RESEARCH ARTICLE

Crop functional diversity drives multiple ecosystem functions during early agroforestry succession

Diego dos Santos[1,2] | Fernando Joner[1] | Bill Shipley[3] | Marinice Teleginski[1] |
Renata Rodrigues Lucas[1] | Ilyas Siddique[1]

[1]Applied Ecology Lab (LEAp), Center of Agrarian Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil

[2]Federal University of Fronteira Sul (UFFS) campus Laranjeiras do Sul, Laranjeiras do Sul, PR, Brazil

[3]Département de Biologie, Université de Sherbrooke, Sherbrooke, QC, Canada

Correspondence
Diego dos Santos
Email: diegosantos@uffs.edu.br

Abstract

1. We still lack practical guidelines for diversifying cropping systems that consider both yield and regulating functions of agroecosystems. Recent studies have suggested that maximizing functional diversity (FD, the distribution of species with different functional traits in the niche space) may lead to agroecosystems with greater multifunctionality due to niche complementarity. Therefore, scientists are now advocating the use of a trait-based approach to develop multifunctional sustainable agroecosystems.

2. In order to assess the effects of crop FD on key agroecosystem functions and to consider trade-offs and synergies among them, we established, in late 2016, permanent experimental field plots of successional agroforestry systems (SAFS), in humid subtropical Southern Brazil. The experiment represents a gradient of plant functional trait diversity (designed FD based on leaf nitrogen concentration), while maintaining crop species richness constant across all treatments. Our hypothesis is that the observed FD of crops (hereafter, crop FD) drives multiple agroecosystem functions.

3. We determined the observed FD by quantifying traits of crops and weeds (maximum plant height, leaf area, specific leaf area) and performed two data collections (March and September 2017) at the end of the summer and winter crops respectively. We used structural equation modelling to test a hypothetical causal model to explain how crop FD affects three functions: weed suppression, soil protection (soil cover by either crops or weeds) and crop yield.

4. Our results support the hypothesis that high crop FD drives agroecosystem processes and contributes to the provision of multiple ecosystem functions. We found that with greater crop FD in SAFS, crop plants occupied a large niche space, thereby increasing the total photosynthetic light intercepted in the agroecosystem, that in turn, increased crop yield. Additionally, greater FD increased soil protection by crops and decreased weed cover. This greater FD also reduced the FD of the weed community.

5. *Synthesis and applications.* Crop mixtures based on complementary plant traits can increase the multifunctionality of agroecosystems through their sustainable use. A more heterogeneous structure and projection of crop leaf area drives greater

resistance to competition with weeds and produces higher crop yields in young diversified crop mixtures.

KEYWORDS

agroecological trade-offs, agroecosystem, ecological intensification, multifunctionality, plant traits, polycultures, syntropic agriculture

1 | INTRODUCTION

Modern cropping systems attempt to maximize one ecosystem service (e.g. food provision from a single crop), but this results in a reduction of biodiversity and ecosystem services related to ecosystem regulation (Power, 2010). In fact, some have argued (Isbell et al., 2017) that a loss of biodiversity affects ecosystem functioning and reduces its stability at the small spatial and temporal scales in which species interact with each other. If so, then an increase in agrobiodiversity could contribute significantly to the delivery of multiple ecosystem services (ES) (Wood et al., 2015).

If this claim is true then agricultural activity could jointly produce both commodity outputs (food, fuel and fibre) and improve certain other functions such as the management of biodiversity, soil protection and contribution to the socio-economic viability of rural areas— the so-called multifunctional agriculture (MFA) (Renting et al., 2009). Therefore, recent studies on biodiversity and ecosystem functioning have focused on multifunctionality (Allan et al., 2015; Blesh, 2018; Lefcheck et al., 2015). Otherwise, our understanding of this cause–effect relationship may be limited or biased if trade-offs or synergies among processes and functions are ignored (Blesh, 2018; Byrnes et al., 2014).

In order to link biodiversity and ecosystem processes in (semi-) natural systems, researchers are advocating the use of a trait-based approach to develop multifunctional agroecosystems, usually under the umbrella concept of 'ecological intensification' (Abalos et al., 2019). Recent studies have suggested that maximizing functional diversity (FD, the diversity of functional traits) may lead to agroecosystems with greater multifunctionality (Blesh, 2018; Finney & Kaye, 2017; Martin & Isaac, 2015; Storkey et al., 2015). However, implementation of this approach is hampered by our limited understanding of the relationship between plant traits and agroecosystem processes (Abalos et al., 2019). Furthermore, very few published studies have manipulated FD variation and tested for its effect on agroecosystem functions independently of species richness (Tobner et al., 2016).

We therefore use a running experiment to test the hypothesis that FD of crops affects multiple agroecosystem functions and leads to greater multifunctionality. In order to identify trade-offs and synergies among processes and functions we used structural equation modelling (SEM). This statistical method allows scientists to use data collected in the field to simultaneously test hypotheses about several, interconnected causal relationships of ecosystem functioning (Shipley, 2016). The core of the experiment consists of species mixtures having identical species richness but varying in FD. As in other long-term biodiversity experiments, for example, Jena Experiment (Roscher et al., 2004), this one was designed to be a long-term experiment that aim to understand the ecological mechanisms that underlie the biodiversity–ecosystem functioning. Here, we present the first results of this experiment, 1 year after its establishment. This is a critical period for several applications ranging from perennial agriculture, silviculture to ecological restoration, during which weed–crop competition strongly influences crop establishment, but also soil protection with potential cascading impacts on crop yield.

2 | MATERIALS AND METHODS

2.1 | Study site

The study site is located on the Ressacada Experimental Farm at the Federal University of Santa Catarina, Florianópolis, Brazil (27°41′7″S, 48°32′28″W). The region has a humid subtropical climate (Cfa— Köppen climate classification). Mean annual temperature is $21 \pm 4°C$ with a mean annual precipitation of $1,415 \pm 435$ mm (mean $\pm$ standard deviation, based on a 2008–2016 dataset from the adjacent meteorological station of Florianópolis International Airport). The soil is classified as Aquic Quartzipsamments (Soil Survey Staff, 2014) and consists of a 60–50 cm deep sandy layer (10% clay; pH 5.4 and 3.7% organic matter analysing 0–20 cm deep soil sample). The study site is a flat agricultural field at 5m above sea level that had been managed for rice production for several years before the establishment of the experiment. An area of approximately 0.15 ha was weeded using a rotating hoe and then crop species mixtures were hand planted (perennials) and hand sown (summer annuals) from 28 October 2016 to 24 November 2016 and from 10 May 2017 to 12 May 2017 (winter annuals). The establishment and management in the experimental area are detailed in Table S1.

2.2 | Experimental design

Agroforests were planted in square plots of 9 m × 9 m. The border around the outermost 0.5 m of each plot was not considered for sampling to avoid edge effects and interplot interactions. The species pool of crop plants comprised 19 species (Table 1).

We designed the experiment maintaining crop species richness constant across all treatments, and varying a single trait to choose the species composition of each treatment, that is, the leaf nitrogen concentration (LNC) of crops (hereafter '*designed FD*', that is,

TABLE 1 Names and additional information of species planted in the experiment

Scientific name[a]	Common name	Family name[a]	LNC[b]	Growth forms[c]	Life history	Growing season[d]
Arachis pintoi Krapov. & W.C. Greg	Pinto peanut	*Leguminosae*	H	Herb.	Perennial	n/a
Cajanus cajan (L.) Millsp.	Pigeonpea	*Leguminosae*	H	Woody	Perennial	n/a
Canavalia ensiformis (L.) DC.	Jackbean	*Leguminosae*	H	Herb.	Annual	Summer
Crotalaria spectabilis Roth	Showy rattlebox	*Leguminosae*	H	Herb.	Annual	Summer
Cymbopogon citratus (DC.) Stapf	Lemongrass	*Poaceae*	L	Herb.	Perennial	n/a
Inga semialata (Vell.) C. Mart.	Guabilla	*Leguminosae*	H	Woody	Perennial	n/a
Ipomoea batatas (L.) Lam.	Sweet potato	*Convolvulaceae*	H	Herb.	Perennial	n/a
Musa paradisiaca L.	Banana	*Musaceae*	H	Herb.	Perennial	n/a
Pachira glabra Pasq.	Saba nut	*Malvaceae*	L	Woody	Perennial	n/a
Pennisetum purpureum Schumach.	Elephant grass	*Poaceae*	L	Herb.	Perennial	n/a
Psidium cattleianum Afzel. ex Sabine	Strawberry guava	*Myrtaceae*	L	Woody	Perennial	n/a
Saccharum officinarum L.	Sugarcane	*Poaceae*	L	Herb.	Perennial	n/a
Sorghum bicolor (L.) Moench	grain sorghum	*Poaceae*	L	Herb.	Annual	Summer/Winter
Tetragonia tetragonioides (Pall.) Kuntze	New Zealand spinach	*Aizoaceae*	L	Herb.	Perennial	n/a
Trema micrantha (L.) Blume	Guacimilla	*Cannabaceae*	H	Woody	Perennial	n/a
Zea mays L.	Maize	*Poaceae*	L	Herb.	Annual	Summer
Raphanus raphanistrum L. Domin	Wild radish	*Brassicaceae*	H	Herb.	Annual	Winter
Vicia sativa L.	Common vetch	*Leguminosae*	H	Herb.	Annual	Winter
Avena strigose (Schreb.)	Black oat	*Poaceae*	L	Herb.	Annual	Winter

[a]According to The Plant List (http://www.theplantlist.org/) accessed in August 2018.

[b]Leaf nitrogen concentration used for designing trait diversity: H = high, LNC > 25 mg/g; L = low, LNC < 25 mg/g.

[c]Growth forms according to Pérez-Harguindeguy et al. (2013): herbaceous plants have either no or at most modest secondary growth, with stem and root tissues that are rather soft compared with typical wood.

[d]n/a = not applicable.

FD imposed by the experimental design). We chose LNC as criterion because its value as a proxy of tissue quality is widely recognized by practitioners for being a key driver of mulch decomposition, which represents a trade-off of a given amount of available biomass for mulching between nutrient release of high LNC (low C:N) residues on the one hand, and soil protection/weed prevention of low LNC (high C:N) residues on the other (Erenstein, 2003; Finney et al., 2016). Due to the widespread use of categorical criteria of species choice by practitioners that simplify tissue quality (Finney & Kaye, 2017), we applied a binary dichotomy of crops with high versus low LNC, with which we composed three intercrop compositions with constant species richness. Thus, our designed FD was based

on plot treatments with crop mixtures of (a) eight species with high LNC (>25 mg/g), (b) eight species with low LNC (<25 mg/g) and (cd) a combination of four species with high and four species with low LNC (called medium LNC).

However, we measured a number of traits to calculate a field-established community-weighted, *observed FD* per plot (hereafter 'crop FD'), used for all subsequent analyses, in order to capture a substantial portion of trait variation relevant to short-term crop–weed competition and agroecosystem functioning and performance. Observed crop FD was positively related to designed, community-level mean leaf N concentrations, that is, plot treatments based on broad categories of LNC relevant for practical adoption by land

managers (Figure S1). In our experiment, all crop legumes were putative N-fixers (Sprent, 2001).

Each community was replicated six times in a randomized complete block design with 18 plots. Within plots, species were planted in four strips according to their growth form and life history (see details in Figure S2). Within strips, a two species mixture was planted at the density according to agronomic recommendations (see details in Figure S3a–f). The distribution of plant growth forms within plots was identical in all six blocks. Plants were fertilized with compost produced from urban organic residues at a constant rate of 4 L/m planting row. For details regarding species, plot type and strips see Tables S2 and S3.

2.3 | Data collection

We collected data on strips within the plots, at the full flowering stage of annual herbaceous plants, around 100 days after planting. Besides using this stage as reference due to management necessities, all crops were assessed. Specificities of each variable are described below. We assessed the following agroecosystem variables: (a) Leaf area index (LAI), (b) crop yield, (c) soil cover by crops (hereafter crop cover), (d) soil cover by weeds (hereafter weed cover). The last two variables combined were considered surrogates for the competitive ability of crops against weeds (weed suppression, i.e. crop cover – weed cover) and for potential soil protection (i.e. crop cover + weed cover).

Leaf area index (LAI; m^2/m^2) was indirectly determined using a Ceptometer AccuPAR LP-80 (Garrity, 2014). We positioned the equipment above and below the canopy of crops, always above the weeds, in order to exclude the contribution of weeds and obtain the crop LAI in both rows and interrows. We collected data in two periods (March and September 2017) on all strips within the plots, at the full flowering stage of annual herbaceous species, around 100 days after planting. As mentioned above, within plots, species were planted in four strips according to their growth form and life history; therefore, in order to estimate one average value of LAI for each plot we calculated the weighted mean, considering the proportion of area occupied by each strip in the plot: (a) erect annuals, occupied 50% of the plot; (b) shrubs, 25%; (c) basal herbs, 12.5%; (d) shrubs/giant herbs, 12.5%.

2.3.1 | Crop yield

We sampled crop yield according to growth form and planting density: (a) basal herbs inside a 0.5 m × 0.5 m quadrat; (b) erect annual herbs along a 0.5 m linear transect (and seeds in a 8 m transect); (c) shrubs/giant herbs in a 1 m linear transect along the crop row. From these samples we collected data of crop products that humans use for food or green manuring, that is, above-ground biomass of the species that was achieved at the beginning of its reproductive phase, except for jackbean (*Canavalia ensiformis* (L.) DC.), grain sorghum

(*Sorghum bicolor* (L.) Moench) and showy rattlebox (*Crotalaria spectabilis* Roth), for which we collected seeds because of the demand for their germplasm (see details in Table S1). The biomass was dried to constant mass at 80°C. First, we collected two randomized samples of each species at the beginning of its reproductive phase in each plot. These samples were extrapolated to square metres. Second, the mean of these two samples was used in order to sum the crop yield in each strip within plots, since each strip was a mixture of two species. Finally, we used this value to calculate the weighted mean (considering the proportion of each strip occupied by that species in the plot) to estimate the average in the plot. Due to different scales of measurement among plant species, we standardized yield data to standard deviation.

$$\text{Crop yield} = \sum_{i=1}^{4} C_i p_i,$$

where C_i is the crop yield of each strip within plots and p_i represents the area proportion that each strip occupied within plots (erect annuals = 0.5; shrubs = 0.25; basal herbs = 0.125; shrubs + trees = 0.125).

$$C = \sum_{j=1}^{2} W_j,$$

where W_j is the standardized crop yield of each species within strips.

$$W = \frac{\frac{x_1}{s} + \frac{x_2}{s}}{2},$$

where x is the crop yield of each sample by species (in kg/m^2) and s is the standard deviation of crop yield considering each species.

For example, considering one plot planted with a mixture of *Pennisetum purpureum* and *Cajanus cajan*, we measured the above-ground biomass in a 1 m linear transect along the crop row, twice, for each species. The mean of the samples (extrapolated to kg/m^2) was standardized, summed and multiplied by the proportion of 0.25 (considering that the strips of shrubs/giant herbs occupied 25% of the plot).

2.3.2 | Crop cover and weed cover

The vegetation composition, species abundance and proportion of soil cover (crop cover and weed cover) were assessed in 0.5 m × 0.5 m quadrats. All plants (crops and weeds) inside the quadrat were identified and their abundance estimated visually using a cover percentage (Lavorel et al., 2008) in the following categories: less than 1%, between 1% and 5% and subsequently in intervals of 10% from 10 to 100 (Londo, 1976). Within plots, two samples were taken, randomly, per type of strip, totalling eight samples per plot. These two samples were used to calculate the mean relative abundance for each plant species within strips, and the mean of weed cover and crop cover within strips. Therefore, the mean of each two quadrats was used

for calculating the weighted mean to estimate the average at the plot scale. We collected data in two periods (March and September 2017) on strips within the plots at the full flowering stage of annual herbaceous plants, around 100 days after planting. In all, 36 sample units were used for data analysis (18 plots and 2 sample periods).

We used standardized protocols (Pérez-Harguindeguy et al., 2013) to determine three plant traits (see Table S4) that are related to the agroecosystem processes assessed in this work (Table 2; Bàrberi et al., 2018; Díaz et al., 2016; Gaba et al., 2017). We measured traits in all crop species on the most frequent weed species (observed in >50% of sample quadrats) and the most abundant weed species (that collectively, i.e. consider all species, made up at least 80% of cumulative relative abundance in each sample quadrat). This threshold was established to ensure a satisfactory description of community properties in relation to biogeochemical cycles in ecosystems (Garnier et al., 2004; Pakeman & Quested, 2007). We determined traits in 19 of 40 weed species present in the experiment in the first period, and in 38 of 76 weed species present in the experiment in the second period (see Table S5).

2.4 | Measuring functional diversity (FD)

Functional diversity (FD) were assessed for both crop and weed communities. This index was calculated using 'SYNCSA' (Debastiani & Pillar, 2012) package in R (R Core Team, 2018). Rao's quadratic entropy (Rao, 1982) has been considered an appropriate measure for FD (Botta-Dukát, 2005; Pillar et al., 2013):

$$FD_Q = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij} p_i p_j,$$

where d_{ij} is a measure of distance between species i and j based on a set of specified functional traits.

Rao's quadratic entropy (Rao, 1982) is an index of functional diversity that incorporates both the relative abundances of species and a measure of the pairwise functional distance between species (Botta-Dukát, 2005). In order to obtain a dissimilarity matrix with Euclidean metric properties, the default function built into 'SYNCSA' package calculates the square root of the one-complement of Gower's similarity index (which ranges from 0 to 1; Debastiani & Pillar, 2012; Pillar et al., 2013).

2.5 | Data analysis

Multivariate analysis in the form of piecewise structural equation modelling (SEM) was conducted in the R software using the packages 'NLME' (Pinheiro J et al., 2018) and 'PIECEWISESEM' (Lefcheck, 2016). A data exploration protocol was used (Zuur et al., 2010) and performed with packages 'LATTICE' (Sarkar, 2008), 'GGPLOT2' (Wickham, 2009) and 'GGCORRPLOT' (Kassambara, 2016).

An initial general hypothetical model (Figure 1) was formulated in order to be tested using SEM. The hypothetical model was used as a framework to fit the collected data. After fitting and parameter estimation, not all initial variables were still part of the model, due to statistical non-significance ($p > 0.05$; Shipley, 2009). Additionally, considering the experimental design, the six blocks and the two periods were considered random factor variables in the model. Therefore, we applied mixed effects modelling and restricted maximum likelihood (REML) estimation method (Shipley, 2013; Zuur et al., 2009).
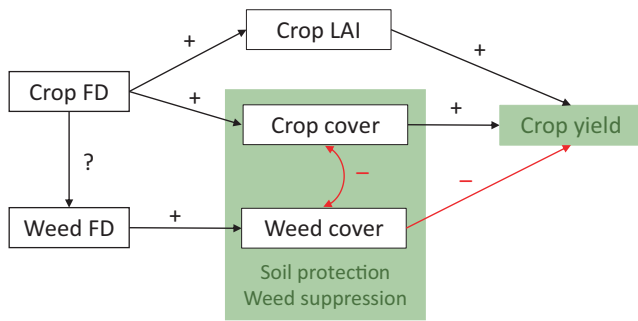
When the initial model was fitted, Fisher's C statistic was calculated to evaluate the model goodness-of-fit. If the data are generated following the causal hypothesis specified in the fitted SEM, this C statistic will follow a chi-squared distribution. If the chi-square is statistically non-significant ($p > 0.05$) the existing deviation between the model and the data is consistent with random sampling variation and so the model is not rejected (Lefcheck, 2016; Shipley, 2009).

Exploratory path analysis was conducted by adding or removing arrows from the hypothesized relationships. In order to account for relations not initially considered between response and predictor variables we used the 'missing paths' command built into the 'piecewise SEM', which provides information supported by statistical significance to improve the initial model (Lefcheck, 2016; Stenegren et al., 2017). If this new relationship was statistically significant ($p < 0.05$) and had biological significance, it was included and the model was tested again. Subsequently, non-significant parameters were excluded from the model. We used graphical tools in order to validate the assumptions of each final SEM. Normality and homogeneity of variances were verified on standardized residuals (Zuur et al., 2009).

3 | RESULTS

Our initial hypothesized model (Figure 1) was not rejected by the data ($C = 14.229$, $df = 14$, $p = 0.43$) but we proceeded an exploratory analysis in order to account for relations not initially considered and to remove non-significant parameters. In our final model ($C = 14.984$,

TABLE 2 List of plant functional traits and their ecological functions directly relevant to growth, survival and reproduction (Díaz et al., 2016; Pérez-Harguindeguy et al., 2013). The quantitative traits were determined from the mean of five replicate individual plants (10 for plant height), collected in the experimental plots or nearby

Trait (unit)	Functions
Maximum plant height (m)	Corresponds with the ability to pre-empt light resources and disperse diaspores
Leaf area (mm^2)	It has important consequences for leaf energy and water balance
Specific leaf area (mm^2/mg)	It is often positively related to potential relative growth rate across species

FIGURE 1 General hypothetical model exploring the effects of agroforest crop functional diversity on weed functional diversity, soil protection and crop productivity. Boxes represent measured variables. Green rectangles represent agroecosystem services. Arrows represent unidirectional relationships among variables and double-headed arrows represent relationships between variable pairs whose causal linkage is not specified
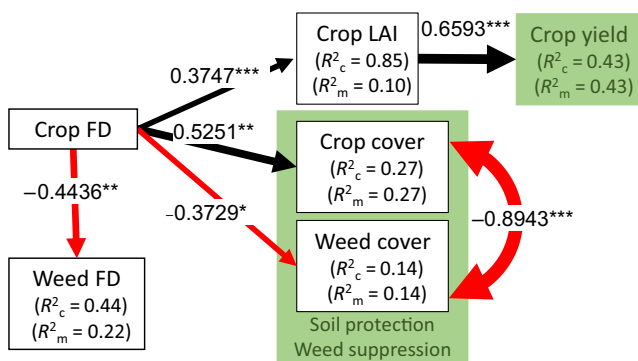
FIGURE 2 Final structural equation model describing the effects of crop functional diversity on weed functional diversity, soil protection and crop productivity during early agroforest succession. Boxes represent measured variables. Green rectangles represent agroecosystem functions. Arrows represent unidirectional relationships among variables and double-headed arrows represent relationships whose causal links are not specified. Black arrows denote positive, and red arrows negative, relationships. The thickness of the significant paths (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$) has been scaled based on the magnitude of the standardized regression coefficient or correlation coefficient, given next to the arrows. The marginal and conditional R^2 (based on the variance of the fixed and both the fixed and random effects respectively) for component models are given in the boxes of response variables. $N = 36$. AIC = 64.984. Fisher's $C = 14.984$ with p-value = 0.663 and on 18 df

were negatively correlated (magnitude of -0.8943, $p < 0.01$), crop FD increases weed suppression as well as soil protection through crop cover, as initially hypothesized. The relationship between crop cover and weed cover was modelled as a free covariance rather than unidirectional causal one since both can affect one another due to reciprocal crop–weed competition.

The marginal R^2 values (in Figure 2) describe the proportion of variance explained by the fixed factors. The conditional R^2 values (in Figure 2) describe the proportion of variance explained by both fixed and random factors. For example, crop functional diversity (fixed factor), experimental blocks and periods (random factors) explain 85% of the variance in LAI of crop ($R^2 = 0.85$). Scatter plots illustrating relationships confirmed by structural equation modelling are in Figure S4.

4 | DISCUSSION

Designing crop mixtures with high FD may be particularly effective for increasing multifunctionality and agroecosystem sustainability (Blesh, 2018). Our study suggests that crop mixtures designed with complementary plant traits could increase the multifunctionality of agroforests. We found that the higher the FD at the agroforest plot scale, the greater the amount of photosynthetic light it intercepts. Greater light interception, in turn, increases agroforest yields. Additionally, agroforests with high crop FD protect a greater proportion of soil by live crop cover and reduce weed cover. Although our results may partly result from other mechanisms (e.g. below-ground niche differences), architectural differences, temporal niche differences and morphological plasticity are the main ones responsible for enhancing the amount of light in mixtures of trees (Sapijanskas et al., 2014), and hence most likely contributed to explain the positive indirect effect of crop FD and crop yield in our agroforestry mixtures.

Functional trait diversity can provide multiple benefits in agroecosystems (Martin & Isaac, 2015). However, there are also potential trade-offs among functions in which increasing functional diversity can enhance some and decrease others (Blesh, 2018). In our study, for example, crop FD enhanced soil cover by crops and decreased both weed FD and weed cover. Weeds are part of agroecosystems and contribute in different ways to their functioning (Guerrero et al., 2014; Marshall et al., 2003). Therefore, some advantages of ecological intensification and agroecosystem multifunctionality are hampered by extirpating weed diversity from agroecosystems. Our observed reduction in weed FD was probably due to crop resource pre-emption, which can drive weed suppression (Baraibar et al., 2018; Mwangi et al., 2007). When crops occupy more niches, weeds that would occupy similar niches are suppressed by the system. Therefore, increasing crop diversity is an effective weed management strategy and can reduce reliance on external agricultural inputs by several mechanisms (Gaba et al., 2017; Storkey & Neve, 2018). Although weeds can compete with crop plants and reduce yield (Oerke, 2006; Park et al., 2003), our data did not show

$df = 18$, $p = 0.66$), crop FD is the only variable that drives responses in agroecosystem functions (Figure 2). It has a direct effect on (a) weed FD (raw regression coefficient, $b = -0.5026$, $p < 0.01$); (b) weed cover (raw regression coefficient, $b = -0.5717$, $p = 0.03$); (c) crop cover (raw regression coefficient, $b = 0.9299$, $p < 0.01$) and; (d) LAI of crops (raw regression coefficient, $b = 4.8921$, $p < 0.01$). In turn, the LAI of crops directly affects the productivity of crops (raw regression coefficient, $b = 1.0579$, $p < 0.01$). As crop cover and weed cover

any direct causal linkage between crop or weed cover and crop yield. Presumably, the weed community in our systems has not reached a level that affects crop yield. Our review of the applied literature in agriculture, forestry and restoration, as well as dialogue with decision makers suggests that the choice of diverse species mixtures by practitioners is more easily adopted if based on recognizable categories (e.g. high versus low) of LNC than on complex a priori calculations of gradients of species mean FD. Therefore, in order to produce a functional diversity gradient we mixed different species in three species compositions. In this way, it is possible that our results are dependent on the identity of groups of plants, such as grasses (for high yielding of carbon-rich biomass) and legumes (N-rich species). We are aware that legume species may be an important driver in diversity experiments (Hooper et al., 2005; Taylor et al., 2020) and influence results as source of misinterpretation (Huston & McBride, 2002)—that is, by the sampling effect for grass–legume combinations, rather than the niche effect among many species. For this reason, more recent biodiversity–ecosystem functioning experiments disentangle legume effects from pure diversity effects through appropriate experimental design (Roscher et al., 2018) or by excluding legume species from the experimental design (Ebeling et al., 2014). However, the plant species selected for this experiment are widely used in Brazil and the use of legumes is pervasive in agroforestry throughout the world (Duchene et al., 2017). Therefore, we consider our results to be applicable to many agroecological practices and designs. Furthermore, one of the reasons for an increased the number of research papers using the trait-based approach in recent years is the promise of its generality, synthesis and predictive ability, despite the idiosyncratic details of each site, taxonomic assemblage or geographical location (Shipley et al., 2016). Besides the inherent limitations of an experiment, the so-called trait-based biodiversity experiments (TBEs) are an important next step in order to directly analyse not only trait contributions to specific processes, but also the relative importance of selection and complementarity effects on multiple ecosystem processes (Ebeling et al., 2014).

Although further investigations should address the effect of functional diversity on community multifunctionality in a range of agroecosystems over time, our results suggest that agroecosystem design should include plants with complementary trait values. Therefore, in order to integrate principles of ecological intensification in agriculture, which implies an increase in plant diversity, it is worth intercropping species that could provide a heterogeneous canopy structure and leaf area projection. This design could promote a more weed-resistant and productive cropping system, with greater soil protection.

The most likely method of improving the environmental sustainability of agriculture is designing agroecological cropping systems that increase biodiversity and simultaneously generate multiple agroecosystem services (Damour et al., 2018). In this way we hypothesize that by using a trait-based management and by providing a high FD of crops, it is possible to provide high levels of multifunctionality in agroecosystems (Figure 3). We can achieve high FD levels at the field, farm or landscape scale, allowing agroecosystems to
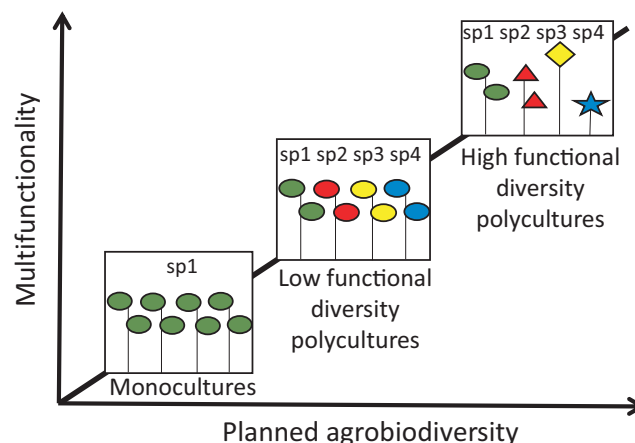
FIGURE 3 Conceptual framework to explain the functional diversity hypothesis: Increasing planned agrobiodiversity along a functional diversity gradient provides greater multifunctionality in agroecosystems. Bottom left: Equal shapes and colours represent a single crop species. Centre: Plants with equal shapes but different colours represent different crop species with similar functional traits. Top right: Different shapes and colours represent species that vary greatly in their functional traits

meet the demands of new community values, such as resilience to climate change (Berthet et al., 2019). In order to validate the functional diversity hypothesis (Figure 3), future studies should simultaneously assess FD and several relevant ecosystem functions along a broad gradient of both taxonomic and functional diversity of agroecosystems, from commercial monocultures to diversified agroforestry systems. By exploring whole-ecosystem responses to changes in biodiversity, long-term experiments such as 'The Jena Experiment' have had an inestimable role in biodiversity–ecosystem functioning research (Weisser et al., 2017); however, on-farm experiments not only reflect realistic environmental and socioeconomic contexts, but can also involve farmer participation in order to define functional diversity management based on goals directly relevant to farming practice (Blesh, 2018). Practitioner-scientist collaborative research could help elucidate ecologically meaningful thresholds along continuous functional trait gradients (such as leaf N or C:N ratio), to separate functional groups of crops into an agronomically practical classification, without suffering from the shortfalls of crop functional groups highlighted by Finney and Kaye (2017). Such agroecologically meaningful functional groupings could then serve as simplified decision tool for the choice of intercrop compositions with sufficient designed FD, an important research gap that is directly relevant to the practical application of our functional diversity hypothesis.

AUTHORS' CONTRIBUTIONS

I.S. and F.J. originally formulated the idea, conceived and designed the experiment; D.d.S., M.T. and R.R.L. conducted fieldwork; B.S. contributed to methodology and data analysis; D.d.S. analysed the data, wrote the first draft of this paper and coordinated the subsequent contributions made by the others authors. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Dataset and R code are available via the Zenodo http://doi.org/10.5281/zenodo.4025187 (Santos et al., 2021).

ORCID

Diego dos Santos https://orcid.org/0000-0002-5546-9194
Fernando Joner https://orcid.org/0000-0002-8896-3607
Bill Shipley https://orcid.org/0000-0002-7026-3880
Marinice Teleginski https://orcid.org/0000-0001-8138-0946
Renata Rodrigues Lucas https://orcid.org/0000-0001-9991-4926
Ilyas Siddique https://orcid.org/0000-0002-0519-2086

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SUPPORTING INFORMATION

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