



# THE WEED COMMUNITY AFFECTS YIELD AND QUALITY OF SOYBEAN (GLYCINE MAX (L.) MERR.)

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Wood, Andrew; SIUC, P Young, Bryan; SIUC, De	Plant Biology sity College Dublin, Environmental and oup, UCD School of Mathematical Sciences ity College Dublin, Environmental and oup, UCD School of Mathematical Sciences
Key Words: Soybean, Seed quality a scaling, Weed communi	and quantity, Non-metric dimensional



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1	THE WEED COMMUNITY AFFECTS YIELD AND QUALITY OF SOYBEAN
2	(GLYCINE MAX (L.) MERR.)
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27	Abstract: The relationship between the weed community and soybean (Glycine max (L.)
28	Merr.) seed yield and quality was assessed in two soybean experiments in Illinois, USA.
29	One field was sown with different proportions of target weeds (Ambrosia trifida L.,
30	Amaranthus rudis J. Sauer, Setaria faberi F. Herrm), and the other was naturally infested
31	with these and other weeds. The composition of the weed communities in both fields
32	were compared to final yield and quality (% protein, oil, and water) of the crop using
33	NMDS ordination. Biomass and canopy cover, and seed quality (% protein, relative water
34	content, seed weight) of the crop, were related to the multivariate structure of the weed
35	community in both experiments. Lower quality soybeans were harvested from plots
36	dominated by the target weeds and a suite of subordinate volunteers. Analysis restricted
37	to the volunteer weed community was also significantly related to seed protein and seed
38	weight. Similar results from the two experiments lend generality to the findings and
39	indicate that soybean producers need to manage the composition of the weed community.
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41	Keywords: Soybean; Seed quality and quantity, Non-metric dimensional scaling; Weed
42	community.
43	Running heading: Weed community effects on soybean.
44 45	INTRODUCTION
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47	Understanding the effects of weeds through competitive interactions on crop
48	plants has concerned agroecologists since the work of de Wit and colleagues in the
49	1960s. <sup>1</sup> However, most investigations have focused upon the interaction between the crop
50	and a single weed. <sup>2</sup> In reality, competitive interactions in communities are diffuse
51	involving multiple interactions among several species. <sup>3</sup> The composition of the weed

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community is sensitive to the management conditions under which a crop is grown,<sup>4</sup> and 52 53 can have a significant effect on the crop that transcends the effect of a single dominant weed.<sup>5</sup> The interactions among the multiple species of a weed community are likely non-54 55 additive because the effects of all the species in a community is more than simply the sum of individual pairwise interactions.<sup>6</sup> There is, indeed, a high degree of 56 unpredictability of the outcome of multispecies competitive interactions<sup>7</sup> that can lead to 57 58 uncertainty in making the correct weed management decisions. The implication of non-59 additivitity and diffuse competition in crop-weed systems is that crop yield loss can arise 60 from the complex interactions among the different species of the weed community rather 61 than simply the overriding effect of a single, dominant weed or the additive combination 62 of a mixture of weeds.

63 Soybean (Glycine max (L.) Merr.) is one of the most widely planted and economically important annual crops in North America.<sup>8</sup> Extensive soybean yield losses 64 can occur from weed competition<sup>9</sup> and consequently, herbicides were applied to 98% of 65 the soybean production areas in the United Stated in 2005.<sup>10</sup> Yield loss can occur 66 67 following multispecies interference from some weeds, e.g., pigweed (Amaranthus L. spp.) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.).<sup>11</sup> Economic thresholds for 68 weed control in response suites of weeds have been developed,<sup>12</sup> but the decision-support 69 software is based upon simple additive models.<sup>13</sup> In addition, most weed control efforts 70 71 in soybean are directed at understanding yield loss, and not necessarily seed quality, i.e., 72 the seed oil and protein content. Seed quality is an increasingly important parameter in determining the economic value and return from soybean<sup>14</sup>. There is a need to better 73 74 understand the multispecies nature of the weed community in soybean fields and the

75	extent to which they are related to both yield and seed quality. We report here on two
76	experiments conducted to assess the relationship between multispecies weed
77	communities and soybean yield and seed quality. We show significant yield and seed
78	quality losses related to a suite of weeds viewed as the whole community.
79 80 81 82	<b>MATERIALS AND METHODS</b> Two parallel experiments were established in 2005, both in soybean fields. The
83	two experiments allowed us to assess the effect of comparable weed communities one
84	sown experimentally (the mesocosm experiment) and the other (the natural experiment)
85	arising entirely through volunteer establishment on crop yield and quality. In the
86	mesocosm experiment, three target weeds common waterhemp (Amaranthus rudis J.
87	Sauer), giant ragweed (Ambrosia trifida L.) and giant foxtail (Setaria faberi F. Herrm)
88	were sown experimentally to obtain plots with a range of weed densities. In the natural
89	experiment, the same target weeds were allowed to volunteer into a soybean field and
90	plots were located that would include the full range of weed densities planted in the
91	mesocosm experiment.
92 93 94	The mesocosm experiment
95	The experiment was established in a 0.3 ha agricultural field (37°70' N, 89°23'
96	W) at the Southern Illinois University, Agronomy Research Center, Carbondale, IL,
97	USA. The field site was previously used for agronomic crop production with intensive
98	weed management practices to reduce indigenous weed infestations. Furthermore, the
99	weed species investigated in this research were not common to the site. Soybean (Glycine
100	max cv. 'Asgrow 4403') was sown on May 18, 2005 using a commercial grain drill (John

101 Deere 750) with a row spacing of 17.5 cm at three densities (247,100, 423,425, and

102 617,750 seed ha<sup>-1</sup>) as six randomly allocated strips in each of two 35 m wide x 18 m long 103 blocks.

104 Two rows of five 4.6 m x 3.7 m plots were established in each density of soybean 105 (n = 60 plots per block). Mixtures of the three target weeds were sown into each plot on 106 May 18, 2005. Seed were hand broadcast into the plots at one of three levels of total 107 weed density (0, 80,000 and 600,000 seed ha<sup>-1</sup>). Seeds of A. trifida and S. faberi were 108 sown first after which the plots were lightly raked. Amaranthus rudis seed was broadcast 109 last and the plots where then the soil was lightly tamped. The weed seed for A. trifida 110 and A. rudis was collected from mature plants in autumn 2003 from naturally infested 111 fields at the Belleville Research Center. Seed for S. faberi was collected from mature 112 plants in autumn 2003 from naturally infested fields at the Agronomy Research Center. 113 The relative abundances of weeds in the weed mixture were manipulated using a Simplex design.<sup>15, 16</sup> Weed mixtures were either monocultures (one weed only sown), 114 115 each weed represented equally, or intermediate points, with one weed being subordinate 116 (10% proportionally) and the other two being equally represented (45%). Densities of 117 weeds sown for each weed species were based on equivalents between weed species supplied by Weedsoft **®**.<sup>13</sup> The weeds were oversown by 100% to account for low 118 germination rates or seedling establishment. 119 120 The plots were weeded to remove non-target weeds once over the course of the 121 experiment, on June 27-28 between sample 1 and sample 2. 122 Natural experiment

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125	This experiment was established in a 0.4 ha agricultural field (38 ° 51' N, 89° 84'
126	W) at Belleville Research Center, Belleville, Illinois, USA, 104 km NNW of the
127	mesocom experiment. Soybean (Glycine max cv. 'Asgrow 4403') was sown on May 17,
128	2005 using a commercial grain drill (Great Plains) with a row spacing of 17.5 cm at three
129	densities (247,100, 423,425, and 617,750 seed ha <sup>-1</sup> ) as 15 randomly allocated strips in
130	each of two 46 m wide x 46 m adjacent blocks. Each soybean row was divided into ten 3
131	m wide x 4.5 m long plots ( $n = 150$ per block). Weeds were allowed to naturally
132	volunteer in these plots and we identified 69 plots on June 17-18 that contained the same
133	relative proportions of the target weeds that were sown in the mesocosm experiment (i.e.,
134	monocultures of each weed, and mixtures of the target weeds). Non-target weeds were
135	hand-removed from the plots on June 17-18 prior to the first survey.
136	
130 137 138 139 140	<b>Data collection</b> Data were collected during four surveys of the plots in both experiments on June
137 138 139	
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137 138 139 140 141	Data were collected during four surveys of the plots in both experiments on June 8, August $1 - 3$ , September 17, and October $7 - 30$ from the mesocosm, and June 17, July
137 138 139 140 141 142	Data were collected during four surveys of the plots in both experiments on June 8, August $1 - 3$ , September 17, and October $7 - 30$ from the mesocosm, and June 17, July 26-27, September 10-11, and October 23-25 from the natural experiment. The first three
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150	flags to allow exact relocation for subsequent surveys. Aboveground biomass of
151	individual soybean plants was estimated by harvesting an average-sized individual from
152	outside of the quadrat survey areas on each survey date. The harvested individual was
153	oven dried to constant weight at 60 ° C and weighed. Final standing crop of soybean and
154	each target weed was determined by harvesting pooled weights (oven dry basis) of all
155	plants from within the two $0.5 \text{ m}^2$ quadrats. Total yield was obtained by stripping and
156	weighing the soybeans from the soybean plants obtained from each plot. The biomass of
157	a random subsample of soybeans from each plot was used to calculate 100-seed weight.
158	Relative Water Content (RWC) was obtained by sampling one leaflet from the upper 1/3
159	of a soybean plant in each plot and placing it in a tarred vial filled with deionized water.
160	Fresh weight (FW) of the leaflet was obtained by subtracting the weight of the tarred vial
161	from the vial with leaf. The vial with leaflet was left overnight at 4°C in the dark, then
162	weighed to obtain turgid weight (TW). The leaflet was then dried at 60°C, and weighed
163	to obtain dry weight (DW). RWC was calculated by the following equation:
164	RWC=100*(FW-DW)/(TW-DW)
165	Percent water, oil, and protein was determined from whole seed samples using a Zeltex
166	ZX-50 portable grain analyzer (Zeltex, Inc., Hagerstown, Maryland).
167 168	
169	Data analyses
170	A multivariate approach was used to quantify the relationship between the weed
171	community and soybean seed yield and quality. <sup>18</sup> Canopy cover data from the two
172	experiments were analyzed using non-metric dimensional scaling NMDS: <sup>19</sup> , a non-
173	parametric ordination method that has been shown to be a robust technique for

174	multivariate analysis <sup>20</sup> , using the program DECODA. <sup>21</sup> Separate ordinations were
175	undertaken for each of the two experiments. The canopy cover data were standardized to
176	adjust species to unit maxima prior to analysis based on the Bray-Curtis dissimilarity
177	coefficient. Canopy cover of the crop Glycine max was not included in the data matrix
178	for ordination analysis. Twenty random starting configurations were initiated running up
179	to 200 iterations to obtain $1 - 4$ dimensional solutions. The minimum number of
180	dimensions necessary to obtain a useful interpretation of the data was retained after
181	inspection of stress plots, minimum stress with R-values, and plots of significant vectors
182	and species centroids (see below).
183	The relationship of the abundance of species to the retained ordination solution
184	was assessed by calculating species scores for each species in the NMDS space. The
185	species scores were calculated as the weighted average of the abundance scores of the
186	samples in which the species occurred in for each dimension. These weighted averages
187	were used to plot species as points in the NMDS ordination and are referred to as species
188	centroids because they show the center of the species' distribution with respect to the
189	ordination axes.
190	The relationship between the ordination solution and independent variables,
191	including time, experimental block, initial proportions and sowing densities of the
192	planted weeds (mesocosm experiment only), density and evenness calculated as
193	Simpson's Evenness <sup>22</sup> , of the target weeds, and crop yield and quality variables, was
194	investigated by fitting vectors of maximum correlation. Vector significance was assessed
195	following permutation tests to generate correlation values. Vectors significantly

196 correlated with the ordination were retained for plotting in ordination space relative to the

197	ordination centroid. The soybean seed yield and quality variables were measured at final
198	harvest, later than the last canopy cover survey. The relationship between these variables
199	and the ordination was assessed in two ways; i) the values for each variable were used to
200	calculate vectors in a single analysis by assessing the values versus the plots for each
201	survey separately and ii) by repeating the values versus the plots for each survey in one
202	analysis. Significant vectors obtained from the first procedure were retained for
203	interpretation except when a single significant vector for the single analysis adequately
204	represented the three vectors calculated independently. The abundance of species in plots
205	arranged along vectors of particular interest (e.g., those for seed protein) were examined
206	by constructing two-way ordered tables.
207	The relationship between a priori defined groups (i.e. time and block) and the
208	weed community in each experiment was tested using Analysis of Similarity ANOSIM:
209	<sup>23</sup> in DECODA. ANOSIM compares within- versus among-group similarity based upon
210	the Bray-Curtis dissimilarity coefficient by 1000 random permutations of group
211	membership to calculate an R-value constrained to a range of -1 to 1 where a value of 1
212	indicates that all plots within a group are more similar to each other than to members of
213	another group. The significance of the R-value is determined as the proportion of
214	permuted R-values greater than or equal to the original.
215 216 217 218 219 220	

225 226 227	RESULTS
228	Crop yield and quality
229	In the mesocosm plots, soybean yield was $538.0 \pm 54.0$ kg ha <sup>-1</sup> (n = 114; 543.0 ±
230	54.0 kg ha <sup>-1</sup> in 11 plots planted as soybean monocultures), mean one-hundred seed
231	weight was $10.0 \pm 0.3$ g (n = 103), water content $5.1 \pm 0.1$ %, protein content $40.2 \pm 0.2$
232	% and oil content $21.5 \pm 0.1$ % (n = 93 for water, protein and oil content).
233	In the natural experiment, soybean yield was $694.0 \pm 46.0$ kg ha <sup>-1</sup> (n = 68), mean
234	one-hundred seed weight was $12.8 \pm 0.1$ g (n = 65), water content $5.2 \pm 0.1$ %, protein
235	content 39.1 $\pm$ 0.1 % and oil content 22.5 $\pm$ 0.1 % (n = 68 for water, protein and oil
236	content).
237	
238	Mesocosm experiment
239	The planted weeds dominated the weed flora in the mesocosm plots with
240	Ambrosia trifida having the highest abundance and Setaria faberi being most frequent
241	(Table 1). The mean number of species per plot, including soybean, ranged from 4 to 7.2
242	over the three surveys, with 24 volunteer weeds colonizing the plots. Some of the
243	volunteer weeds were common (e.g., Mollugo verticillata L.100% at survey 1, Ipomoea
244	hederacea (L.) Jacq. 55% at surveys 1 and 2) with four achieving 37.5% canopy cover in
245	at least one plot (i.e., Cyperus esculentus L., Cardamine parviflora L., Digitaria
246	sanguinalis (L.) Scop., Sorghum bicolor (L.) Moench.).
247	There was a strong relationship between the weed community and sample date,
248	with time being a significant discriminating variable among groups of plots especially

249	between survey 1 and survey 2 (ANOSIM: $R = 0.30$ , $p < 0.0001$ , survey 1 vs survey 2, R
250	= 0.44, survey 1 vs 3 R = 0.46, 2 vs 3 R = 0.06, all $p < 0.0001$ ). There was a weak, albeit
251	significant, difference between the weed community among the two experimental blocks
252	(ANOSIM, $R = 0.05$ , $p < 0.0001$ ).
253	
254	(Figure 1 location)
255	
256	A 3-dimensional NMDS solution was retained for interpretation (stress = $0.17$ )
257	and the structure of the ordination strongly reflected survey date (Fig 1). The centroids of
258	the distributions of the three planted weeds were separated in the ordination. A group of
259	early season volunteer weeds characterized plots at survey 1, including Sida spinosa L.,
260	Mollugo verticillata, Chamaesyce humistrata (L.) Small, Lamium amplexicaule (L.)
261	Small, Poa annua L., Solanum carolinense, Ranunculus abortivus L., and Oxalis stricta
262	L. (Table 1, Fig 2). Later in the season following emergence after the plots were weeded,
263	the plots were characterized by some of the same species along with a new suite of
264	volunteers including Sorghum bicolor, Paspalum leave Michx., Conyza canadensis (L.)
265	Cronq., and Xanthium strumarium L Species such as Cardamine parviflora, Digitaria
266	sanguinalis, and Ipomoea hederacea were frequent throughout the season.
267	
268	(Figure 2 location)
269	
270	Significant vectors related to the 3-dimensional solution indicated that the number
271	of species per plot decreased through time following weeding between survey 1 and 2,

273original sown proportions of target weeds and evenness of their canopy cover were274related to the ordination, reflecting the later dominance of the weed flora by the target275weeds. Evenness of the target weeds also formed an obtuse angle with total yield, i.e.276increase in evenness is somewhat associated with reduced total yield. Total seed yield of277the crop, seed water content, one-hundred seed weight, and protein content were related278to the composition of the weed community. The vectors for these crop yield and quality279components were aligned away from the plots where the weed flora included high280abundance of <i>A. trifida</i> , and, to a lesser extent, the other planted weeds. Some weeds such281as the planted <i>S. faberi</i> were most abundant in the plots where <i>A. trifida</i> was at a low282abundance aligned with the seed quality (protein), seed water content and one-hundred283seed weight. Specifically, the vector for seed protein related to the weed community at284survey 1 showed that plots associated with the lowest seed protein were those with the285highest canopy cover of the planted <i>S. faberi</i> , the volunteer <i>Ipomea hederacea</i> and286 <i>Chamaesyce humistrata</i> . By contrast, plots associated with the highest evenness of the target289weeds.290(Figure 3 location)291(Figure 3 location)292An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS293solution, stress = 0.15), i.e., without the planted target weeds, also showed a significant	272	and as crop canopy cover and biomass increased through the season (Table 2, Fig 3). The
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280abundance of A. trifida, and, to a lesser extent, the other planted weeds. Some weeds such281as the planted S. faberi were most abundant in the plots where A. trifida was at a low282abundance aligned with the seed quality (protein), seed water content and one-hundred283seed weight. Specifically, the vector for seed protein related to the weed community at284survey 1 showed that plots associated with the lowest seed protein were those with the285highest canopy cover of the planted A. trifida and volunteer Ipomea hederacea and286Chamaesyce humistrata. By contrast, plots associated with the highest seed protein had287the highest abundance of the planted S. faberi, the volunteer Mollugo verticillata,288Digitaria sanguinalis at survey 1 (App. 1), and overall the highest evenness of the target290weeds.291(Figure 3 location)292An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS	278	to the composition of the weed community. The vectors for these crop yield and quality
<ul> <li>as the planted <i>S. faberi</i> were most abundant in the plots where <i>A. trifida</i> was at a low</li> <li>abundance aligned with the seed quality (protein), seed water content and one-hundred</li> <li>seed weight. Specifically, the vector for seed protein related to the weed community at</li> <li>survey 1 showed that plots associated with the lowest seed protein were those with the</li> <li>highest canopy cover of the planted <i>A. trifida</i> and volunteer <i>Ipomea hederacea</i> and</li> <li><i>Chamaesyce humistrata</i>. By contrast, plots associated with the highest seed protein had</li> <li>the highest abundance of the planted <i>S. faberi</i>, the volunteer <i>Mollugo verticillata</i>,</li> <li><i>Digitaria sanguinalis</i> at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	279	components were aligned away from the plots where the weed flora included high
282abundance aligned with the seed quality (protein), seed water content and one-hundred283seed weight. Specifically, the vector for seed protein related to the weed community at284survey 1 showed that plots associated with the lowest seed protein were those with the285highest canopy cover of the planted A. trifida and volunteer Ipomea hederacea and286Chamaesyce humistrata. By contrast, plots associated with the highest seed protein had287the highest abundance of the planted S. faberi, the volunteer Mollugo verticillata,288Digitaria sanguinalis at survey 1 (App. 1), and overall the highest evenness of the target290veeds.291(Figure 3 location)292An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS	280	abundance of A. trifida, and, to a lesser extent, the other planted weeds. Some weeds such
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<ul> <li>survey 1 showed that plots associated with the lowest seed protein were those with the</li> <li>highest canopy cover of the planted <i>A. trifida</i> and volunteer <i>Ipomea hederacea</i> and</li> <li><i>Chamaesyce humistrata</i>. By contrast, plots associated with the highest seed protein had</li> <li>the highest abundance of the planted <i>S. faberi</i>, the volunteer <i>Mollugo verticillata</i>,</li> <li><i>Digitaria sanguinalis</i> at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	282	abundance aligned with the seed quality (protein), seed water content and one-hundred
<ul> <li>highest canopy cover of the planted <i>A. trifida</i> and volunteer <i>Ipomea hederacea</i> and</li> <li><i>Chamaesyce humistrata</i>. By contrast, plots associated with the highest seed protein had</li> <li>the highest abundance of the planted <i>S. faberi</i>, the volunteer <i>Mollugo verticillata</i>,</li> <li><i>Digitaria sanguinalis</i> at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	283	seed weight. Specifically, the vector for seed protein related to the weed community at
<ul> <li><i>Chamaesyce humistrata.</i> By contrast, plots associated with the highest seed protein had</li> <li>the highest abundance of the planted <i>S. faberi</i>, the volunteer <i>Mollugo verticillata</i>,</li> <li><i>Digitaria sanguinalis</i> at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	284	survey 1 showed that plots associated with the lowest seed protein were those with the
<ul> <li>the highest abundance of the planted <i>S. faberi</i>, the volunteer <i>Mollugo verticillata</i>,</li> <li><i>Digitaria sanguinalis</i> at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	285	highest canopy cover of the planted A. trifida and volunteer Ipomea hederacea and
<ul> <li>Digitaria sanguinalis at survey 1 (App. 1), and overall the highest evenness of the target</li> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	286	Chamaesyce humistrata. By contrast, plots associated with the highest seed protein had
<ul> <li>weeds.</li> <li>(Figure 3 location)</li> <li>An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS)</li> </ul>	287	the highest abundance of the planted S. faberi, the volunteer Mollugo verticillata,
290291292293An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS	288	Digitaria sanguinalis at survey 1 (App. 1), and overall the highest evenness of the target
<ul> <li>291 (Figure 3 location)</li> <li>292</li> <li>293 An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS</li> </ul>	289	weeds.
<ul><li>292</li><li>293 An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS</li></ul>	290	
293 An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS	291	(Figure 3 location)
	292	
solution, stress = 0.15), i.e., without the planted target weeds, also showed a significant	293	An NMDS ordination restricted to the volunteer weeds (3-dimensional NMDS
	294	solution, stress = $0.15$ ), i.e., without the planted target weeds, also showed a significant

relationship between the multivariate structure of the weed communities and seed quality, including protein content at survey 2 (r = 0.36, p = 0.012, n = 88) and one-hundred seed weight at survey 1 (r = 0.39, p = 002, n = 103). Similarly, an NMDS ordination restricted to only the planted weeds (3-dimensional NMDS solution, stress = 0.09) was related significantly to one-hundred seed weight (r = 0.37, p < 0.0001, n = 296) and total yield (r = 0.42, p < 0.0001, n = 329), but not seed oil or protein content.

301

302 Natural experiment

303 The target weeds dominated the weed communities that volunteered into the 304 natural experiment with A. trifida having the highest canopy cover, exceeding that of the 305 crop, and occurring in 100% of the plots (Table 3). The mean number of species per plot, 306 including soybean, ranged from 5.1 to 6.9 over the three time periods, with 20 unplanted 307 weeds colonizing the plots. In addition to the three target weeds, *Ipomoea hederacea*, 308 Abutilon theophrastii Medic, and Panicum dichotomiflorum Michx. occurred in > 50% of 309 the plots during at least one survey, with Amaranthus retroflexus L. reaching 15% canopy 310 cover in at least one plot.

There was a strong relationship between the weed community and sample date, with time being a significant discriminating variable among groups of plots especially between survey 1 and survey 3 (ANOSIM: R = 0.20, p < 0.0001, survey 1 vs survey 2, R= 0.17, survey 1 vs 3 R = 0.32, 2 vs 3 R = 0.15, all p < 0.0001). There was only a weak difference between the weed communities among the two experimental blocks (ANOSIM, R = 0.07, p < 0.0001). 318 (Figure 4 location)

319

320	A 2-dimensional NMDS solution was retained for interpretation (stress = $0.27$ )
321	and the structure of the ordination strongly reflected survey date (Fig 4, Table 4). The
322	centroids of the distributions of the three target weeds were centrally located in the
323	ordination (Fig 5). A group of early season volunteer weeds characterized plots at survey
324	1, including Amaranthus retroflexus, Cyperus esculentus, Ampelamus albidus (Nutt.)
325	Britt., and Sida spinosa with a different suite of species characterizing the plots later in
326	the season including Persicaria pensylvanicum, Xanthium strumarium, Hordeum pusilum
327	Nutt., and Panicum dichotomiflorum, and (Table 3, Fig 5). Ipomoea hederacea and
328	Ambrosia artemisiifolia L. were frequent throughout the season.
329	
330	(Figure 5 & Figure 6 location)
331	
332	Significant vectors related to the 3-dimensional solution indicated that the number
333	of species per plot decreased through the season as crop biomass increased (Table 4, Fig
334	6). Soybean biomass and canopy cover, relative water content, and seed protein content
335	of the crop were related to the composition of the weed community. Although not
336	planted, the density and biomass of the two of the target weeds (Amaranthus rudis,
337	Setaria faberi) were significantly related to the 2-dimensional solution. The vectors for
338	crop seed quality (relative water content and protein content) were aligned towards plots
339	containing the highest density of Setaria faberi, and high frequency and abundance of
340	Solanum carolinense and Digitaria sanguinalis, and away from plots with high amounts

341	of Amaranthus rudis and weeds of minor importance including Eragrostis trichodes
342	(Nutt.) Wood (Fig 6). The vector for seed protein and the weed community at survey 3
343	indicated that the highest levels of seed protein were associated with plots in which the
344	weed community was characterized by the Ambrosia artemisiifolia and Ipomoea
345	hederacea and low amounts of the target weed Amaranthus rudis (App. 2). Soybean yield
346	components were unrelated to the 2-dimensional weed community ordination. Seed oil
347	content and 100 seed weight were related to a 3-dimensional solution (not presented), but
348	little extra interpretative value with respect to the importance of the weed community was
349	attributed to this solution.
350 351 352 353	DISCUSSION
354	
354 355	Together the two experiments confirm that the abundance (density, biomass,
	Together the two experiments confirm that the abundance (density, biomass, cover), and seed yield and quality of soybean were related to the composition of the weed
355	
355 356	cover), and seed yield and quality of soybean were related to the composition of the weed
355 356 357	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the
355 356 357 358	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the mesocosm experiment) and in plots allowed to become naturally infested with weeds (the
355 356 357 358 359	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the mesocosm experiment) and in plots allowed to become naturally infested with weeds (the natural experiment). The mesocosm experiment was planted at a site where the three
<ul> <li>355</li> <li>356</li> <li>357</li> <li>358</li> <li>359</li> <li>360</li> </ul>	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the mesocosm experiment) and in plots allowed to become naturally infested with weeds (the natural experiment). The mesocosm experiment was planted at a site where the three target weeds ( <i>Amaranthus rudis, Ambrosia trifida</i> , and <i>Setaria faberi</i> ) were not expected
<ul> <li>355</li> <li>356</li> <li>357</li> <li>358</li> <li>359</li> <li>360</li> <li>361</li> </ul>	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the mesocosm experiment) and in plots allowed to become naturally infested with weeds (the natural experiment). The mesocosm experiment was planted at a site where the three target weeds ( <i>Amaranthus rudis, Ambrosia trifida</i> , and <i>Setaria faberi</i> ) were not expected to occur, allowing their planting densities to be controlled. The natural experiment was
<ul> <li>355</li> <li>356</li> <li>357</li> <li>358</li> <li>359</li> <li>360</li> <li>361</li> <li>362</li> </ul>	cover), and seed yield and quality of soybean were related to the composition of the weed community. This consistent result was obtained both in plots planted with weeds (the mesocosm experiment) and in plots allowed to become naturally infested with weeds (the natural experiment). The mesocosm experiment was planted at a site where the three target weeds ( <i>Amaranthus rudis, Ambrosia trifida</i> , and <i>Setaria faberi</i> ) were not expected to occur, allowing their planting densities to be controlled. The natural experiment was conducted at a site that had a prior history of high abundance of these three weeds so that

366	elsewhere). Indeed, A. trifida is one of the most aggressive weeds in soybean fields						
367	reducing yield at less than two plants per 9 m of soybean row. <sup>24</sup>						
368	Variation in soybean seed quality affects its economic value as a crop. <sup>14</sup> Both seed						
369	protein and oil content can vary among cultivars and in response to environmental						
370	conditions. <sup>25-27</sup> Seed protein content varies more than seed oil content, although the two						
371	are inversely related to each other. <sup>28</sup> Our experiments showed a relationship between seed						
372	protein content and the weed community, but no relationship to seed oil content.						
373	Numerous studies with a variety of crops and weed species have clearly established that						
374	increasing competition negatively impacts yields. <sup>29</sup> However, the impact of weed						
375	competition upon seed quality (i.e. protein and oil content) has not been extensively						
376	studied. Previous research with soybean has demonstrated that the protein content of soy						
377	seeds was unaffected by altered densities of the weedy species Trianthema						
378	portulacastrum L. <sup>30</sup> However, in the legume Lathyrus sativus L., protein content did						
379	increase within plots containing a mixture of weedy species (i.e. Chenopodium album L.,						
380	Avena fatua L. and Setaria viridis (L.) P. Beauv.), and the increase was attributed to						
381	decreased seed size (i.e. dry matter content). <sup>31</sup> In the experiments described here, the						
382	protein content of soybean seeds was altered in both mixed species competition and						
383	single species competition. Protein content increased under high weed density conditions						
384	with both Amaranthus rudis and Setaria faberi, while decreasing under high weed						
385	density conditions with Ambrosia trifida (Figures 2,3,5 & 6).						
386	Results of the mesocosm experiment suggested that soybean yield was most						
387	closely related to the abundance of the target weeds (planted density and evenness: Fig						
388	3), whereas seed quality, specifically protein content, was related to the composition of						

389	the volunteer weed community. The target weeds were more abundant that the volunteer
390	weeds and so might be expected to reduce soybean yield. That the community of less
391	abundant volunteers was also related to soybean seed quality suggests a more subtle
392	relationship with the soybean plants than that exerted by the target weeds in reducing
393	yield. There appears to be a diversity/synergy interaction among members of the weed
394	community affecting soybean. <sup>32</sup> Potential resources known to affect seed protein content
395	that the volunteer weed community may have been competing for with soybean for
396	include soil moisture and nutrients. <sup>33, 34</sup> The low canopy cover of the volunteer weeds
397	suggests that aboveground competition for light was unlikely to have affected seed
398	protein. Within-field variation in seed protein content of a similar magnitude as we
399	observed (i.e., $< 2\%$ or 2 g kg <sup>-1</sup> ) has been previously attributed to spatial variation in soil
400	nitrogen; <sup>33</sup> which itself may vary in response to competition with the weed community
401	and may have affected the weed community particularly in the natural experiment.
402	Significant genotype by environment interactions can affect soybean seed protein content
403	to a similar extent, <sup>35, 36</sup> although the environmental component has not previously been
404	attributed to the effects of weed competition.

405 Soybean yield and seed quality were related to the weed community in different 406 ways in the natural experiment. The occurrence of these differences suggests that the 407 soybean simultaneously had to compete with different groups of weeds, more than likely 408 for a different suite of resources. In this case, aboveground competition for light with 409 large weeds such as *Ambrosia trifida* was reducing soybean yield, while belowground 410 competition for soil resources with minor weeds including *Solanum carolinense* and 411 *Digitaria sanguinalis* was reducing seed quality. Both *S. carolinense* and *D. sanguinalis* 

> 17 http://mc.manuscriptcentral.com/jsfa-wiley

Page 18 of 42

412 are known infest soybean fields and their growth forms are more conducive for

- 413 belowground than aboveground competition with soybean.<sup>37, 38</sup> An effect of these two
- 414 species on soybean seed quality is previously unreported.

415 There was also a temporal dynamic to the weed-crop relationship as the weed 416 communities changed through the season with spring emerging plants dominating early 417 on being replaced with later season weeds towards the end of the season. The relationship 418 between the early season weed community (i.e., at the first survey) and final soybean 419 seed water and protein content in the mesocosm experiment suggests that early season 420 interactions between non-reproductive soybean (V2-V3 stages) and weeds are 421 sufficiently important to manifest their effects late in the season. Previous studies has shown that early season weed infestation can significantly reduce soybean yield <sup>38</sup>, but 422 423 less is known about how these early season factors affect seed quality. By contrast, the 424 natural experiment suggested that the effects of the weed community on soybean 425 biomass, yield and seed quality (protein) were the results of only late season interactions 426 (i.e., when the soybeans were at the R2-R3 stage). Overall, the implication is that the 427 magnitude and importance of different types of competitive interactions vary through the season.<sup>39</sup> Previous studies have indicated that there are critical times important for weed 428 removal in soybean to minimize yield losses <sup>40, 41</sup> supporting this observation. 429

430

#### 431 Conclusion

432

The multivariate analytical approach that we use here is circumstantial and
 retrospective,<sup>42</sup> however, it allows inferences to be made regarding the mechanistic basis

435	for the patterns observed because of the experimental nature of the mesocosm
436	experiment. We have interpreted the relationships between the crop and the weed
437	communities in terms of competitive interactions. Alternatively, the weed communities
438	may be reflective of environmental spatial heterogeneity in the crop fields to which both
439	the crop and the weeds were responding. In other words, the weed community may be an
440	indicator of conditions, such as areas of droughty or degraded soil, that is directly
441	affecting both the weed community and crop seed yield and quality. <sup>5, 43</sup> Nevertheless,
442	decision-support software can accurately predict yield loss in soybean from weeds, <sup>44</sup> but
443	does not provide an integrated and multivariate or non-additive account of mixed-weed
444	communities. Our approach has shown that the diffuse nature of the weed community
445	may be of importance for understanding both yield and seed quality loss in soybean. The
446	weed communities in crops are likely to increase in diversity and complexity as reduced
447	tillage practices are increasingly adopted <sup>45</sup> making an understanding of the relationship
448	between the weed community and the crop particularly important. <sup>46</sup>
449	
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**Table 1.** Species abundance from the mesocosm. Glycine max, Ambrosia trifida,

Amaranthus rudis, and Setaria faberi were planted, other species (below dotted

line) were volunteers (ordered by frequency at survey 1, and survey 2,

respectively).

	Mean						
	canopy	y cover		Max			
	(%)			cover	Frequency	/ (% of 120	) plots)
Species/survey	1	2	3	(%)	1	2	3
Glycine max (L.) Merr.	2.8	15.1	5.1	85.0	100	98	97
Ambrosia trifida L.	3.5	27.4	20.1	97.5	58	69	64
Amaranthus rudis J. Sauer	0.3	0.7	3.2	37.5	44	34	62
Setaria faberi F. Herrm	1.5	9.8	10.1	97.5	85	76	70
Mollugo verticillata L.	0.7	0	0	3.0	100	0	0
Chamaesyce humistrata (L.) Small	0.4	0.004	0	0.5	78	1	0
Ipomoea hederacea (L.) Jacq.	0.6	0.8	0.1	15.0	55	55	12
Digitaria sanguinalis (L.) Scop.	0.9	0	2.8	37.5	54	0	23
Sida spinosa L.	0.2	0	0	0.5	36	0	0
Oxalis stricta L.	0.2	0.004	0.1	3.0	31	1	10
Cyperus esculentus L.	0.2	0.004	0.9	37.5	31	1	3
Chenopodium album L.	0.6	0.7	0	15.0	24	19	0
Cardamine parviflora L.	0.04	0.01	1.4	37.5	8	3	13
Amaranthus retroflexus L.	0.02	0.3	0.2	15.0	3	17	6

Ambrosia artemisiifolia L.	0.05	0.008	0	3.0	3	2	0
Poa annua L.	0.008	0.2	0	15.0	2	3	0
Cerastium vulgatum L.	0.008	0.004	0.025	3.0	2	1	1
Xanthium strumarium L.	0.004	0	0.3	15.0	1	0	12
Lamium amplexicaule L.	0.004	0	0	0.5	1	0	0
Ranunculus abortivus L.	0.004	0	0	0.5	1	0	0
Solanum carolinense L.	0.004	0	0	0.5	1	0	0
Sorghum bicolor (L.) Moench.	0	2.1	0.3	37.5	0	46	16
Galium aparine L.	0	0.3	0	15.0	0	11	0
Physalis subglabrata Mack. & Bush.	0	0.07	0	3.0	0	9	0
Festuca arundinacea Schreb.	0	0.06	0	3.0	0	8	0
Paspalum leave Michx.	0	0.02	0.2	15.0	0	3	8
Conyza canadensis (L.) Cronq.	0	0	0.01	3.0	0	0	5
Persicaria pensylvanicum (L.) Small	0	0	0.004	0.5	0	0	1
Average No. spp per plot*	7.2	4.6	4.0				
* including G. max.			9	2			

Table 2. Correlations (R) and probability (P) of significant environmental vectors with 3dimensional NMDS ordination of mesocosm cover data.

Variable	n	R	Р
Time	360	0.80	< 0.0001
Block	360	0.24	< 0.0001
Sown Proportion Ambrosia trifida <sup>1.</sup>	360	0.60	< 0.0001
Sown Proportion Amaranthus rudis <sup>1.</sup>	360	0.37	< 0.0001
Sown Proportion Setaria faberi <sup>1.</sup>	360	0.63	< 0.0001
Planting density target weeds	360	0.37	< 0.0001
Simpson's Evenness target weeds	347	0.53	< 0.0001
Soybean density	240	0.48	< 0.0001
Soybean biomass	359	0.51	< 0.0001
Soybean cover	360	0.35	< 0.0001
Soybean final density (at harvest) <sup>3.</sup>	360	0.25	< 0.0001
Soybean standing crop final biomass <sup>4.</sup>	342	0.30	< 0.0001
Number of species	360	0.72	< 0.0001
% seed protein <sup>5.</sup>	279	0.21	0.008
$\%$ seed water survey $1^{4.}$	93	0.30	0.004
Soybean total yield <sup>4.</sup>	342	0.26	< 0.0001
100-seed-weight	309	0.31	< 0.0001

<sup>1.</sup> Vectors for planting densities of the three target weeds were also significant and aligned in the same direction as those for sown proportions.

<sup>2.</sup> Vector for protein at survey 1, R = 0.27, P = 0.09, n = 93 was aligned close to this composite vector.

<sup>3.</sup> Vectors for soybean final density were also significant for surveys 1 - 3 and were closely aligned to that of the composite vector shown.

<sup>4.</sup> Vectors for separate surveys were also significant with the composite vectors aligned close to those from survey 3 for Soybean total yield and survey 2 for soybean standing crop final biomass and 100-seed-weight, respectively.

<sup>5.</sup> A significant vector was obtained only for the relationship between the weed communities from survey 1 and % seed water.



### Table 3

Species abundance from plots in the natural experiment. The crop *Glycine max* was planted, *Ambrosia trifida, Amaranthus rudis*, and *Setaria faberi* were target volunteers, other species (below dotted line) were volunteers (ordered by frequency at time 1, and time 2, respectively).

				Max			
	Canopy c	cover		Frequency (% of 69 plots)			
Species/survey	1	2	3	(%)	1	2	3
<i>Glycine max</i> (L.) Merr.	4.1	9.6	7.1	37.5	100	100	100
Ambrosia trifida L.	13.8	29.8	23.4	62.5	100	100	100
Amaranthus rudis J. Sauer	2.3	2.5	1.9	15.0	87	99	87
Setaria faberi F. Herrm	1.7	2.5	2.4	37.5	95	83	70
Ipomoea hederacea (L.) Jacq.*	1.3	1.0	0.9	3.0	84	88	57
Abutilon theophrastii Medic	0.7	0.5	0	3.0	53	58	0
Ampelamus albidus(Nutt.) Britt.	0.5	0.1	0	3.0	48	25	0
Amaranthus retrflexus L.	1.3	0	0	15.0	40	4	0
Cyperus esculentus L.	0.2	0.03	0	0.5	29	6	0
Ambrosia artemisiifolia L.	0.2	0.4	0.3	3.0	24	33	22
Persicaria pensylvanicum (L.) Small	0.2	0.01	0.1	3.0	8	3	10
Sida spinosa L.	0.03	0	0	0.5	7	0	0
Digitaria sanguinalis (L.) Scop.	0.03	0	0	0.5	7	0	0

Chenopodium album L.	0.02	0.01	0	0.5	5	3	0
Oxalis stricta L.	0.01	0	0	0.5	2	0	0
Solanum carolinense L.	0.01	0	0	0.5	2	0	0
Taraxacum officinale Weber	0.01	0	0	0.5	2	0	0
Xanthium strumariumi L.	0	0.2	0.2	3.0	0	23	13
Hordeum pusilum Nutt.	0	0.01	0	0.5	0	2	0
Panicum dichotomiflorum Michx.	0	0	0.5	3.0	0	0	48
Eragrostis trichodes (Nutt.) Wood	0	0	0.01	0.5	0	0	3
Average No. species per plot <sup>†</sup>	6.9	6.3	5.1				

\* Includes some *I. lacunose* L.

† including G.max

## Table 4

Correlations (R) and probability (P) of significant environmental vectors with 2-

dimensional NMDS ordination of weed species cover data from the natural experiment.

Variable	n	R	Р
Time (survey date)	200	0.71	< 0.001
Block	200	0.22	0.006
Soybean biomass (per plant)	196	0.54	< 0.001
Soybean canopy cover	200	0.26	0.001
Setaria faberi density survey 1	131	0.22	0.044
Amaranthus rudis density survey 1	131	0.25	0.035
Amaranthus rudis final density	200	0.26	0.002
Amaranthus rudis standing crop biomass	188	0.25	0.009
Number of species	200	0.65	< 0.001
RWC	119	0.32	0.002
% seed protein time 3	68	0.34	0.045

2

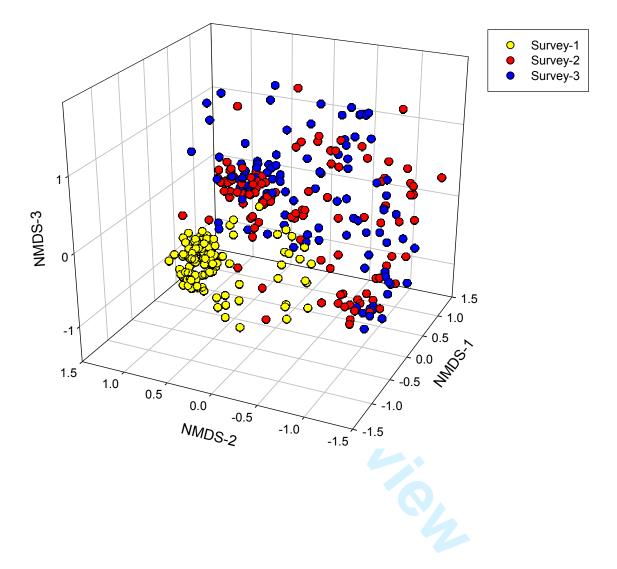
#### **Figure legends**

- Fig 1. Three-dimensional nonmetric dimensional scaling ordination of the weed community from the mesocosm experiment. Each circle represents a plot from one of the three surveys.
- Fig 2. Species centroids from the 3-dimensional ordination from the mesocosm experiment. Species abbreviations: AMAR = *Ambrosia artemisiifolia*, AMRE = *Amaranthus retroflexus*, AMRU = *Amaranthus rudis*, AMTR = *Ambrosia trifida*, CAPA = *Cardamine parviflora*, CEVU = *Cerastium vulgatum*, CHHU = *Chamaesyce humistrata*, CHAL = *Chenopodium album*, COCA = *Conyza canadensis*, CYES = *Cyperus esculentus*, DISA = *Digitaria sanguinalis*, FEAR = *Festuca arundinacea*, GAAP = *Galium aparine*, IPHE = *Ipomoea hederacea*, LAAM = *Lamium amplexicaule*, MOVE = *Mollugo verticillata*, OXST = *Oxalis stricta*, PALA = *Paspalum leave*, PEPE = *Persicaria pensylvanicum*, PHSU = *Physalis subglabrata*, POAN = Poa annua, RAAB = *Ranunculus arbortivus*, SEFE = *Setaria faberi*, SISP = *Sida spinosa*, SOCA = *Solanum carolinense*, SOBI = *Sorghum bicolor* XAST = *Xanthium strumarium*.
- Fig 3) Significant vectors associated with the 3-dimensional ordination of the mesocosm experiment. Soy-bio, Soy-can, Soy-d, Soy-fden, Soyscfb, Totyield, Protein,
  Seedwater, and 100swht = mean individual biomass, canopy cover, density, final density, standing crop final biomass, total seed yield, % seed protein, % seed water, and 100 seed weight, respectively, of *Glycine max*. P\_AMRU = sown seed density of *Amaranthus rudis*, P\_AMTR = sown seed density of *Ambrosia trifida*,
  P\_SEFA = sown seed density of *Setaria faberi*, AD\_Weeds = total density of

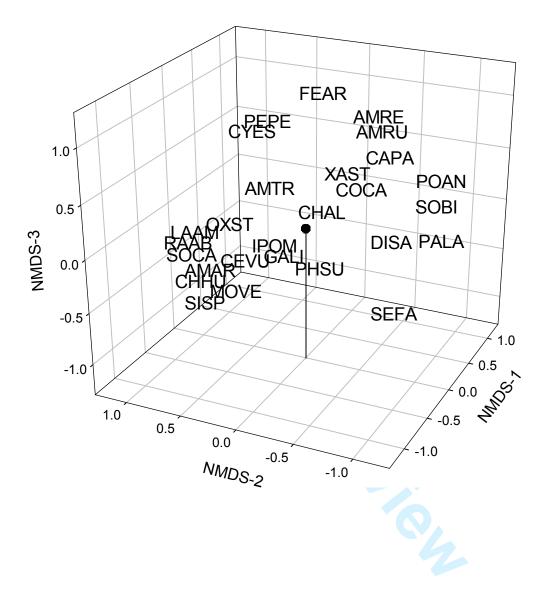
sown weeds, No spp = number of species per plot, RWC = soybean relative water content, SFdt1 = *Setaria faberi* density at survey 1, Time = survey date, Even = Simpson's Evenness of target weed canopy cover.

- Fig 4) Nonmetric dimensional scaling ordination, 2-dimensional solution of the natural experiment. Each circle represents a plot from one of the three surveys.
- Fig 5) Species centroids from the 2-dimensional ordination from the natural experiment.
  Species abbreviations as in Fig 2 plus: ABTH = Abutilon theophrastii, AMAL = Ampelamus albidus, ERTR = Eragrostis trichodes, HOPU = Hordeum pusilum,
  IPHE = Ipomoea hederacea/lacunosa, PADI = Panicum dichotomiflorum, TAOF = Taraxacum officinale.
- Fig 6) Significant vectors associated with the 2-dimensional ordination of the natural experiment. ARd/b represents the average position of 3 vectors associated with the density and biomass of *Amaranthus rudis* (all were aligned between the Block and Soycov vectors), No spp = number of species per plot, RWC = soybean relative water content, Soybio = soybean biomass, Soycov = soybean canopy cover, SFdt1 = *Setaria faberi* density at survey 1, Time = survey date.

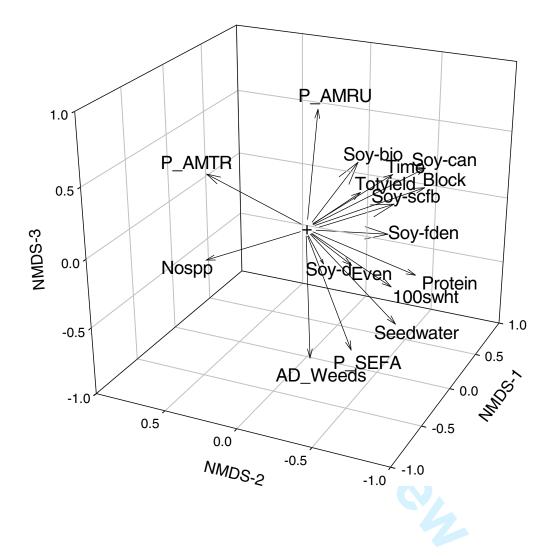




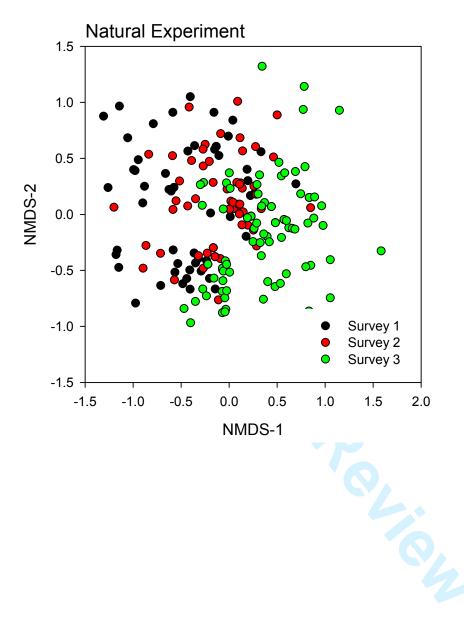




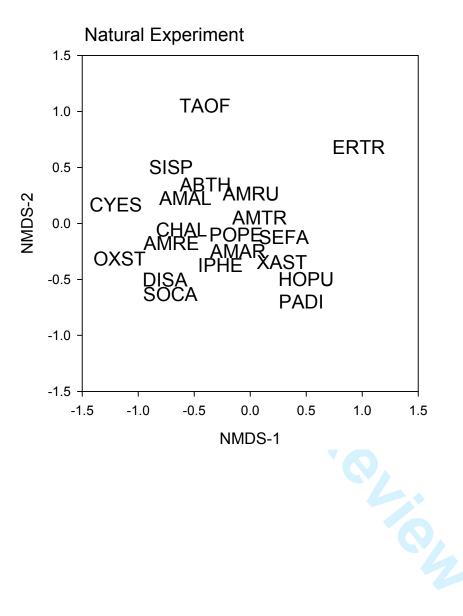




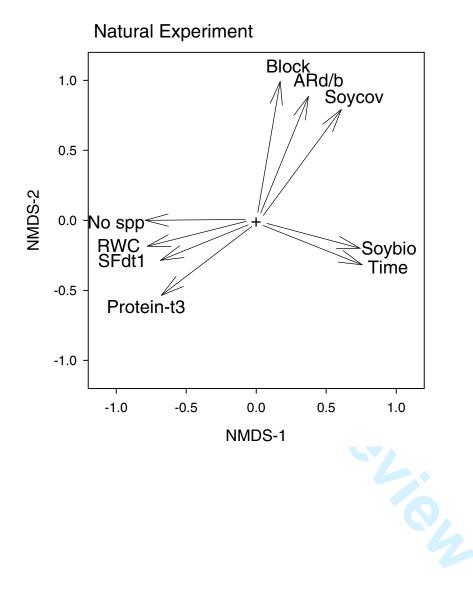








**Fig 6**)



Appendix 1. Two-way table of mesocosm plots and species ordered by ascending position (left to right and top to bottom) along the survey 1 protein vector from 3D-NMDS ordination. Species abbreviations as in legend to Fig 2. The data for each species were standardized to unit maxima, and placed into one of four categories; - = absent, 1 = 0.1 - 0.25, 2 = 0.26 - 0.50, 3 = 0.51 - 0.75, 4 = 0.76 - 1.00.

	396335865215555111143238147235644188421727364322794191113311519916715292538956996814767144128877182798116167111811111496 116289193105 4700609689218844636 0450395 0 553953338800207378440 071267715390425281217 5710296241006671111 9101 210104 8 3 70 5 42 0 1 1 6 49 3 792 08658
17 LAAM	4
19 RAAB	- 4
20 SOCA	4
12 AMAR	4
1 AMTR	44441444444444411111444411111-4111-4-11-1-1111-1-1-1111-11-
8 IPHU	$11414114 \cdot 1144 \cdot \cdots 1 \cdot 111144 \cdot \cdots \cdot 1411 \cdot 1111 \cdot 1 \cdot 1 \cdot 111 \cdot 14 \cdot \cdots \cdot 4 \cdot \cdot 1 \cdot 1111 \cdot 4 \cdot 11 \cdot 1 \cdot 1111 \cdot 1 \cdot $
6 CHHU	4 - 4444 - 44444 - 44444444444444444444
7 OXST	44
15 CAPA 16 XAST	4 - 4 - 4 4 4
2 AMRU	1411111-1-111111-411111-14-11111111111-1-1-1-1-1-1-1-1-1-
14 CHAL	
11 AMRE	44
5 SISP	
18 POAN	4 - 4
10 CYES	4-4-44-4-4-4
13 CEVU 4 MOVE	- 4 4
4 MOVE 3 SEFA	111111111111111111111111111111111111
9 DISA	-11
5 51511	

Appendix 2. Two-way table of natural experiment plots and species ordered by ascending position (left to right and top to bottom) along the survey 3 protein vector from 2D-NMDS ordination. Species abbreviations as in legend to Figs 2 and 5. The data for each species were standardized to unit maxima, and placed into one of four categories; - = absent, 1 = 0.1 - 0.25, 2 = 0.26 - 0.50, 3 = 0.51 - 0.75, 4 = 0.76 - 1.00.

	111112121111112111111212111111121111111
ERTR	44
AMRU	1114 - 1 - 14111 - 11111114111111111111
SOHA	1111-14111411111111144
AMTR	11111422422222222222244444442422242222444444
XAST	1
SEFA	-211-11121-1-11-1-111-111-111111111
POPE	1414
AMAR	11141-44411-41-
IPHE	
	ERTR AMRU SOHA AMTR XAST SEFA POPE AMAR IPHE