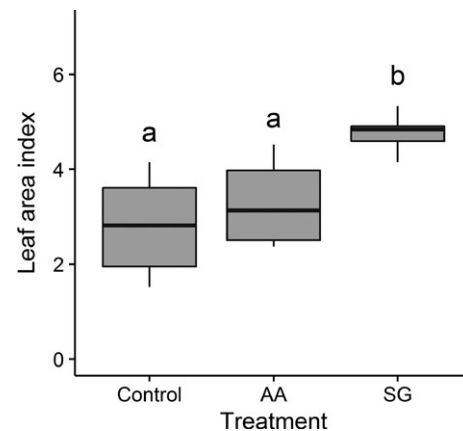


invasive species (*A. artemisiifolia*:  $F = 60.5$ ,  $p < .0001$ ; *S. gigantea*:  $F = 239.9$ ,  $p < .0001$ ; Fig. 3). For both invasive species, there was a lower above-ground biomass under competition with the community designed to suppress *S. gigantea*, followed by the AA community and the monoculture of the invasive species.

#### 4 | DISCUSSION

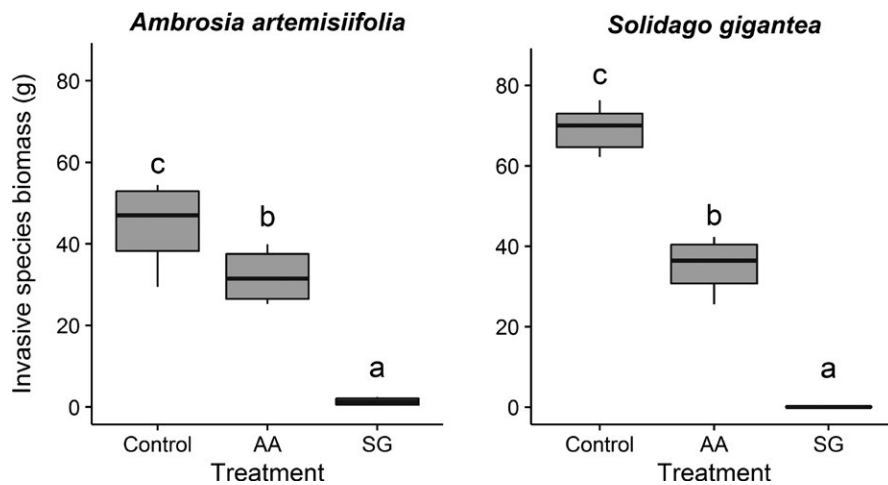
In our experiment, the composition of sown communities of native species significantly affected the early establishment of two invasive alien plant species. The community designed to suppress *Solidago gigantea* (SG) had a higher suppression effect over the two tested invasive species than the community designed for *Ambrosia artemisiifolia* (AA), compared to monocultures of both invasive species. Although it could be argued that our results partially support the limiting similarity hypothesis for *S. gigantea*, the SG community successfully suppressed both invaders and thus points to other predictors of resistance. In our experiment biotic resistance is mainly explained by native plant density instead of multi-trait limiting similarity using the method proposed by Laughlin (2014). A potential explanation for the lack of support is that the method can only maximize trait similarity within the trait space delineated by the chosen pool of species. In our case, the choice of traits available for community assembly was restricted due to our aim to use species that are frequent in our regional calcareous grasslands. This limitation is not a problem created by the method itself, but resulting from practical constraints and considerations. For instance, if a project seeks to restore a site to a grassland community, a proportion of the native species pool will be grasses, even if the invaders are not, thus not matching them in that characteristic (Karrer & Milakovic, 2016). The target native vegetation type for the project should, therefore, be considered in the context of the most significant invasive species potentially threatening the restoration project.

On the same lines, the limited support for the limiting similarity hypothesis in our experiment could be related to the use of traits obtained from trait databases. While these are measured on adult



**FIGURE 2** Community density measured as leaf area index, eight weeks after sowing, was different among monocultures of the invasive species (control) and communities designed for suppression of *Ambrosia artemisiifolia* (AA) or *Solidago gigantea* (SG). Letters indicate differences among treatments ( $p < .05$ )

plants, our experiment only covered the establishment phase. With the exception of seed mass, the used traits might not portrait resource acquisition or competitive ability during establishment (Hooper & Dukes, 2010; Yannelli, Koch et al., 2017). For instance, plant height, or traits related to leaf size could be useful as plant 'competitive tools' in fully established communities, but are perhaps less relevant at a very early phase of establishment. Yet, seed size provided a hint on the early competitive ability of the sown species, given that it is positively correlated with seedling height (Moles & Westoby, 2004). Another issue is related to trying to obtain trait information for all niche dimensions during the whole plant development (Funk et al., 2016). We might have failed to include juvenile traits related to competition that could explain our results in terms of limiting similarity at this early stage. Although the use of traits obtained from databases can help making inferences, in some cases the conditions in which they have been measured are not clear. Often the plant traits hold for one single population of adults measured at a single point in time, and seasonal, spatial or ontogenetic variation



**FIGURE 3** Eight weeks after sowing aboveground biomass of the two invasive species differed between monocultures of the invasive species (control) and the communities designed for suppressing *Ambrosia artemisiifolia* (AA) or *Solidago gigantea* (SG). Letters indicate differences among treatments ( $p < .05$ )

is not considered (Funk et al., 2016). Hence, if the limiting similarity hypothesis is used for designing native communities, we recommend utilizing trait information pertinent to the restoration phase, preferably gained at the respective site with a trial experiment.

In the SG community trait similarity was mostly determined by seed size (Table 2), resulting in a higher sowing density of natives matching a higher seeding density of *S. gigantea* compared to *A. artemisiifolia*. Although invasive plants responded to early competition by investing more into growing tall to escape the already developing native canopy, at the end of the experiment both invaders were intensively suppressed by the SG community. This suppression was most likely due to a density effect, as indicated by the larger LAI in SG communities compared to the other treatments. Higher sowing density of native species increases their chances of establishment due to the higher propagule numbers, which in turn translates into higher rates of resource acquisition (Lockwood, Cassey, & Blackburn, 2005). The higher biomass found for the SG community compared to AA also supports this idea, since it reflects the better ability to capture the available resources (Lulow, 2006). Similar results were found by Yannelli, Hughes et al. (2017) for the same invasive species when testing similarity in terms of seed size. In this study a higher suppression effect resulted from small-seeded native species at high density. Density of native grassland species also explained biomass decrease of *A. artemisiifolia* during the restoration of an abandoned quarry (Gentili, Gilardelli, Ciappetta, Ghiani, & Citterio, 2015). Overall, our study is in agreement with Byun, de Blois, and Brisson (2015), showing that a dense native cover resulting from high seed sowing density is effective for developing early successional communities resistant to invasions. Nevertheless, as noted by Byun et al. (2015), even if the native community exerts high suppression, future recruitment of the remaining invaders might still be possible. Another practical issue to take into account is that appropriate native seeds can be expensive, so sowing density should be carefully planned to avoid wasting seed material (Török, Vida, Deák, Lengyel, & Tóthmérész, 2011).

The identity and characteristics of the most abundant species of the seed mixtures, later dominant in the community, might also have contributed to the strong suppressive effect. In the mixture for the SG community, due to its small seeds, the forb *A. millefolium*

was seeded at high densities and later dominated the community. This species also had fast germination and a juvenile growth form with multi-leaved rosettes, which contributed to the formation of a very dense and strongly competitive canopy (F. A. Yannelli, personal observation). Furthermore, *A. millefolium* is native also to North America, where is one of the few species reported to have a competitive effect on the invasive perennial grass *Holcus lanatus* in coastal prairies of California (Deck, Muir, & Strauss, 2013). Similar results were reported by Milakovic and Karrer (2010) using mixtures dominated by *Lolium perenne* to suppress *A. artemisiifolia*, whereby a dense canopy of the tussock grass prevented the early establishment of this invader. Therefore, when choosing species for restoration starting from bare ground, the identity and early traits of the most abundant species should be taken into account.

## 5 | CONCLUSIONS AND IMPLICATIONS FOR MANAGEMENT

Even though our results could be interpreted as supporting the limiting similarity hypothesis at least for *Solidago gigantea*, the high suppression effect of the SG community on both invasive species points to other factors. We suggest that mainly the higher sowing density resulting from the smaller seeds included in the SG mixture, and the dominance of one competitive native forb are better predictors of biotic resistance against both invasive species. The lack of support for the limiting similarity hypothesis in our experiment using a system of linear equations to design resistant communities might have methodological reasons. We propose that the pool of native species that is used for designing the seed mixtures can prevent the identification of mixtures with maximum trait similarity to an unwanted invasive. Therefore, choosing appropriate natives for the species pool, and also using trait information related to their juvenile stage should increase the usefulness of the method. Based on our findings, an effective way to suppress invasive species during the early stage of restoration can be to increase seeding density and to include species with fast development of a competitive canopy.

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## ORCID

Florencia A. Yannelli  <http://orcid.org/0000-0003-1544-5312>

Johannes Kollmann  <http://orcid.org/0000-0002-4990-3636>

Tina Heger  <http://orcid.org/0000-0002-5522-5632>

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