Abstract

Plant monocultures growing for extended periods face severe losses of productivity. This phenomenon, known as 'yield decline', is often caused by the accumulation of above- and belowground plant antagonists. The effectiveness of plant defences against antagonists might help explaining differences in yield decline among species. Using a trait-based approach, we studied the role of 20 physical and chemical defence traits of leaves and fine roots on yield decline of 18-year old monocultures of 27 grassland species.

We hypothesized that yield decline is lower for species with high defences, that root defences are better predictors of yield decline than leaf defences, and that in roots, physical defences better predict yield decline than chemical defences, while the reverse is true for leaves. We additionally hypothesized that species increasing the expression of defence traits after long-term monoculture growth would suffer less yield decline. We summarized leaf and fine root defence traits using principal component analysis and analysed the relationship between defence traits mean as a measure of defence strenght and defence traits temporal changes of the most informative components and monoculture yield decline.

The only significant predictors of yield decline were the mean and temporal changes of the component related to specific root length and root diameter (e.g. the so called collaboration gradient of the root economics space). The principal component analysis of the remaining traits showed strong trade-offs between defences suggesting that different plant species deploy a variety of strategies to defend themselves. This diversity of strategies could preclude the detection of a generalized correlation between the strength and temporal changes of

defence gradients and yield decline. Our results show that yield decline is strongly linked to belowground processes particularly to root traits. Further studies are needed to understand the mechanism driving the effect of the collaboration gradient on yield decline.

Keywords

antagonists, collaboration gradient, functional traits, mutualists, performance change, trait plasticity.

Introduction

Most crop monocultures growing in the same field for extended periods of time face severe losses of productivity (Bennett et al. 2012, Zhao et al. 2020). In agricultural settings, this phenomenon is known as 'yield decline' (Bennett et al. 2012). Recently, it has also been observed for non-crop species in several grassland diversity experiments (Guerrero-Ramírez et al. 2017), including the Jena Experiment (Meyer et al. 2016, Dietrich et al. 2020). In biodiversity research, monoculture yield decline is one of the reasons why experiments find increased positive biodiversity–ecosystem functioning relationship through time (Eisenhauer et al. 2012, Meyer et al. 2016). One of the major drivers of yield decline is the accumulation of below- and aboveground plant antagonists through time (Bennett et al. 2012, Benitez et al. 2021), which has been well documented in agricultural (Bennett et al., 2012) and experimental plant-soil-feedback studies (Mills and Bever 1998, Maron et al. 2011, Schnitzer et al. 2011, Latz et al. 2012, Kulmatiski et al. 2012, van der Putten et al. 2013, Cortois et al. 2016, Wang et al. 2019). These antagonists include pathogenic fungi, bacteria, and protists (Petermann et al. 2008, Hilton et al. 2013, 2021, Xu et al. 2015, Neupane et al. 2021, Li et al. 2022) as well as herbivores, such as plant-feeding nematodes (Jones et al. 2013, Grabau and Chen 2016, Wilschut et al. 2019) and arthropods (Brust and King 1994, Spencer et al. 2014). To counteract the effects of aboveground and belowground antagonists, plants evolved a plethora of defence strategies (reviewed in Hanley et al. 2007, Raguso et al. 2015, Moore and Johnson 2017). Despite the majority of species experience yield decline, the extent of yield decline differs substantially between species (Bennett et al. 2012, Marquard et al. 2013, Eisenhauer et al. 2019, Zhao et al. 2020, Dietrich et al. 2020). The differences in type and strength of plant

52 defence strategies in leaves and fine roots could be one mechanism to explain the differences
53 in yield decline observed between plant species (Figure 1 panel A). In plant ecology, defence
54 strategies are generally divided into physical and chemical defences and often characterised
55 using plant functional traits, such as tissue toughness or the presence of toxic compounds
56 (Poorter et al. 2004). Using a trait-based approach, we aim to study the importance of physical
57 and chemical defence traits in leaves and fine roots for yield decline of 18-year old
58 monocultures of 27 grassland plant species.

59 Both below- and aboveground antagonists have detrimental effects on plant performance and
60 thus contribute to yield decline (Bennett et al. 2012). However, in grassland systems, the
61 effect of belowground antagonists on plant fitness often exceeds aboveground effects
62 (Stanton 1988, Rasmann and Agrawal 2008). Root herbivores, such as plant-feeding
63 nematodes and insect chewers are among the most abundant and effective antagonists
64 (Andersen 1987, Ingham and Detling 1990, Zvereva and Kozlov 2012, Johnson et al. 2016b,
65 van den Hoogen et al. 2019) and are often the cause of yield decline for several crop species
66 (Bennett et al. 2012). The importance of root antagonists in long-term monocultures is further
67 amplified by their lower mobility compared to aboveground antagonists, which move more
68 easily between hosts in the canopy (Brown and Gange 1990). In the short term, the reduced
69 mobility of root antagonists decreases the probability of a belowground attack compared to
70 an aboveground one. However, in long-term monocultures, once a suitable host is found, root
71 antagonists likely enforce a stronger and more persistent pressure on the plant compared to
72 aboveground antagonists (Johnson et al. 2016a). This effect could be amplified by the longer
73 plant-feeding life stages of many root insect herbivores (Brown and Gange 1990) or the quick

74 growth of root-feeding nematodes populations. Thus, in long-term monocultures, the
75 probability and severity of a belowground attack increases over time, while it may not as much
76 aboveground. According to optimal defence theory, allocation to defence depends on the
77 value of the plant tissue, the benefit from defence, and the probability of attack (Stamp 2003).
78 Even though tap or coarse root are more vulnerable to chewing herbivore attacks, a great
79 number of root antagonists, such as nematodes and pathogens, prefer to feed on fine roots
80 (Tsunoda and van Dam 2017). A higher probability and severity of belowground attack should
81 therefore support a higher allocation of resources to root defences to counteract the
82 accumulation of root antagonists (Figure 1 panel C-1). However, the benefit of defence
83 depends on the efficiency of protection against the most important antagonists. Physical
84 defences, such as tissue toughness, are known to be a major defence, especially against insect
85 plant chewers (Hanley et al. 2007, Caldwell et al. 2016, Johnson et al. 2016b, Hervé and Erb
86 2019, Freschet et al. 2021b). For example, Johnson et al. (2010) found that root toughness
87 significantly reduced the ability of wireworms to feed on modified tobacco roots. Plant
88 physical defences are strongly associated with compounds embedded in the cell wall, such as
89 cellulose, lignin, or silica (Moore and Johnson 2017). In addition to increasing the mechanical
90 strength of a tissue, they also reduce tissue palatability for many invertebrates (Cooke et al.
91 2016, Moore and Johnson 2017). Another strategy to counteract negative effects of
92 belowground antagonists is to collaborate with mutualists. Along the recently defined root
93 economics space (Bergmann et al. 2020), this is captured by the collaboration gradient,
94 defined by a trade-off between specific root length and root diameter, which is positively
95 related to the presence of mycorrhizal fungi. In grassland species, the presence of arbuscular
96 mycorrhizae (AMF) can reduce herbivory rates from several groups of root antagonists

97 (Rasmann et al. 2011) due to competition for space and resource with nematodes and
98 pathogens, by promoting plant tolerance and by inducing plant defences (reviewed in Frew et
99 al., 2021). For instance, two recent studies showed that the fine roots of species on the
100 outsourcing side of the collaboration gradient, thus with high root diameter and low specific
101 root length and potentially high mycorrhization rates, harbour fewer root-feeding nematodes
102 than species on the 'do-it-yourself' side of the gradient with thinner roots (Otfinowski and
103 Coffey 2020, Dietrich et al. 2021). Overall, species that invest in belowground physical defence
104 strategies and on collaboration with mutualists, such as AMF should be able to counteract the
105 accumulation of major belowground antagonists and reduce yield decline in long-term
106 monocultures (Figure 1 panel C-2).

107 Aboveground, yield decline has primarily been linked to invertebrate herbivores and leaf spot
108 diseases caused by fungi or protists (Fernandez et al. 1998, Bailey et al. 2001, Jalli et al. 2021).
109 Antagonist accumulation over time is mostly associated with soil- or litter-bound larval or
110 dormant stages (Reavey and Gaston 1991, Johnson et al. 2006, Judelson 2008, Jain et al. 2019).
111 However, during their aboveground life stages, antagonists are more mobile and thus more
112 likely to find a suitable plant host or to change the host in shorter intervals (Johnson et al.
113 2016a). In addition, aboveground insect herbivores are expected to have a higher species
114 richness and feeding guild diversity than their belowground counterparts (Rasmann and
115 Agrawal 2008). As a consequence, aboveground plant canopies face a more diverse antagonist
116 community than plant roots, and attacks aboveground may be more frequent, but potentially
117 less severe (Rasmann and Agrawal 2008, Zvereva and Kozlov 2012). This calls for a more
118 diverse and flexible defence strategy aboveground. Plants harbour an extremely diverse

arsenal of defensive phytochemicals (Wetzel and Whitehead 2020). These can act either directly as toxins or indirectly through the attraction of natural enemies (Raguso et al. 2015), potentially providing a quick and effective defence against the multitude of aboveground antagonists. Whitehead et al. (2021) found that the number of apple antagonist species that are negatively affected by a mixture of phenolics in bio-assays increases with the structural diversity and richness of phenolics in the mixture. This suggests that phytochemical diversity is an important dimension of plant defence when plants are facing a quick turn-over of multiple antagonist species. Leaf physical defences, effective protection against leaf chewers (Hanley et al. 2007, Loranger et al. 2012, Muiruri et al. 2019), may be less effective to cope with the high diversity and quick turn-over of aboveground antagonists. Thus, aboveground a strategy relying on the diversity of defensive phytochemicals seems to be more promising than physical defences to cope with aboveground antagonists and forestall yield decline in long-term monocultures (Figure 1 panel C-3).

Plant functional traits provide a quantitative framework that might help to understand the relationship between plant fitness and the environment by quantifying plant morphological, physical, and phenological characteristics (Violle et al. 2007). Plant defence traits are those traits that promote plant fitness in the presence of antagonists relative to when antagonists are absent (Didiano et al. 2014). The type and intensity of defence can vary substantially across species (Moles et al. 2013). Species investing in a high mean expression of specific defence traits, which are well-suited against dominant antagonists may be able to maintain a high yield in monocultures over time (Figure 1 panel A). However, plant defence traits show high phenotypic plasticity in response to current selective pressure through antagonists, even

141 within short time frames (i.e. one growing season) (Poorter et al. 2019, Ojha et al. 2022). Given
142 more time, strong selection by antagonists can result in altered plant defence trait expression
143 through microevolution (Didiano et al. 2014). Indeed, plant species growing either in
144 monoculture or mixture for eleven years showed genetic and epigenetic trait divergence in
145 the Jena Experiment (van Moorsel et al. 2018, 2019). Thus, if the accumulation of antagonists
146 is a major selective pressure in monocultures, and an increased level of defence promotes
147 fitness, long-term monocultures should express higher levels of defence traits compared to
148 young monocultures (delta defence, Figure 1 panel B). Overall, plant species with either a high
149 mean expression of specific defence traits or species able to increase their defence in response
150 to the accumulation of antagonists over time, should show lower levels of yield decline in long-
151 term monocultures (Figure 1 panel C-4).

152 In this study, we measured 20 physical and chemical defence traits (summarised in Table 1) of
153 27 grassland plant species growing in monocultures for 4 (young monocultures) and 18 years
154 (old monocultures) in the Jena Experiment. For each species, we estimated total above- and
155 belowground physical and chemical defences by summarizing the 20 individual defence traits
156 with principal component analyses. We used the scores of the most informative principal
157 components to calculate species-specific mean defences for old and young monocultures
158 together as a measure of defence strength and the difference (delta defences) between old
159 and young monocultures as a measure of defence temporal changes. We estimated yield
160 decline for each species using the monoculture aboveground biomass temporal trend over 17
161 years. We then use mean and delta defences to explain different levels of yield decline across
162 species. We tested the following hypotheses:

1 Fine root defences are a stronger predictor of monoculture yield decline than leaf defences (Figure 1 panel C-1).

2 Fine root physical defences and mutualistic collaboration are stronger predictors of monoculture yield decline than fine root chemical defences (Figure 1 panel C-2).

3 Leaf chemical defences are a stronger predictor of monoculture yield decline than leaf physical defences (Figure 1 panel C-3).

4 Defence strenght and temporal changes (difference in defence between old and young monocultures) of fine roots and leaves are both important predictors of yield decline (Figure 1 panel C-4).

Material and methods

Study site and experimental design

The monocultures sampled in this study are part of a large grassland biodiversity experiment, the Jena Experiment. The experiment is located along the Saale River's floodplain near Jena (Thuringia, Germany, latitude 50.95, longitude 11.62, altitude 130 m a.s.l.). The regional mean annual air temperature is 9.9°C, and annual precipitation is 660 mm (1980–2010) (Hoffmann et al. 2014). In 1960, the experimental site was converted from grassland to a highly-fertilized arable field until the start of the experiment. Sixty species of the Arrhenatherion mesophilic grassland type (Ellenberg 1988) belonging to four functional groups were selected for the experiment. The classification of functional groups was based on above- and belowground

functional traits and differentiates grasses (16 species), legumes (12 species), small herbs (12 species), and tall herbs (20 species) (Roscher et al. 2004). For each of the sixty species, two monocultures were established randomly within the four blocks of the experiment in 3.5x3.5 m plots. Monocultures were sown in May 2002 using 1000 viable seeds per m^2. In November 2002, species with no or spare cover were re-sown (Roscher et al. 2004, Heisse et al. 2007). After that, no additional sowing was done. In 2008, one of the two monoculture replicates was abandoned, and in 2009 the plots were reduced to 1x1 m. We hereafter refer to these monocultures as 'old monocultures'.

In 2016, additional monoculture plots of 1 m^2 for all sixty species, hereafter called 'young monocultures', were established randomly within the four blocks of the experiment in soil not previously conditioned by the target species. To reproduce the original soil conditions at the start of the Jena Experiment, the top 30 cm of the soil were removed and replaced with soil from an adjacent field (north of the site). A 30 cm deep plastic sheet barrier was placed around the plots to avoid contamination of the new soil from the area outside the plot. The young soil had been under the same management regime as the experimental site prior to the start of the Jena experiment. Laboratory analysis of the young soil confirmed that the soil structure, carbon content, and nutrient content closely resemble conditions of the soil in 2002 (Vogel et al. 2019). Seeds from the same supplier as in 2002, were sown in the young soil using the same approach used for the old monocultures in 2002.

Both, old and young monocultures, were maintained by weeding of non-target species two to three times per year in spring, summer, and autumn (Weisser et al. 2017). Plots were mowed

204 in June and September every year, and the biomass removed to simulate the common hay
205 meadow management of the region.

206 At the time of sampling for this study, in May 2020, the old monocultures were 18 years old
207 while the young monocultures were 4 years old and thus 14 years younger than the old
208 monocultures. In the young monocultures, plant-soil feedback effects should not yet be well
209 established or strong enough to promote yield decline and in turn phenotypic or genotypic
210 plant functional trait responses. The comparison between old and young monocultures allows
211 us to use the spatial domain instead of the temporal domain to investigate the effect of time
212 on plant-soil feedback effects and yield decline. The advantage of this experimental design is
213 that our analysis is independent of different climatic conditions between years.

214 ## Yield decline

215 To estimate yield decline, we used the annual aboveground biomass of the old monoculture
216 in the period from 2003 to 2020. Within this period, aboveground biomass was measured
217 twice a year: end of May and end of August. From 2003-2009 two biomass samples were
218 harvested using a 0.2x0.5 m frame in a random position within the central part of each plot
219 (excluding 0.5 m margin). From 2010-2020, one biomass sample was collected with the same
220 frame and only if no individual of target species was present within the frame the harvest area
221 was doubled. Plants were cut at 3 cm above the ground, and the harvested material was dried
222 at 70°C for 48 h before weighing. The annual aboveground biomass was calculated as the sum
223 of the biomass of the two harvests per year extrapolated to 1 m^2.

Yield decline was estimated with species-specific linear regressions using scaled plant annual aboveground biomass as response variable and the year since the start of the experiment as explanatory variable. Aboveground biomass scaling was done by dividing the annual aboveground biomass of each species by the mean annual aboveground biomass of that species over the full period. The *scaled annual aboveground biomass* accounted for differences in plant biomass across species. Without the scaling, linear regression slopes would be primarily influenced by species mean biomass. With the scaling, the slope is expressed as unit distance to the mean of species biomass, which allows for comparison across species. Scatterplots of linear regressions for the sampled species are shown in Supporting information. The slopes of those regressions (x) were multiplied by '-1' and are hereafter called yield decline: $scaled\ aboveground\ biomass \sim -Yield\ decline * year + b$. This was done to transform negative slope values into positive numbers so that high values indicate species with high yield decline (more negative slopes), simplifying the interpretation of the results. Yield decline affected all the sixty species of the Jena Experiment except one (*Ajuga reptans*). Due to extinction or low cover of some old or young monocultures, only twenty-seven full species pairs with viable old and young monocultures out of the sixty species of the Jena Experiment could be included in this study. The distribution of yield declines for the sampled species does not represent the yield decline distribution for all the 60 species (Supporting information): the extinction of several species led to a strongly skewed yield decline for all the sixty species, with the majority of the species undergoing stronger yield decline than the sampled species. Thus, our sample represents a conservative estimate of potential effects of yield decline. Among the sampled species, the extent of yield decline varies

246 substantially between species and is independent of plant functional group identity ($F_{3,23}$ =
247 0.395, p = 0.76; Figure 2).

248 ## Leaf and fine root sampling

249 The sampling campaign was conducted from May 18th to June 5th 2020, after the plots were
250 weeded. Sampling was restricted to the morning from 7 to 11 am to minimize chemical trait
251 shifts during the day. Twenty-seven species were sampled in both monoculture types (young
252 and old) for a total of 54 plots. In each of the monocultures, we sampled the above- and
253 belowground part of 3 to 5 individuals to account for intraspecific trait variation. We first
254 harvested the aboveground plant part by cutting the stem 1-2 cm above the ground. Each
255 plant individual was stored in a separate, sealed plastic bag with a wet paper towel to ensure
256 leaves rehydrated to full potential before trait measurements (Pérez-Harguindeguy et al.
257 2013). We then sampled the roots of each individual by collecting a 5x10 cm (diameter x
258 depth) soil core with the remaining part of the stem in the centre of the core. The cores of
259 individual root systems were stored together in a sealed plastic bag. All sampled material was
260 stored in a dark cooling box. Samples were stored at 4°C in the lab for a maximum of 6 h after
261 sampling. Sample processing started 6 h after the collection of the first sample and ended
262 within 26 h. Above- and belowground samples were processed in parallel.

263 ## Measurements of leaf morphological traits and leaf antagonists damage

264 All fully-expanded and undamaged leaves of each individual were separated from the rest of
265 the aboveground portion of the plant, and rachis and petioles were removed. One or a few
266 leaves (depending on leaf size) attached between the 3rd and 5th internode from the top of

267 each individual were processed separately. For grasses without flowering stems, this was not
268 possible, and random leaves were taken instead. The rest of the leaves were pooled at the
269 plot level and used to measure the fresh weight and leaf area with a flatbed Epson Expression
270 11000XL scanner at 600DPI resolution (EPSON Tokyo, Japan). Leaves were then frozen in liquid
271 nitrogen and stored at -80°C until the end of the sampling campaign. Leaf dry weight was
272 measured from freeze-dried samples. We calculated leaf mass per area (LMA; g/m^2) as dry
273 weight divided by the leaf area and leaf dry matter content (LDMC; g/g) as the dry weight
274 divided by the fresh weight (Pérez-Harguindeguy et al. 2013). We measured leaf damage (%)
275 caused by antagonists as the proportion of damaged leaf area (damaged or infested leaf area
276 / undamaged leaf area) using leaf scans in imageJ (v. 1.53a; Schneider et al. 2012). The
277 proportion of leaf damage was estimated separately for chewers, miners and raspers and
278 pathogen infestation (leaf spot and rust diseases). Due to difficulties of differentiating damage
279 caused by miners and raspers, the two categories were grouped together (Meyer et al. 2017).
280 To estimate the undamaged area, we summed the leaf area from the scan with the leaf area
281 lost due to chewing damage.

282 The separated leaves from each individual were used to measure leaf water repellency, hair
283 density, and mean hair length as well as leaf toughness. We measured those traits on one leaf
284 per individual in the widest part of the lamina between the main vein and the leaf edge.

285 We assessed water repellency (WR; deg.) as a proxy for epicuticular waxes by measuring the
286 left and right contact angle of a 10 or 5 µl water droplet on the leaf adaxial and abaxial surface
287 of one leaf per individual (Pérez-Harguindeguy et al. 2013; for additional details see
288 Supporting information). All values (left and right, adaxial and abaxial and individuals) were

289 averaged at the plot level. High contact angle values and thus high water repellency is
290 associated with crystalline waxes (Barthlott and Neinhuis 1997), which are known to reduce
291 attachment of plant antagonists to the leaf surface (Gorb and Gorb 2017).

292 To measure leaf hair density and mean hair length, we collected images of the adaxial and
293 abaxial surface using a dissecting microscope equipped with a camera at 4.5 X magnification
294 (Di-Li 2009-16). To keep the leaf flat during the collection of images, we gently pressed
295 microscope slide on the top of the leaf. We used ImageJ (v. 1.53a; Schneider et al. 2012) to
296 count all the hairs within the image frame, measured the length of ten random hairs and
297 calculated the area of the leaf image. Hair density was calculated as the number of hairs
298 divided by the leaf area (N^o. of hairs/mm^2) and the hair length as the mean of the 10
299 measurements (mm). All values (adaxial and abaxial and individuals) were averaged at the plot
300 level.

301 We measured leaf toughness on each leaf with the shearing test (Pérez-Harguindeguy et al.
302 2013). Leaves were mounted on a motorized vertical test stand equipped with a Sauter FH 50
303 dynamometer and a surgical blade type 24. The motorized vertical test stand was operated at
304 a constant speed of 15 mm/min. One cut per leaf was done perpendicular to the main vein
305 and towards the edge of the leaf avoiding the main vein. The maximum force registered was
306 recorded and divided by the thickness measured with a digital calliper at the side of the cut.
307 Leaf toughness was calculated as maximum force to shear to the thickness (N/mm), and values
308 were averaged at the plot level.

Fine root morphological traits and root mycorrhizal colonisation

We washed roots from the soil by soaking soil cores in cold water for 15 min. We then removed the soil by gently massaging the core inside a bucket filled with water to avoid the rupture of roots. We refreshed the water in the bucket by filtering the water with soil debris into a sieve and collected fine root fragments. We repeated this procedure until the roots were completely free of soil particles. Only fine roots attached to the stem of the correct species or large fine root fragments that were unequivocally identified as being from the same species using dissecting microscopes were kept for further processing. We bulked the fine roots of each individual at the plot level and discarded all coarse roots with a diameter larger than 2 mm. Fine roots with a diameter lower than 2 mm were separated into three random subsamples: (1) one subsample was used to measure morphological traits, (2) a second subsample was stored in 75% ethanol at 4^{o}C for the quantification of arbuscular mycorrhizal (AMF) colonisation rate (Freschet et al. 2021a), (3) the remaining fine roots were frozen in liquid nitrogen and stored at -80°C to be used for chemical analyses.

For the morphological trait measurements, we scanned fine roots (flatbed Epson Expression 11000XL) at 600dpi and measured the fresh weight after carefully drying the roots with a paper towel. We then dried the scanned fine roots for 48 h at 70°C. We used WINRHIZO (Regent Instruments Inc., Quebec City, Canada) to retrieve root length and mean root diameter (RD; mm). We calculated specific root length (SRL; m/g) by dividing root length by the root dry weight and root dry matter content (RDMC; g/g) by diving the dry weight by the fresh weight (Freschet et al. 2021a). We measured root toughness on five random root fragments with the shearing test using a similar approach as for leaves. Root fragments were

cut perpendicular to the length, and root thickness was measured at the edge of the cut. Root toughness was calculated as maximum force to shear to the thickness (N/mm), and values were averaged at the plot level. We additionally measured AMF colonisation rate as a proxy of plant mutualist collaboration using the method developed by Trouvelot et al. (1986); additional details on the measurement of AMF colonisation rate can be found in Supporting information.

Leaf and fine root chemical analyses and untargeted metabolomics

We freeze-dried and ground the samples for chemical analyses with a zirconium kit in a ball mill (MM400, Retsch, Haan, Germany). To avoid overheating, samples were shaken at 30 Hz for 1 min and cooled at -20°C for 1 or 2 min. The procedure was repeated until the samples were reduced to powder. The samples were then frozen at –80°C and freeze-dried once again before further measurements.

We measured nitrogen content (N, % of dry weight) on 10 mg of each sample with an elemental analyser (VarioEL II, Elementar, Hanau, Germany), at the RoMA laboratory of the Max-Planck-Institute for Biogeochemistry in Jena, Germany. We quantified cellulose content (% of dry weight) on 10 mg of sample by sulfuric acid digestion and anthrone solution dye (Viles and Silverman 1949), with a spectrophotometer (V730, Jasco, Gross-Umstadt, Germany) at 630 nm (for additional details see Supporting information). Due to limitations in sample material, N (24% of samples, 5 leaf and 17 fine root samples) and cellulose content (14 % of samples, 5 leaf and 10 fine root samples) were predicted using near-infrared spectra measured with a Multi-Purpose FT-NIR-Analyzer (MPA, Bruker Corporation, Billerica, USA) coupled with a bootstrapped CARS-PLSR models procedure calibrated with the rest of the data.

353 This was done following the procedure developed by Elle et al. (2019) with minor
354 modifications as described in Volf et al. (2022). Model validation statistics confirmed the high
355 accuracy of both models (R^2 = 98% for nitrogen content and R^2 = 75% for cellulose content). A
356 detailed description of the procedure and validation statistics is reported in Supporting
357 information.

358 We extracted silicon (Si; % of dry weight) by adding 30 ml of alkaline solution of 0.1 M Na_2CO_3
359 to 30 mg of sample material. The sample was incubated in a water bath at 85°C for 5 h and
360 shaken every 30 min (Katz et al. 2021). We filtered the extract with a 0.45 µm syringe filter
361 and analysed the extract with an ICP-OES (IRIS Intrepid II XSP, Thermo Fischer Scientific,
362 Dreieich, Germany).

363 We measured protease inhibitor activity against trypsin (nmol/mg; nmol inhibited trypsin per
364 mg of extracted protein) using the radial diffusion assay as described in Jongsma et al. (1993,
365 1994). Protein extracts from 10 mg of sample material were tested for trypsin-inhibiting
366 activity in gel diffusion assays stained with Fast Blue B salt (scbt, Dallas, USA) and N-acetyl-DL-
367 phenylalanine-beta-naphthyl ester (APNE; Sigma-Aldrich, Darmstadt, Germany). The full
368 description of the method is provided in Supporting information.

369 We measured phytochemical diversity using an untargeted metabolome analysis by
370 calculating the feature richness (number of features) in each sample. Polar metabolites were
371 extracted using methanol (75% v/v) and water acetate buffer (25% v/v) extraction. The
372 untargeted metabolome analysis was performed using an ESI-UHR-Q-ToF-MS (maXis impact,
373 Bruker Daltonics, Hanburg, Germany) in positive mode, following the procedure described in
374 Weinhold et al (2022) with some minor modifications. The full description of the method is

reported in Supporting information. The raw data were processed in Bruker Compass MetaboScape Mass Spectrometry Software (V 5.0.0; Build 683; Bruker Daltonics, Hanburg, Germany). The MetaboScape's T-ReX algorithm was used to perform mass recalibration, peak alignment, peak picking, region complete feature extraction, grouping of isotopes, and adduct and charge states (all settings are reported in Supporting information). After features from blanks (2,149) were removed, our final data matrix contained 16,330 features and was used to calculate the number of features in each sample.

Soil phosphorus availability measurement

To evaluate the role of nutrient depletion on yield decline we measured soil available phosphorus with the calcium-acetate-lactate extract (PCAL) according to Schüller (1969). In each plot we collected and pooled 3 soil cores of 5 x 2.5cm (diameter x depth). Soil cores were quickly stored in a cooling box and frozen at -20 °C upon arrival to the laboratory. We freeze-dried and sieved the soil to remove root fragments and homogenize it. For the extraction we used 1 mg of dry soil. As a proxy of phosphorus depletion we calculated the delta between the old and new monoculture.

Missing value imputation and variable reduction (PCA)

To avoid missing values in our trait data matrix due to limitation of sample material (Si) and errors during the measurements of some sample (WR, SRL, RDMC, N and features richness), we imputed those missing values with a phylogenetically informed missForest algorithm ('missForest' R package; v. 1.4; Stekhoven & Buhlmann, 2012) as those traits could not be well predicted with the NIR procedure. Except for the Si dataset, with 12% of missing data points,

the remaining traits had only 1 to 3 missing data points (overview of missing data points is shown in Supporting information). Prior to the imputation, we added the first three phylogenetic eigenvectors to the full trait matrix (11 leaf and 9 fine root traits) as described in Debastiani et al. (2021). We obtained the phylogenetic tree (Supporting information) with the 'V.Phylomarker' R package and the 'GBOTB.extended.tree' as backbone (v. 0.1.0; Jin & Qian, 2019).

We summarised plant defence traits for leaves and fine roots separately by running two principal component analyses (PCAs). To increase interpretability of the fine root trait PCA, we applied a varimax rotation, so that traits with the highest loading lay parallel to the rotated components (R package psych 2.2.3; Revelle 2022). The full list of traits included in the two and their roles in plant defence is reported in Table 1. We then extracted the scores of the first two principal components (PCs) of the leaf defence traits PCA and the first two rotated components (RCs) of the fine root defence traits varimax rotated PCA, and, for each species, we calculated the mean scores between old and young monocultures, hereafter called 'mean defence', and the delta score calculated as the difference between old and young monocultures, hereafter called 'delta defence'. We used the mean defence as a proxy of the overall species defence strenght and the delta defence as the proxy of temporal change in defence response between 18- and 4-year old monocultures. Positive values of delta defences indicate an increase, while negative values indicate a reduction along the components.

Statistical analysis

All statistical analyses were performed in R (v. 4.1.1; R Core Team 2021). We validated the effect of the two leaf trait PCA components as defence by testing the correlation between the

two leaf defence components against foliar damage caused by chewers, miners and raspers, and pathogen infestation. To meet linear model assumptions, variables were log (chewers, miners and raspers) or arcsine square root transformed (pathogen infestation). Similarly, we tested the correlation between mutualists and antagonists and the two varimax rotated component of fine root traits PCA by regressing AMF colonisation rate and abundance of root-feeding nematodes collected in 2014 in the old monocultures (previously published in Dietrich et al. 2020). In this case, we used only the PC scores of the old monocultures, as nematode data for the new monoculture was not available.

We tested the effect of mean and delta defences for both leaves and fine roots (eight variables) on yield decline using multiple linear regressions and assessed significance levels with ANOVA type II sum-of-squares ('car' R package v. 3.0-12 Fox and Weisberg 2019). We additionally performed a commonality analysis ('yhat' R package v. 2.0-2; Nimon et al. 2020) to decompose the variance explained by each predictor in unique and common fractions to interpret the relative contribution of each defence variable on yield decline (Ray-Mukherjee et al. 2014).

Given the strong link between the collaboration gradient and AMF (Bergmann et al. 2020) we tested if the potential effect of the collaboration gradient on yield decline is mediated by AMF, using a linear regression with yield decline as response variable and the mean AMF colonisation rate in old and young monocultures as independent variable. We additionally tested if the potential effect of root trait gradients or AMF on yield decline is driven by their role on nutrient uptake rather than protection against antagonists. This was done using a

linear regression with yield decline as response variable and the delta of soil phosphorus availability as independent variable.

Results

Relationships between leaf defences and antagonists

The first and second component of the leaf trait PCA explained 35% and 19% of the variation in leaf traits, respectively (Figure 3 panel A; Supporting information). The first component was characterised by a trade-off between physical (toughness and leaf dry matter, cellulose and silicon content) and mostly chemical defences (leaf feature richness but partly also hair length), hereafter referred to as 'leaf physical vs chemical defence trade-off'. This first component was positively correlated with foliar damage caused by chewers (R^2=17%, p=0.0016) as well as raspers and miners (non-significant) and negatively to damage caused by pathogen infestation (R^2=24%, p=0.0002; Figure 3 panel B; Supporting information). Thus, leaves with high leaf toughness and silicon, cellulose and dry matter content and with low feature richness were less damaged by chewers, but had higher pathogen infestation. The second component was characterised by a negative correlation between leaf mass per area (LMA) and leaf surface defence defined by leaf N, hair density and length, and water repellency. We named this second component 'leaf surface defence and palatability'. The leaf damage caused by chewers and raspers and miners along this component was slightly higher for plant species with low palatability (high LMA and low nitrogen content) and lower for plant species with high surface defence (high hair length and density and water repellency). However, both trends were not significant (Figure 3 panel B; Supporting information).

Relationships between root defences and antagonists and mutualists

The varimax rotated root-trait PCA explained 36% and 26% of the variation in fine root traits by the first and second component, respectively (Figure 3 panel A; Supporting information). Comparable to the leaf PCA, the first component of the fine root PCA showed a trade-off between physical and chemical defences, hereafter referred to as 'root physical vs chemical defence trade-off': species with high fine root toughness, dry matter, silicon and cellulose content (but also high proteinase inhibitors) had lower feature richness. This component was marginally negatively correlated with the abundance of root-feeding nematodes measured in 2014 (R^2=11%, p=0.09), and positively with AMF colonisation rate, as measured in this study (R^2=8%, p=0.04; Figure 3 panel B; Supporting information). Thus, the abundance of plant feeding nematodes in 2014 was lower for species with high fine root physical defences and lower for fine roots with high feature richness. On the other hand, the abundance of AMF was higher in species with high fine root feature richness and lower in fine roots with high physical defences. The second component of the root PCA showed the 'collaboration gradient' of the recently defined root economics space (Bergmann et al. 2020) with a negative correlation between root diameter (RD) and specific root length (SRL). This component was significantly positively correlated with AMF colonisation rate (R^2=16%, p=0.003; Figure 3 panel B; Supporting information). Thus, in line with the root economics space, outsourcing species with high fine root diameter and low specific root length had higher AMF colonisation rates than DIY species (Bergmann et al. 2020).

Effect of mean and delta leaf and root defences on yield decline

Testing the effect of the mean and delta defences of the four main PCA axes of leaf and fine root defence traits on yield decline revealed significantly negative effects for the mean and delta of the root collaboration gradient (Table 2; Figure 4 panel A). The negative effect of the mean collaboration gradient on yield decline indicates that species on the outsourcing side of the root economics space, and thus with high fine root diameter and low specific root length, experienced lower yield decline than species on the DIY side of the root economics space. The negative effect of the delta collaboration gradient on yield decline, indicates that, under long-term selective pressure in monocultures, species that increased fine root diameter and at the same time reduced specific root length, experienced lower yield decline than species that reduced fine root diameter and increased specific root length. The commonality analyses revealed that the mean and delta collaboration gradient uniquely explained 25.9% and 15.1% of yield decline, respectively, and jointly explain 7.9% of the variation in yield decline (Table 2; Figure 4 panel B). The remaining PCA axes, leaf and fine root chemical vs physical defence trade-offs and the leaf surface defences and palatability had no significant effect on yield decline (Table 2; Figure 4 panel B). Our results further showed that both AMF colonisation rate ($p=0.87$) and delta soil available phosphorus ($p=1.00$) had no effect on yield decline (Supporting information), suggesting that AMF do not have a direct effect on yield decline and that yield decline in our system is not driven by phosphorus depletion.

Discussion

In this study, we investigated the predictive power of a comprehensive set of 20 physical and chemical defence traits of leaves and fine roots on monoculture yield decline of 27 grassland plant species. Our aim was to compare the effects of differing aboveground vs belowground defence strategies and their changes through time on yield decline using principal components of leaf and root traits. Our results revealed that none of the expected leaf and root physical or chemical defence trait gradients were significant predictors of monoculture yield decline. Instead, fine root anatomical traits defining the root collaboration gradient of the root economics space, as well as their change over 14 years of selection in a monoculture, strongly explained changes in monoculture performance over time, highlighting the importance of belowground mechanisms in this grassland system.

Yield decline response to the collaboration gradient and its temporal changes

The key results of our study thus support our first hypothesis that plant root traits should be stronger predictors of monoculture yield decline than leaf traits. In addition, our results support our fourth hypothesis that both, differences in defence strength and their temporal changes under long-term selective pressure in monocultures, as indicated by the mean and delta defences parameters, were important predictors of monoculture yield decline. We were able to show that plant species with low specific root length and high root diameter, and thus species on the 'outsourcing' side of the root collaboration gradient of the root economics space, experienced substantially lower monoculture yield decline over 18 years than species on the 'do-it-yourself' (DIY) side of the gradient.

Additionally, we could show that not only the mean expression of specific root length and root diameter was important, but also their temporal changes under long-term selective pressure in monocultures: species that increased root diameter and reduced specific root length over time (delta collaboration gradient), experienced yield decline to a similar extent as species that were on the outsourcing side of the collaboration gradient in the first place (mean collaboration, Figure 4 panel A). These species-specific shifts along the collaboration gradient highlight that long-term monoculture growth exerts a strong selective pressure against DIY species. Despite these trait temporal changes may be due to phenotypic plasticity (Ojha et al. 2022), the genetic and epigenetic trait divergence previously found in the same monocultures of this study (van Moorsel et al. 2018, 2019) suggests that the shift along the collaboration gradient may be partially due to plant microevolution in response to belowground processes, such as a potential accumulation of root antagonists (Didiano et al. 2014). Moreover, the missing link between leaf defence gradients and yield decline, suggests that belowground antagonists or other belowground processes are more important drivers of monoculture yield decline than aboveground processes (Bennett et al. 2012, Benitez et al. 2021).

Yield decline response to leaf and root physical and chemical defences

Our second hypothesis was only partly supported by our data. Despite the fact that root collaboration predicted monoculture yield decline, there was no indication that root physical defences were more important than root chemical defences. Similarly, the lack of correlation between any of the leaf defence trait gradients and yield decline does not support our third hypothesis of higher importance of chemical compared to physical defences aboveground. The first components of both the root and leaf PCA showed a similar trade-off between

physical and chemical defences (Figure 3 panel A), highlighting that while some species are primarily defended through physical barriers other species are rather defended through chemical compounds (Eichenberg et al. 2015). The second component of the leaf PCA showed a gradient from non-palatable species (high leaf mass per area and low nitrogen content) to palatable species (low leaf mass per area and high nitrogen content) that are well defended through leaf surface barriers including hair density, hair length, and water repellency (Figure 3 panel A). The two extremes of this gradient, non-palatable and non-defended species to palatable but well defended species reflect two of the defence syndromes identified by Agrawal and Fishbein (2006) in 24 milkweeds species (*Asclepias* spp.). Overall, these defence trade-offs may suggest that either different plant species can deploy different defence strategies to cope with similar antagonists (Agrawal 2007, Moore and Johnson 2017, Hervé and Erb 2019, Whitehead et al. 2021) or that plant species use different defence strategies to cope with different groups of antagonists. Our analysis on foliar damage showed that each defence strategy was effective against only a restricted group of antagonists but not against other groups of antagonists. This suggest that plant species that deploy different defence strategies may suffer from the accumulation of different groups of antagonists. For instance, plant species with pronounced leaf physical defences were well protected against foliar chewers, but at the same time they were more susceptible to foliar pathogens, while the opposite was true for species with high leaf chemical defences (Supporting information). Similarly, species with high root physical defences and low chemical defences may be well protected against root chewers (Hanley et al. 2007, Johnson et al. 2010, Caldwell et al. 2016, Freschet et al. 2021b), but not against other groups of root antagonists. Thus, some species would need to invest more in physical defences, while for others chemical defences might be

565 more advantageous; yet, the variety of different options might preclude strong trait-based
566 responses of either individual or combined trait axes.

567 ## Possible drivers of yield decline and the role of the collaboration gradient

568 Our results suggest that belowground processes related to the root collaboration gradient of
569 the root economics space may be key to drive yield decline. In a previous study on the same
570 site, Dietrich et al. (2020) found soil nematodes to be a strong driver of monoculture yield
571 decline, thus supporting knowledge about nematodes as key antagonists in several crop
572 species (Bennett et al. 2012, Jones et al. 2013, Grabau and Chen 2016, Wilschut et al. 2019).
573 In addition, two recent studies found that the abundance of root-feeding nematodes in the
574 soil is higher for species with high specific root length and thus on the DIY side of the
575 collaboration gradient (Otfinowski and Coffey 2020, Dietrich et al. 2021). Similarly, and at the
576 same site, Ristok et al. (2022) found the abundance of root-feeding nematodes to be higher
577 in species with higher root length density, a trait positively correlated with specific root length
578 (Freschet et al. 2021b). However, in our study, the abundance of root-feeding nematodes was
579 not affected by the collaboration gradient (Figure 3 panel B). This suggests that AMF, highly
580 abundant in the roots of outsourcing species (Bergmann et al. 2020; Figure 3 panel B), may
581 not protect plants against root-feeding nematodes but they may promote plant fitness and
582 reduce yield decline through other means.

583 One common other cause for yield decline is, for example, nutrient depletion (Bennett et al.
584 2012). Given the importance of AMF for nutrient uptake (Freschet et al. 2021b), AMF could
585 mediate the positive effect of the collaboration gradient on yield decline as previously shown
586 in a plant-soil feedback study with a similar pool of species (Cortois et al. 2016). When

587 nutrients are limiting, outsourcing AMF to explore the soil and increase nutrient uptake may
588 be more efficient than increasing specific root length (Smith and Read 2010). Despite the fact
589 that we cannot generally exclude the role of nutrient depletion on yield decline, our results
590 showed that soil phosphorus depletion is not driving yield decline, and that AMF colonisation
591 does not mediate the effect of the collaboration gradient on yield decline (Supporting
592 information). Thus, we were not able to link the importance of the collaboration gradient for
593 yield decline to root-feeding antagonists nor to soil phosphorus depletion or indirectly to
594 processes controlled by AMF. We can only speculate on other potential mechanisms driving
595 the defensive role of the collaboration gradient, which all still await further testing.

596 ## Speculations on alternative roles of the collaboration gradient

597 One alternative hypothesis linking the collaboration gradient to yield decline is the possibility
598 that outsourcing species with thicker roots have higher penetration strength through soil and
599 often also deeper roots (Freschet et al. 2021b). This is not related to defence, but might lead
600 to lower yield decline especially after the dry years prior to our study (Rakovec et al. 2022).
601 Another potential alternative mechanism might be that roots with high specific root length
602 explore a larger volume of soil and expose a larger surface per unit carbon than species with
603 low specific root length (Ho et al. 2005). This allows species to explore the soil for nutrients,
604 but it may also increase the chance to encounter root antagonists. The large root surface
605 exposed in DIY species may increase the area available for pathogen (Laliberté et al. 2015) and
606 nematode (Ristok et al. 2022) infection. Thus, higher specific root length could increase yield
607 decline in DIY species. This mechanism would promote the accumulation of any group of root
608 antagonists independently of their taxonomic group or feeding guilds and would be in line

609 with our suggestion that the groups of antagonists responsible for yield decline differ between
610 species with different defence strategies.

611 Conclusion

612 Our study demonstrates that the collaboration gradient and the plastic response of roots
613 along this gradient of the root economics space are significant predictors of yield decline for
614 27 plant species in a long-term grassland experiment. Our study further indicates that plants
615 can deploy a large variety of defence strategies and that each of these strategies may be
616 effective only against a restricted group of antagonists, possibly masking a generalisable
617 relationship between plant defence traits and yield decline. When species are growing in
618 mixtures, this diversity of plant defence strategies may promote defence complementarity,
619 which could support the increasingly positive biodiversity effect on ecosystem functioning
620 through time. Defence complementarity might also help to counteract yield decline in
621 agricultural settings, e.g. via increased genetic diversity in crops or a diversification of crop
622 rotation or increased spatial diversity of different crops. While the mechanism relating the
623 collaboration gradient to yield decline is still obscure, the present findings stimulate research
624 on the relationship between root traits and different groups of plant antagonists and
625 mutualists in natural or seminatural systems.

626

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Figures captions

Figure 1. Graphical illustration of antagonist accumulation in response to defence strength (mean defences; A) and defence temporal changes (delta defences; B) and our four hypotheses (C; from 1 to 4). Flower colour represents species, plant size represents biomass and the size of the shield represents the defence of each species. The number of aboveground and belowground antagonists indicates the overall pressure of antagonists. Plants on the left side are young monocultures (4 years) while plants on the right side are old monocultures (18 years) of the same species. (A) species with higher mean defence traits calculated as the mean between young and old monoculture have lower yield decline than species with lower mean defence. (B) species with a higher delta defences or increase in defence after 14 years in monoculture, calculated as the difference between defence traits in old and young monoculture, have lower yield decline than species with lower delta defences. For details on the hypotheses 1 to 4 see the main text.

Figure 2. The extent of yield decline for the sampled species in old monocultures. Yield decline is expressed as the slope of a linear regression with scaled aboveground plant biomass as response variable and year as explanatory variable. Biomass scaling (^{a}) was done by dividing species annual biomass by the species mean biomass in the period 2003 to 2020. Slopes were multiplied by '-1', so that higher values depict higher yield decline. For each species a separate linear regression was constructed using old monocultures' data from 2003 to 2020 (year of trait measurement). Shades of grey depict different plant functional groups.

977 **Figure 3. (A) Biplot of the first two components for the leaf trait PCA (on the left) and the**
978 **root trait varimax rotated PCA (on the right). (B) Correlation (Pearson's *r*) heatmap for the**
979 **first two components of the leaf PCA and leaf foliar damage caused by three major classes**
980 **of leaf antagonists (on the left) and the first two components of the root PCA and AMF**
981 **colonisation rate and abundance of root-feeding nematodes (on the right).** Variation
982 explained by each component is reported on axis labels. Note that we applied a varimax
983 rotation to the root PCA and refer to these components as rotated component (RC) rather
984 than principal component (PC). Axes scales on the left and bottom refer to the scores while
985 scales on the right and top refer to the loadings. Note that data on root-feeding nematodes
986 was measured 6 years before the current study. Abbreviations: LMA= leaf mass per area, FR=
987 feature richness, HL= hair length, N = nitrogen content, HD=hair density, PI= protease
988 inhibitor, WR= water repellency, LDMC= leaf dry matter content, T= toughness, Ce= cellulose
989 content, Si= silicon content, RD= root diameter, SRL= specific root length, a= log
990 transformation and b= square root arcsine transformation.

991

992 **Figure 4. (A) Scatterplot of the mean and delta (temporal changes) collaboration gradient**
993 **against yield decline.** Slopes and 95% confidence intervals are reported as solid line and grey
994 band. Significance levels are reported with asterisks: ** $P < 0.01$; * $P < 0.05$. **(B)**
995 **Commonality coefficients for the yield decline against plant mean and delta defences**
996 **linear model**. For each of the four defence components, the unique and common variance of
997 yield decline explained by the mean and the delta defences is depicted in different colours.

998

Tables

Table 1. List of leaf and fine root defence traits selected in this study, their directional effect and role on defence and related references. The symbols '+' and '-' in the column 'Direction' indicate that defences level are respectively increased or decreased, with higher value of the respective trait. Physical and chemical defences are reported in sperate sections. Abbreviations: LMA= leaf mass per area, N = nitrogen, DMC= dry matter content, Si= silicon content, RD= root diameter and SRL= specific root length, PI= protease inhibitor.

Tissue	Trait	Direction	Mechanisms	References
Physical defences				
Leaf	Water repellency	+	Surface barrier: reduced attachment and mobility of antagonists	(Gorb and Gorb 2017) (Hanley et al. 2007)
Leaf	Hair density	+		
Leaf	Hair length	+		
Leaf	LMA	+	Palatability* and mechanical strength	(Hanley et al. 2007, Johnson et al. 2010, Schuldt et al. 2012, Loranger et al. 2012, Caldwell et al. 2016, Hartley and DeGabriel 2016, Moore and Johnson 2017)
Leaf / root	DMC	+		
Leaf / root	N	-		
Leaf / root	Cellulose	+		
Leaf / root	Si	+		
Leaf / root	Toughness	+		
Root	SRL	-	Protection through AMF	(Cortois et al. 2016, Johnson et al. 2016b, Frew et al. 2022)
Root	RD	+		
Chemical defences				
Leaf /root	PI (trypsin)	+	Toxicity	(Johnson et al. 2016b, Moore and Johnson 2017, Whitehead et al. 2021)
Leaf / root	Features richness	+		

* with the term 'palatability' we refer to the nutritional quality of the plant tissue

Table 2. ANOVA table based on type II sum of squares and results from the commonality analysis of the linear regression with yield decline as response variable and plant mean (strength) and delta (temporal changes) defences as explanatory variables. The table reports degree of freedom (Df), beta coefficient (Estimate), F statistic (F) and unique explained variance (U) for each predictor. Variance commonly explained by mean and delta defences of each defence component (C) is also reported. Significance levels are reported with asterisks: *** P < 0.001; ** P < 0.01; * P < 0.05. R^2 and adjusted R^2 for the full model are reported at the bottom.

Explanatory variable (N°=27)	**Df**	**Estimate**		**F**		**U (%)**		**C (%)**
		Mean	**Delta**	**Mean**	**Delta**	**Mean**	**Delta**	
(Intercept)		0.092	-	-	-	-	-	-
Leaf defences								
Physical vs chemical defences trade-off (PC1)	1	0.015	0.006	3.06	0.24	7.6	1.3	- 0.3
Surface defence and palatability (PC2)	1	0.002	-0.007	0.14	0.44	0.3	0.6	- 0.1
Root defences								
Physical vs chemical defences trade-off (RC1)	1	-0.011	-0.011	0.54	0.47	1.4	1.2	0.7
Collaboration gradient (RC2)	1	**-0.039**	**-0.032**	**10.37****	**6.03***	**25.9**	**15.1**	**7.8**
Residuals	18	-		-	-	-	-	-

R^2= 56%; Adjusted R^2=37%