Seedbank and Field Emergence of Weeds in Glyphosate-Resistant Cropping Systems in the United States

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A segment of the debate surrounding the commercialization and use of glyphosate-resistant (GR) crops focuses on the theory that the implementation of these traits is an extension of the intensification of agriculture that will further erode the biodiversity of agricultural landscapes. A large field-scale study was initiated in 2006 in the United States on 156 different field sites with a minimum 3-yr history of GR-corn, -cotton or -soybean in the cropping system. The impact of cropping system, crop rotation, frequency of using the GR crop trait, and several categorical variables on seedbank weed population density and diversity was analyzed. The parameters of total weed population density of all species in the seedbank, species richness, Shannon's H′ and evenness were not affected by any management treatment. The similarity between the seedbank and aboveground weed community was more strongly related to location than management; previous year's crops and cropping systems were also important while GR trait rotation was not. The composition of the weed flora was more strongly related to location (geography) than any other parameter. The diversity of weed flora in agricultural sites with a history of GR crop production can be influenced by several factors relating to the specific method in which the GR trait is integrated (cropping system, crop rotation, GR trait rotation), the specific weed species, and the geographical location. Continuous GR crop, compared to fields with other cropping systems, only had greater species diversity (species richness) of some life forms, i.e., biennials, winter annuals, and prostrate weeds. Overall diversity was related to geography and not cropping system. These results justify further research to clarify the complexities of crops grown with herbicide-resistance traits to provide a more complete characterization of their culture and local adaptation to the weed seedbank.

Nomenclature: Glyphosate; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.

Key words: Community structure, corn, cotton, glyphosate-resistant, multivariate analysis, Nonmetric Dimensional Scaling, Procrustes Analysis, soybean.

The culture of genetically engineered crops has been argued as a recent component in the continuing evolution of crop management practices to achieve greater production efficiency (Young et al. 2013). More specifically, the commercialization of glyphosate-resistant (GR) crops beginning in 1996 in the United States has allowed the widespread use of glyphosate as a weed control strategy in cotton, corn, and soybean production systems (Dill et al. 2008). As a result, U.S. growers have reported a perceived reduction in weed pressure in several cropping systems, especially in no-tillage systems (Kruger et al. 2009). The introduction of glyphosate as an alternative herbicide mode of action for use in GR crops has also been credited with mitigating problems associated with weed populations resistant to the acetolactate synthase (ALS, EC 2.2.1.6)-inhibiting and protoporphyrinogen oxidase (PPO, EC 1.3.3.4)-inhibiting herbicides (Norsworthy et al. 2012) as well as other types of herbicide-resistant weed biotypes (Heap 2013).

A significant volume of research has investigated the influence of planting GR crops on the biodiversity of both plants and animals, in agricultural systems. The premise for the studies has been the

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connection between biological diversity in the ecosystem and the impact on sustainability. More specifically, some research arguments have suggested that agricultural practices that promote greater weed diversity should be regarded as a progressive step towards the development of greater agricultural sustainability (Hyvönen and Salonen 2002). However, there is no conclusive evidence that GR crops have a greater effect on biodiversity in agricultural fields compared with non-GR crops (Cerderia and Duke 2006; Owen 2008). The impacts on biodiversity, as a whole, depends on several factors, including the cropping system, weed management tactics, the specific GR crop, geography, and individual grower tactics (Young et al. 2013). For example, the Farm Scale Evaluations (FSE), conducted in the United Kingdom from 2000 to 2002, were the first large-scale research studies conducted to examine how GR crops contrasted with conventional crops in association with crop management techniques to preserve biodiversity (Champion et al. 2003). This research demonstrated a mixed association of weed density and the culture of GR crops, where weed density was increased or decreased in GR crops depending on the specific crop or the timing of the weed density measurement (Heard et al. 2003). The authors of the FSE research noted that the specific integration and management with the GR crop may further complex the impact on biodiversity (Squire et al. 2003).

There are still only a limited number of studies that have examined shifts in weed communities under GR cropping systems (Gibson et al. 2013; Gulden et al. 2010; Young et al. 2013). Importantly, most of the studies are not conducted at a scale to provide a reasonable representation of the impacts of production agriculture. Furthermore, a specific genetic crop trait such as glyphosate resistance has never been documented to directly impact weed communities. Rather, the specific crop management tactics enabled by the crop trait can lead to weed community shifts (de la Fuente et al. 2006; Owen 2008). Diversifying crop management systems can be an effective weed management tool to reduce weed density by limiting the dominance of some species (Doucet et al. 1999). Varying crop management systems can reduce weed species diversity because such practices allow for more consistent and grower-responsive weed control tactics (Gibson et al. 2013).

The soil seedbank provides a reservoir of weed seeds available for the future colonization of an agricultural field under suitable conditions (Cardina et al. 2002). Because of seed dormancy and viability longevity, buildup of the seeds of problematic weeds can occur (Cardina et al. 2002; Forcella et al. 1992). While annual management practices will readily alter the aboveground weed flora, the soil seedbank is typically slower to respond because of the inherent buffering capacity with seeds present from multiple seasons of seed rains. The importance of the soil seedbank on the future weed management challenges makes it imperative to determine the extent to which the soil seedbank reflects regional patterns of plant abundance or local effects such as the cropping system, particularly the implementation of GR cropping systems. Prior research in other systems have demonstrated an effect of cropping system (Cardina et al. 2002; Harbuck et al. 2009; Legere et al. 2011), including crop sequences of genetically engineered (GE) crops (Bohan et al. 2011) on a regional basis, but these relationships have been limited to broad categorizations of the weed seedbank (e.g., monocotyledons and dicotyledons) and not a multivariate community-based assessment. Outside of row cropping systems, glyphosate applications can cause dramatic shifts in the seedbank composition of managed pastures (Rodriguez and Jacobo 2013). Based upon the results of surveying the emerged weeds in these fields (Young et al. 2013), we expected the weed flora in the soil seedbank across broad regions of the U.S. in agricultural fields to show a signal in response to the rotation of GR traits and crops. We tested the following hypothesis: the impact of using the GE crop trait for glyphosate resistance on the weed soil seedbank is dependent on cropping system and geography.

Materials and Methods

Study Sites. A research protocol was outlined that included 156 commercial field sites across six U.S. states in corn, cotton, and soybean production with a minimum 3-yr field history of the following cropping systems: (1) a single continuous GR crop, (2) a rotation of two GR crops, and (3) a GR crop rotated with a non-GR crop. The inclusion of commercial field sites without a history of GR crops was not possible due to their low frequency in the landscape and the non-random approach that would have been required to identify these sites. A complete list of agronomic crop producers who previously used a GR crop trait in six states, Illinois (IL), Indiana (IN), Iowa (IA), Mississippi (MS), Nebraska (NE), and North Carolina (NC) in the

U.S. was compiled in fall 2005. These states represent the major crop growing regions in the U.S., have a high adoption of the GR crops, and have a diversity of environments and cropping systems for production of corn, cotton, and soybean. Further detail on the procedures and criteria used for field site selection are reported in Shaw et al. (2011).

In spring 2006, each field was divided into two equal sections to establish a side that would be managed by the grower and a side managed by a university specialist who implemented Best Management Practices (BMPs) to manage weeds and deter the evolution of GR weed species. The side utilizing BMPs was not used for the analysis presented here and is addressed elsewhere (Wilson et al. 2011).

Data Collection. The grower managed side of the field was sampled using a W-pattern (Thomas 1985) with one sample point per 0.4 ha for a total of 20 (0.5 by 1 m) quadrats in most fields. The sampling locations were GPS-referenced at the first sample time in order to relocate the sample sites for subsequent data collection. The fields were sampled for emerged weeds, enumerated by species at four time periods during the cropping season: (1) prior to crop planting, (2) just prior to any postemergence herbicide applications, (3) two weeks following the postemergence herbicide application, and 4) at crop harvest. Analysis of the emerged weed data are presented in Gibson et al. (2013) and Young et al. (2013). Soil seedbank samples were collected prior to planting from each quadrat in each field. A commercial bulb sampler was used to extract 6.2-cm diameter by 15-cm deep soil samples (0.003 m^2 of soil surface area) which were then stored at -14 C for at least 6 wk before each sample was thawed, de-aggregated, stirred, and distributed over a permeable plastic barrier covering 1.8 cm of vermiculite in a 10 by 15.25-cm plastic tray. The trays containing the soil samples were placed on a greenhouse bench and sub-irrigated via a capillary mat to ensure uniform watering. Irrigation was electronically controlled to provide two to three 30- to 45-min events each day as needed with the water flow adjusted according to temperature and humidity in the greenhouse. Seedlings were identified and counted over 4 weeks, after which soils were then allowed to dry, and the irrigation and germination cycle repeated. After the second germination cycle, soils were stored at 5 C or frozen prior to a third germination cycle. Efficiency of the germination procedures was tested using seed elutriation from samples followed by a crush test for viability (full details in Borza et al. 2007).

Univariate Analyses. Data analyzed were average population density of species emerging as seedlings per 450 cm^3, which is represented by 33.1 m^2, soil sample per site ($n = 20$ for most sites), population density by functional group, and diversity (number of weed species, Shannon's index H$'$ and evenness) per site and diversity by functional group per site. Species were grouped into the following functional groups: summer annuals, winter annuals, forbs, prostrate herbs, erect herbs, perennials, climbing species, graminoids, C_3 plants, C_4 plants, *Ipomea* spp. and *Amaranthus* spp., and GR species (details in Young et al. 2013).

Sixteen of 139 weed species emerging from the weed seedbank were considered to occur frequently enough (i.e., present in > 15% of the 150 sites from which seedlings emerged) for analyses as individual species. Many species were absent or were infrequent in one or more regions necessitating in some cases, the merging of adjacent regions for the mixed model analysis.

A mixed model analysis was conducted using the MIXED procedure in SAS (Statistical software, SAS Institute, 2002–2008. SAS Software, Version 9.2, SAS Institute, Cary, NC 57513) following Littell et al. (2006) (details in Young et al. 2013). Cropping system (1 = single continuous GR crop, 2 = rotation of two GR crops, 3 = GR crop rotated with a non-GR crop), crop rotation (0 = no rotation, 1 = rotation), and GR trait rotation (0 = no rotation, 1 = rotation) were considered as categorical fixed effects. There were too few sites to allow the development of a complete model, thus three analyses were conducted (1) cropping system by region, (2) crop rotation by region, and (3) GR trait rotation by region. Region (USDA hardiness zone, 4, 5, 6, 7, or 8: (U.S. National Arboretum 2003) was considered as a fixed effect in the three analyses (preliminary analysis with region as a random effect was uninformative). The USDA hardiness zone each site location was included in the analysis rather than U.S. State to avoid the use of political boundaries in the analysis (although the results were qualitatively similar when U.S. State was included). The response variable was log transformed prior to analysis as preliminary analyses indicated that the error variance was proportional to the square of the mean indicating that this was the most appropriate transformation for these data (Shaw et al. 2011). Untransformed variables were

used for means calculations. A first-order autoregressive covariance structure (type = ar(1) in PROC MIXED) was used in the model as it returned the lowest Akaike's Information Criteria (AIC) fit statistic compared with unstructured or compound symmetry. Means separation of significant interactions and, when appropriate, main effects, were based on LSMeans tests.

Multivariate Analyses. Weed species population density was analyzed using Non-Metric Dimensional Scaling (NMDS) based on the Bray-Curtis dissimilarity coefficient among sites, a non-parametric ordination procedure in DECODA (Minchin 1991) that we used similarly in a previous experiment on the weeds invading soybean fields (Gibson et al. 2008) and on the weed emergence data from this study (Young et al. 2013). Data consisted of the presence or absence of each species emerging from soil samples collected from each field with the data matrix double standardized by row and column totals. Analysis based upon weed seedling population densities produced qualitatively similar results. Mean total weed population density of all species, species richness, Shannon's H′ and evenness were calculated in DECODA as independent continuous variables. Of the 156 sites, 150 sites were used in the analysis. Sites with no species emerging from the seedbank were omitted from the analysis, as well as one outlier site that had only a single unique species. One hundred random starting configurations were initiated running up to 200 iterations to obtain 1 to 6 dimensional solutions. The minimum number of dimensions necessary to obtain a useful interpretation of the data was retained after inspection of stress plots, and minimum stress with R values.

The relationship between the ordination solution and independent continuous variables (as listed above, as well as site longitude and latitude) were investigated by fitting vectors of maximum correlation in DECODA. Vector significance was assessed following permutation tests to generate correlation values. Vectors significantly correlated with the ordination were retained for plotting in ordination space relative to the ordination centroid.

The importance of categorical variables (6 states, 2004, 2005, and 2006 crop [cotton, rice (*Oryza sativa* L.), sorghum [*Sorghum bicolor* (L.) Moench], soybean, or wheat (*Triticum aestivum* L.), crop rotation [yes, no], cropping system [three categories], glyphosate trait rotation [yes, no], soil texture [12 categories], irrigation [two categories],

herbicide application [two categories], and USDA Hardiness Zone [nos. 4, 5, 6, 7, and 8] were quantified by testing for differences among *a priori* groups (e.g., Illinois sites versus Iowa sites) using Analysis of Similarity (ANOSIM) in DECODA (Minchin 1991). ANOSIM compares within-versus among-group similarity based upon the Bray-Curtis dissimilarity coefficient (itself based upon the presence/absence of each weed species per site) using random permutations of group membership to calculate an *R*-value that is then tested for significance as the proportion of permutated R-values for sites within a group compared to members of another group.

Soil texture was obtained for each site from the USDA Web Soil Survey (USDA 2013). Sites were classified into 12 soil texture categories using the soil texture classification with the largest percentage of land coverage at each site. Herbicide application categories were based upon whether or not herbicide had been applied to the field post-harvest in the previous year (0 = no, 1 = yes). Irrigation was classified by field as 0 = not irrigated or 1 = irrigated in 2006.

The relationship of individual weed species to the ordination was assessed by calculating species scores for each species in the NMDS space. The species scores were calculated as the weighted average of the abundance scores of the samples in which the species occurred in each dimension. These weighted averages were used to plot species as points in the NMDS ordination and are referred to as species centroids because they show the center of the species' distribution with respect to the ordination axes.

Procrustes analysis (PA: least squares orthogonal mapping) was conducted in DECODA (Minchin 1991) to quantify how well NMDS ordination solutions reported in Young et al. (2013) based on aboveground weed counts from each of four sample dates during the 2006 crop season (i.e., [1] prior to crop planting [t1], [2] just prior to postemergence herbicide applications [t2], [3] two weeks following the postemergence herbicide application [t3], and [4] at crop harvest [t4]) related to the ordinations of the soil weed seedbank. In addition, weed count data were pooled across all four sample dates (tPool) by presence/absence to generate a single ordination for comparison with the belowground data. PA estimates how well ordination scores fall within the same position when a test ordination is rotated and scaled to fit a target ordination (the seedbank ordination) with the same number of dimensions

Table 1. A comparison between the mean frequency (%) and population density (m^{-2}) of the top ten weed species in the soil seedbank and field emergence data.

| Rank | Soil seedbank | | Field emergence[a] | |
	Frequency (%)	Population density (m^{-2})	Frequency (%)	Population density (m^{-2})
1	Carpetweed (44)	Common waterhemp (397)	Giant foxtail (37)	Giant foxtail (9.0)
2	Purslane speedwell (34)	Henbit (364)	Velvetleaf (30)	Henbit (5.9)
3	Prickly sida (32)	Carpetweed (265)	Common lambsquarters (28)	Common waterhemp (5.1)
4	Common lambsquarters (31)	Purslane speedwell (199)	Common waterhemp (25)	Common chickweed (3.2)
5	Henbit (30)	Common chickweed (166)	Prickly sida (25)	Annual bluegrass (3.1)
6	Common waterhemp (29)	Redroot pigweed (132)	Henbit (24)	Redroot pigweed (3.0)
7	Velvetleaf (27)	Green foxtail (99)	Horseweed (23)	Large crabgrass (3.0)
8	Redroot pigweed (27)	Eastern black nightshade (66)	Annual bluegrass (23)	Prickly sida (2.7)
9	Green foxtail (25)	Annual bluegrass (66)	Redroot pigweed (21)	Fall panicum (2.3)
10	Common chickweed (22)	Prickly sida (66)	Large crabgrass (21)	Mouseear chickweed (2.0)

[a] Field emergence data, pooled over four sample periods, were adapted from Young et al., 2013.

(Chandy and Gibson 2009; Schoenemann and Carroll 1970). In this case, the test ordinations were the aboveground weed count ordinations from each of the four sample dates and an ordination based upon summed presences over all dates. The relevant test statistic is the scaled root mean square residual which is calculated by expressing the Procrustes RMS residual (an overall measure of fit between the test and target ordination) as a percentage of the length of the first NMDS axis in the target ordination (seedbank ordination in this case). The Procrustes RMS residuals for each site from the separate aboveground weed flora test ordinations (i.e., tPool, t1, t2, t3, t4) were treated as dependent variables in mixed model analyses testing the effects of region, cropping system, glyphosate trait rotation, and crop rotation in the same way that individual species seedbank densities were tested (described above under univariate analysis). The premise here is that because the weed seedbank is the reservoir from which the aboveground weed flora develops, then if the latter reflects the former, this will be reflected in low Procrustes RMS residuals, and vice versa, and will likely reflect aspects of the environment such as management and/or geography (i.e., region in this case).

Results and Discussion

Floristics and Comparison with Aboveground Weed Emergence. Across all 150 sites, 139 weed species were identified in the soil weed seedbank as evidenced by emergence from the soil samples. Of these, three species were common to all six states, i.e., common lambsquarters (*Chenopodium album* L.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and carpetweed (*Mollugo verticillata* L.)

(data not presented). Seventy-nine weed species were unique to a single state and 46 weed species were unique to a single site. On average, there were 7.4 weed species per site, ranging from 1 to 23 species, with $2,849 \pm 397$ seeds per m^2 (minimum 33, maximum 33,322 seeds) (data not presented). Species richness and seedling population density per site were positively correlated (Spearman's Rank correlation = 0.68, P < 0.0001, df = 149). The ten most frequent weed species represented in the soil seedbank over all sites were carpetweed (44% of 150 sites, 265 seeds m^{-2}), purslane speedwell (*Veronica peregrina* L.) (34%, 199 seeds per m^{-2}), prickly sida (*Sida spinosa* L.) (32%, 66 seeds m^{-2}), common lambsquarters (31%, 33 seeds m^{-2}), henbit (*Lamium amplexicaule* L.) (30%, 364 seeds m^{-2}), common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (29%, 397 seeds m^{-2}), velvetleaf (*Abutilon theophrasti* Medik.) (27%, 33 seeds m^{-2}), redroot pigweed (*Amaranthus retroflexus* L.) (27%, 132 seeds m^{-2}), green foxtail [*Setaria viridis* (L.) Beauv.] (25%, 99 seeds m^{-2}), and common chickweed [*Stellaria media* (L.) Vill.] (22%, 166 seeds m^{-2}) (Table 1).

The soil weed seedbank had 93 weed species in common with the aboveground weed emergence data from the same year (Young et al. 2013), but contained 47 unique weed species. Three weed species recorded in the field emergence data were not observed in the seedbank: wild garlic (*Allium vineale* L.), corn, and common speedwell (*Veronica arvensis* L.). There was a strong correlation between weed species frequency across sites in the soil weed seedbank and field emergence (Spearman's Rank correlation = 0.66, P < 0.0001, df = 93) with some weed species such as purslane speedwell and carpetweed occurring in more sites from the

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seedbank than recorded for field emergence (both > 30% frequency in seedbank, < 10% field emergence).

By contrast, some weed species such as horseweed [*Conyza canadensis* (L.) Cronq.] were identified more frequently during field emergence compared with the soil weed seedbank (21% of sites versus 10%, respectively). The number of weed species recorded from the soil weed seedbank per site was a predictor of the number of weed species found during field emergence at one sample date (prior to planting, positive linear regression $R^2 = 0.37$, P < 0.05; other dates no significant relationship) because field application of herbicides influenced weed emergence after planting. This contrasts with Harbuck et al. (2009), although this similarity varied by location and depended upon which time of the year in relation to crop management that the weed community during field emergence was sampled.

Individual Species and Life Form Relationships. Of 16 weed species that occurred with a frequency of 15% or greater in sites for analysis, 10 showed a relationship between abundance (weed seedbank population density per site) and region (i.e., USDA hardiness zone) as a main effect (five species: carpetweed, henbit, common chickweed, giant foxtail (*Setaria faberi* Herrm.)) or in combination with a management treatment (region × cropping system: redroot pigweed, yellow nutsedge (*Cyperus esculentus* L.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], purslane, annual bluegrass (*Poa annua* L.); region × GR trait: barnyardgrass, green foxtail; or region × crop rotation: barnyardgrass, purslane, yellow nutsedge, Figures 1 and 2). These interactions indicate that management effects on the weed seedbank were generally restricted to either region 5 in the north of the study area (i.e., yellow nutsedge, purslane) or region 8 in the south (i.e., barnyardgrass), or both (i.e., annual bluegrass). This could possibly be due in part to the type of crops produced in each region. For example, in region 8 the management of GR cotton would commonly include some soil residual herbicides, which can maintain weed populations with less frequent glyphosate applications (Givens et al. 2009). Management effects alone without an interaction with region were restricted to three weed species, redroot pigweed (low population density in cropping system 3), annual bluegrass (low population density with glyphosate trait rotation), and green foxtail (high population density with cropping

system 3, and in the presence of GR trait rotation and crop rotation) (Figure 3). This result is of particular importance in efforts to mitigate the evolution of GR weeds since moving away from the GR trait may not alter the composition of weed species. Rather, increasing crop diversity may have a greater impact on deterring the evolution of GR weeds than avoiding the use of the GR crop trait. Crop rotation and the use of multiple herbicide modes of action have been cited as an important component of best management practices to deter the evolution of weed resistance to herbicides (Beckie 2007; Johnson et al. 2009; Norsworthy et al. 2012).

Total soil weed seedbank density, total number of weed species per site (richness) and Shannon's H′ diversity was related to region but not to any management treatment. Highest species richness, diversity, and total soil seedbank density were in USDA hardiness zone 6, with the lowest in USDA hardiness zones 4 and 7 (Figure 4). Weed species evenness was not related to region or management. These findings provide further support to the conclusion that geographical location is an underlying factor that can alter the influence of crop rotation and GR traits on weed diversity in the seedbank. The differences observed in the soil weed seedbank in corn compared with cotton and soybean may be related to the differences in the growth form of the three crops (a tall versus two low growing herbs), crop practices, or both. The use of specific herbicides, which can be used in each cropping systems and their soil residual effects, varies and could also influence the soil weed seedbank. For example, atrazine, which cannot be used in cotton or soybean, can have a long soil residual effect. Factors such as these that are common to the production of a specific crop are difficult to separate from the effect of crop rotation on the long-term effects to the weed seedbank (Cardina et al. 2002).

Parameters associated with geography (longitude, latitude, state, USDA Hardiness Zone) were strongly related to the composition of the weed seedbank which provides support for previous findings on the aboveground flora: the presence of individual weed species and life forms, and their abundance and diversity is primarily due to climatic factors associated with location (Young et al. 2013). This location effect could be an overarching factor that could alter the influence of crop rotation and GR traits on weed diversity. Region was related to both weed seed population density and species
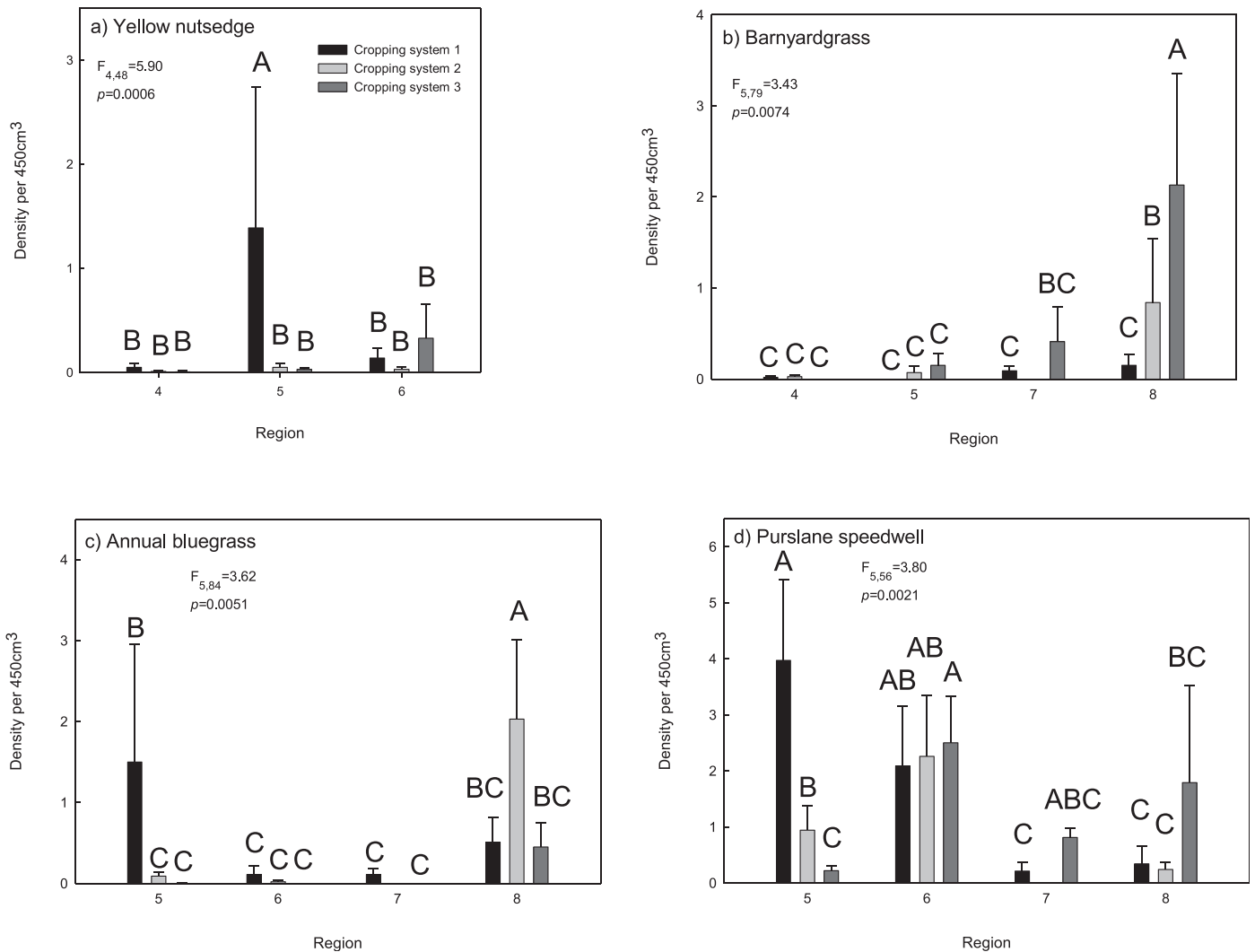
Figure 1. Interaction between USDA Hardiness Zone (region) and cropping system on population density of (a) yellow nutsedge, (b) barnyardgrass (c) annual bluegrass, and (d) purslane speedwell in the weed seedbank. Bars within a panel sharing the same letter are not significantly different (P > 0.05, lsmeans test). Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops.

richness when considered in terms of 11 of the 14 species functional groups. The total population density of *Ipomoea* spp. and the richness of *Amaranthus* spp. and GR weed species were not related to region although their respective richness (*Ipomea* spp.) and population density counts (*Amaranthus* spp. and GR species) were. The composition of the weed seedbank was affected by a hierarchy of filters that reduced the regional species pool (Booth and Swanton 2002; Smith 2006). Geographic location acts as a primary abiotic filter affecting dispersal constraints, thereby limiting the weed species available for colonization at site, while management, crop, and the aboveground weed flora act as a local abiotic and biotic filters. A weed species has to pass through these filters for its seed to be incorporated into the seedbank, which in turn provides the seed source for the emerged weed community (Willand et al. 2013). These filtering effects on the weed seedbank as the source of emerged weed communities were evident at both the individual species level, with the occurrence of 47 unique weed species in the weed seedbank that were not observed during field emergence, and at the community level.

Of the life form groups, the population density of graminoid species was related to the interaction between region and management (Figure 5). The greatest seed density of graminoids occurred in USDA hardiness zone 6 under cropping system 3 with GR trait and crop rotation. These results suggest that while geography is of primary importance, management, including the GR trait, affects only some life forms. The richness of weed species in three life history groups were related to management, but only as a main effect unrelated
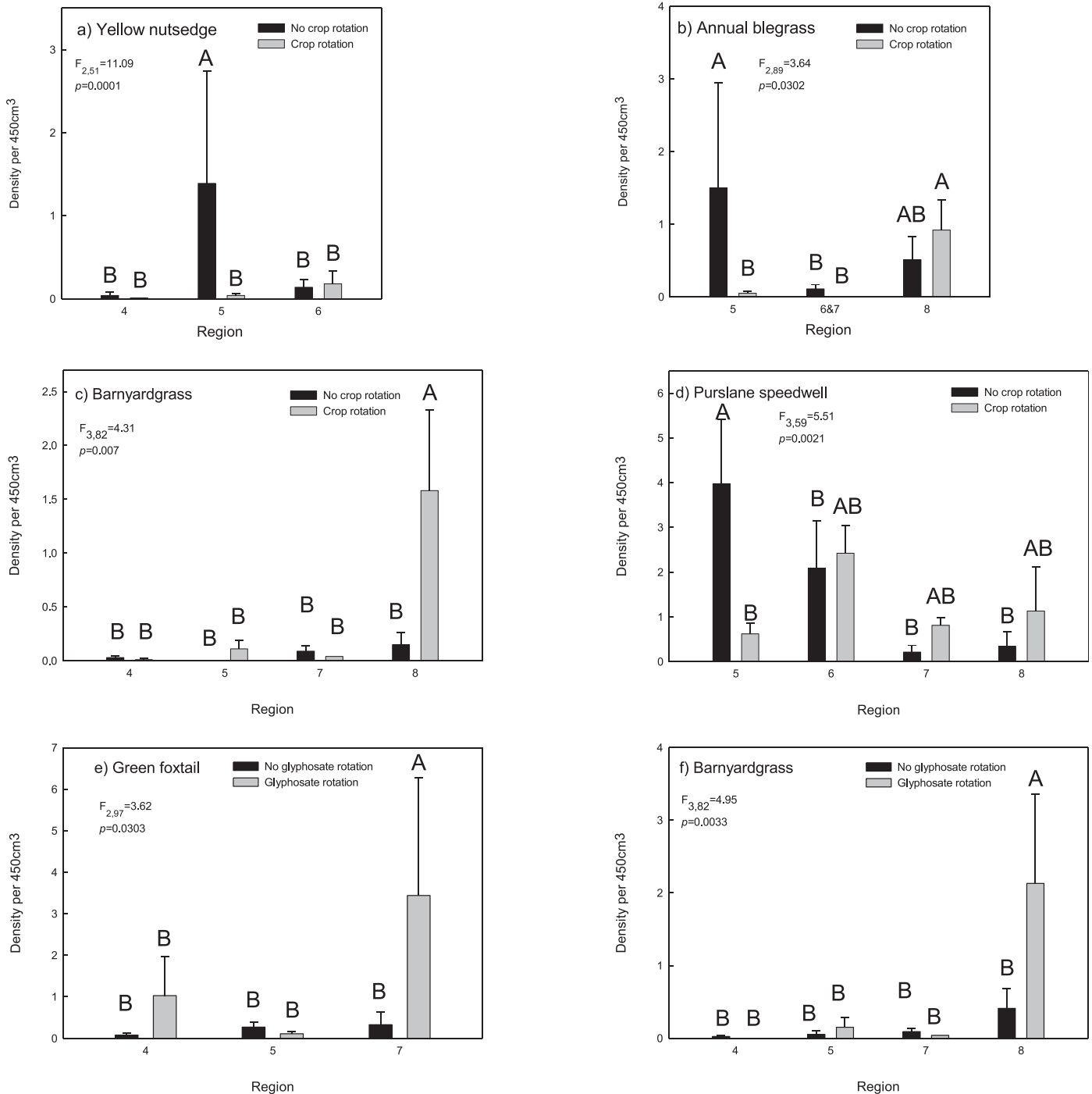
Figure 2. Interaction between USDA Hardiness Zone (region) and crop rotation on population density of (a) yellow nutsedge, (b) annual bluegrass, (c) barnyardgrass, and (d) purslane speedwell in the weed seedbank. Interaction between USDA Hardiness Zone (region) and glyphosate trait rotation on population density of (e) green foxtail and (f) barnyardgrass in the weed seedbank. Bars within a panel sharing the same letter are not significantly different (P > 0.05, lsmeans test).

to region. Richness of biennial weed species was highest in cropping system 1 compared with cropping system 3, and in continuous GR trait use (Figure 6). The presence of biennial weeds may be an artifact of continuous production of GR soybeans using no-tillage practices more than other cropping systems. Richness of winter annual weed

species and prostrate growing weed species were similarly highest in the continuous use of the GR trait.

Multivariate Relationships. A two-dimensional ordination solution was retained for interpretation (stress = 0.185). This analysis showed that the
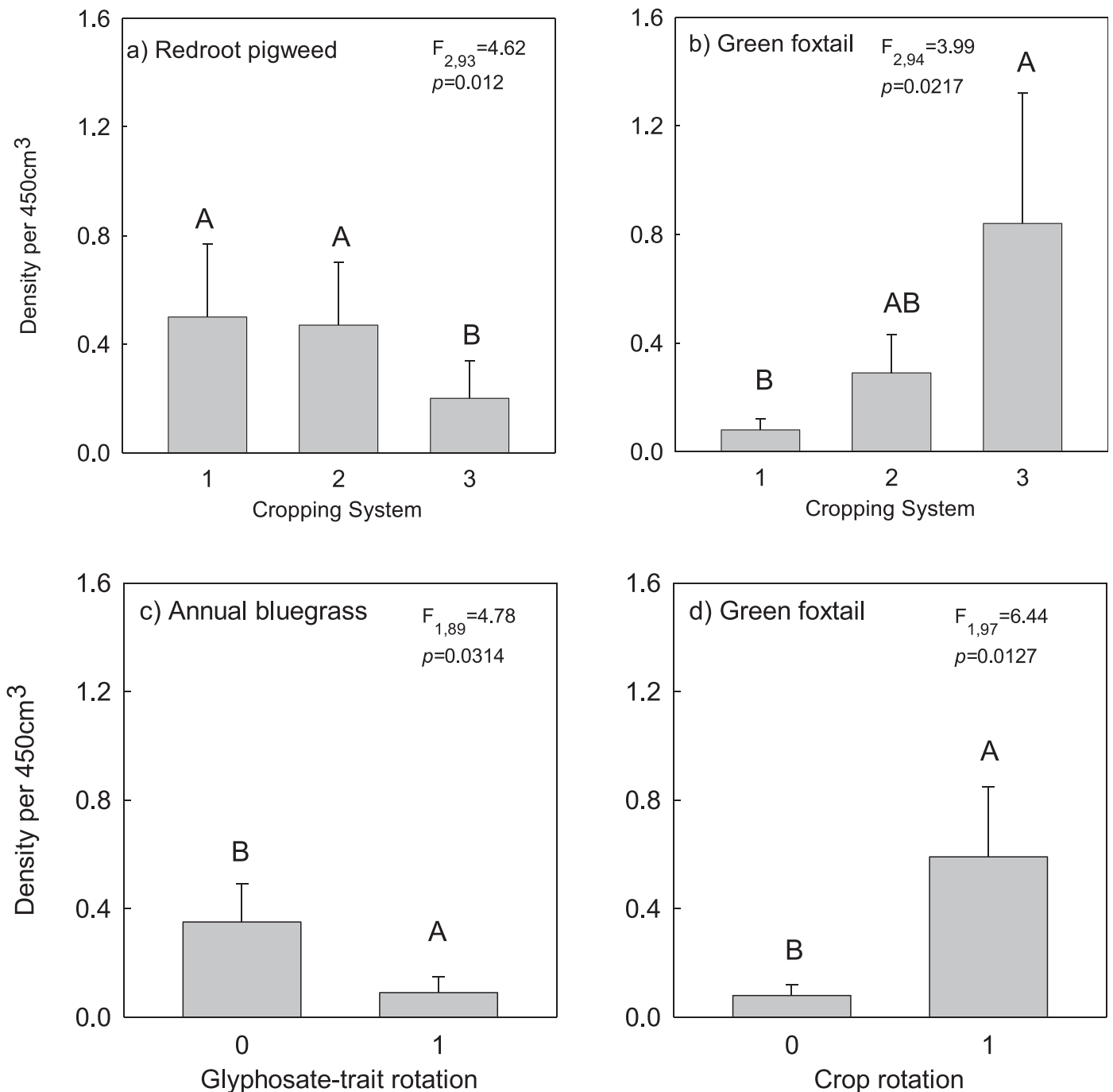
Figure 3. Single main effects on population density in the weed seedbank for: cropping system on (a) redroot pigweed, and (b) green foxtail; glyphosate trait-rotation on (c) annual bluegrass; and crop rotation on (d) green foxtail. Bars within a panel sharing the same letter are not significantly different (P > 0.05, lsmeans test). Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR / non-GR crops. Crop rotation: (0) = continuous cropping; (1) = crop rotation. Glyphosate-resistant (GR) trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait.

composition of the weed flora in the soil weed seedbank was strongly related to location whether expressed as longitude, latitude, state or USDA Hardiness Zone, as well as soil texture and irrigation (Figures 7a and 7b; Table 2). However, there was also a strong signal in these data indicating a relationship to crops in the previous two years, but not three years ago, and cropping system (1 vs.

both 2 and 3, but not 2 vs. 3) and crop rotation, but not GR trait rotation. These relationships suggest that while the weed seedbank is a legacy of recent past conditions, composition can be dynamic reflecting changes in cropping system and management (Bàrberi et al. 1998; Beckie 2006). These changes, however, reflect local rather than regional processes. This relationship could be in part due to
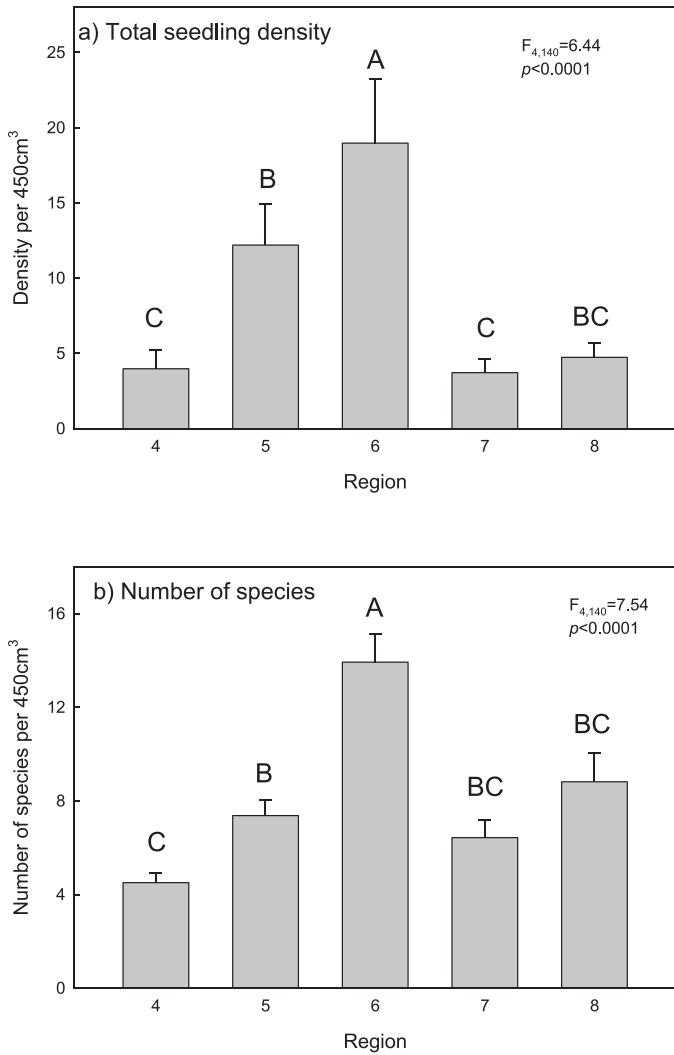
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Figure 4. Effect of USDA Hardiness Zone (region) on a) total population density and b) number of species (Shannon's H′ showed the same pattern; $F_{4,136} = 5.85$, $P = 0.002$) in the weed seedbank. Bars within a panel sharing the same letter are not significantly different ($P > 0.05$, lsmeans test).
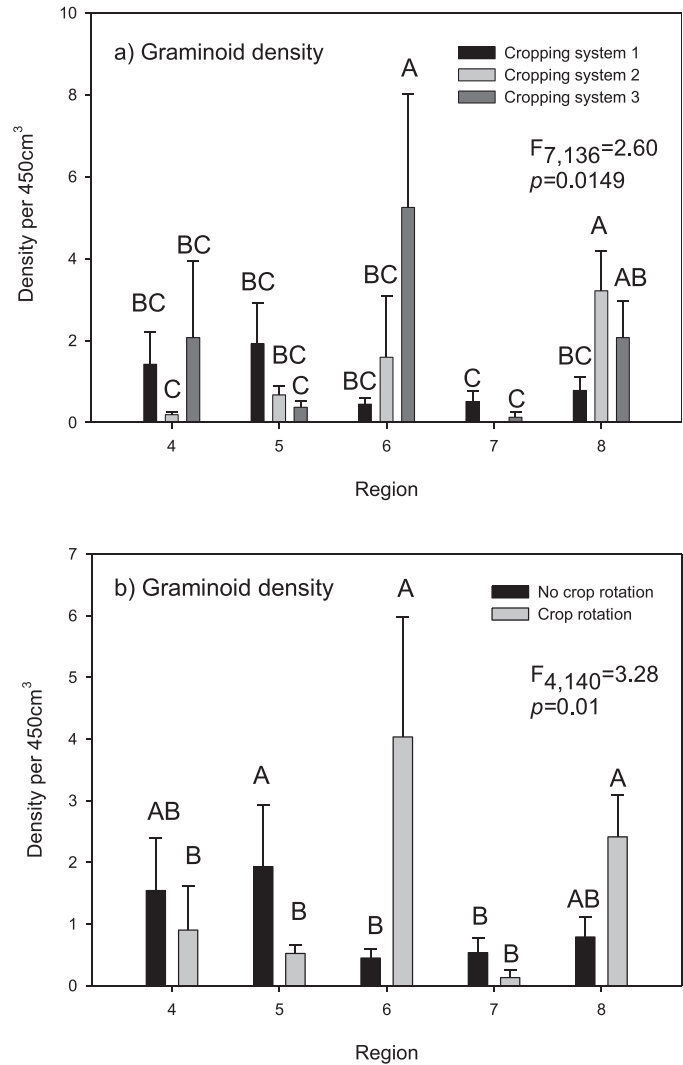

Figure 5. Effect of (a) cropping system and USDA Hardiness Zone (region) on graminoid population density, and (b) crop rotation and USDA Hardiness Zone (region) on graminoid population density in the weed seedbank. Bars within a panel sharing the same letter are not significantly different ($P > 0.05$, lsmeans test). Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops.

the specific weed management tactics implemented by individual growers, which varies regionally (Shaw et al. 2011) and has a significant influence on weed diversity (Wilson et al. 2011; Young et al. 2013). In this study, the continuous production of a single GR crop was associated with the highest weed seedbank diversity (number of species) out of all of the cropping systems for some, but not all, life forms. Continuous production of a single GR crop has also most frequently been associated with the evolution of GR weeds in commercial field sites (Culpepper et al. 2006). Thus, the management practices allowing the highest weed diversity in field populations may also promote the evolution of GR weeds. This may be contrary to common observations in which the evolution of glyphosate-resistant weeds results in a monoculture of that weed species

within the field, essentially the absence of weed diversity. However, the high weed diversity in glyphosate-based herbicide strategies may be a product of the common practice of very few, if any, residual herbicides being used which allows for a diverse weed population to be present prior to the application of glyphosate. Selection pressure on the diverse weed population through repeated glyphosate applications eventually allows for the survival of a dominant, glyphosate-resistant weed species. Cropping systems that integrate GR trait rotation and herbicide rotation may both reduce weed build up in the soil weed seedbank, and evolution of GR weeds (Beckie 2007), even though this system may not
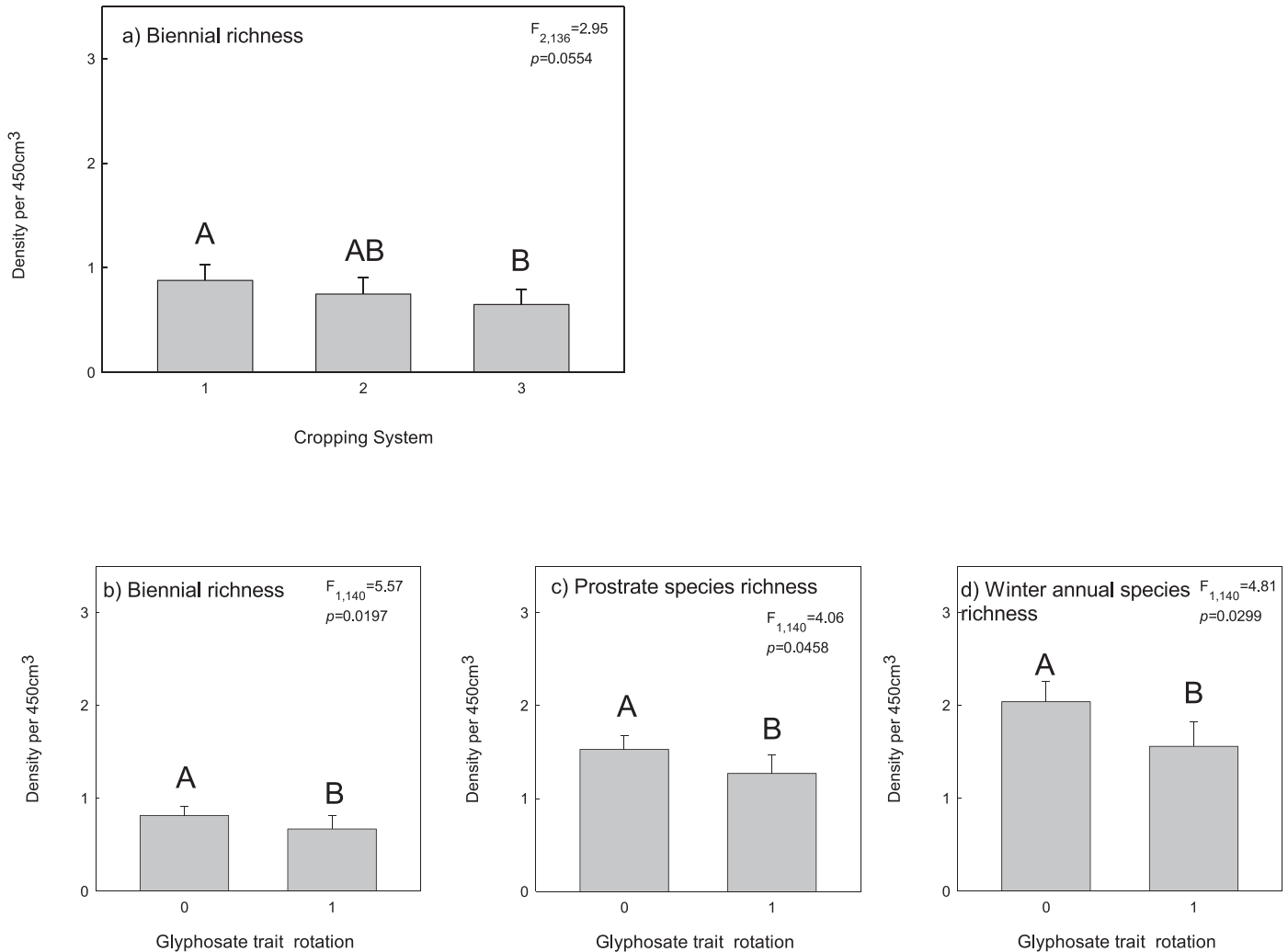
Figure 6. Single main effects of cropping system on (a) biennial species richness and glyphosate trait rotation on (b) biennial species richness, (c) prostrate species richness, and (d) winter annual species richness in the weed seedbank. Bars within a panel sharing the same letter are not significantly different (P > 0.05, lsmeans test). Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops. Glyphosate-resistant (GR) trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait.

provide the greatest weed species diversity within the field which may be thought of as a more sustainable system from an environmental perspective.

Diversity and richness of the weed seedbank were related to the distribution of sites in the ordination (Figure 7a), with the most diverse and species rich sites occurring at low longitudes and latitudes, particularly in USDA hardiness zone 8 where the species centroid plots indicated that redroot pigweed, common lambsquarters, green foxtail, and velvetleaf were most frequent (Figure 7c). Despite the significant difference in the weed seedbank between cropping systems 1 and cropping systems 2 and 3, this distinction was not clearly related to the occurrence of individual species (Figure 7c), rather it reflected the multispecies nature of this floristic difference. Total weed seed population density per

site, and evenness were unrelated to the ordinations (P > 0.05).

Procrustes analysis showed that compositionally, the aboveground weed flora prior to postemergence herbicide application (Young et al. 2013) was most similar to the composition of the weed seedbank compared with other sample dates (i.e., low RMS residual scores expressed as a percent of the first axis of the target seedbank ordination; Table 3). Nevertheless, ordinations of the weed flora from the other sample dates had only 2 to 6% lower RMS residual scores indicating that herbicide suppression of the emerging weeds was having a minor impact on within-season seedbank composition. Overall, the aboveground weed flora based upon summed presences over all dates (tPool) had the best relationship to the seedbank. This result was
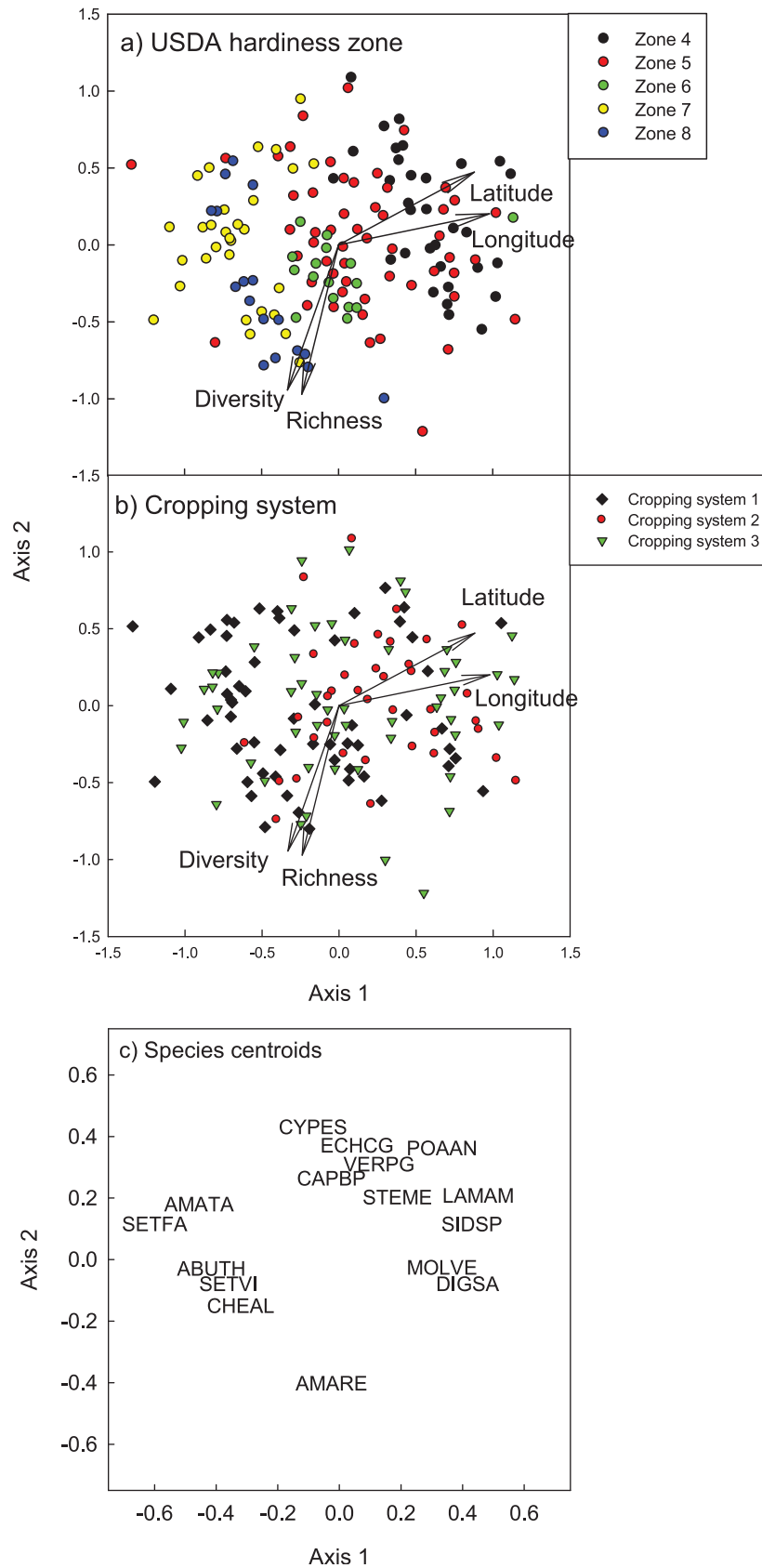
Figure 7. NMDS ordination based upon presence/absence of weed species in the weed seedbank. Plots are labeled according to (a) USDA hardiness zone, and (b) cropping system. Lines with arrow heads show significant (P < 0.05) vectors of maximum correlation of continuous variables longitude ($R = 0.58$, P < 0.0001), latitude ($R = 0.82$, P < 0.0001), richness ($R = 0.46$, P < 0.0001) and diversity (Shannon's H′, $R = 0.40$, P < 0.0001). (c) Species centroids showing the mean weighted average location of species occurring in > 10% of the sites and analyzed in the univariate analyses labeled according to Bayer codes. Note that the axes for the

Table 2. Relationship among a priori groups based upon presence/absence of species emerging from the soil seedbank from 2006. N = number of a priori groups, R is the ANOSIM test statistic. Only significant differences are shown within a variable (e.g., cotton vs. soybean in crops in 2004 was not significant).

Variable	n	R	P
State (all states different to each other)	6	0.63	< 0.0001
USDA Hardiness Zone (all zones different except zones 5 v. 6)	5	0.29	0.0001
Crops in 2003	6	0.008	0.36
Crops in 2004 (corn, cotton, soybean, wheat)	4	0.0815	< 0.0001
Corn vs. cotton		0.32	0.004
Corn vs. soybean		0.07	0.001
Crops in 2005 (corn, cotton, soybean, rice, sorghum, wheat)	6	0.07	< 0.0001
Corn vs. cotton		0.47	< 0.0001
Corn vs. rice		0.25	0.0134
Cotton vs. soybean		0.12	0.022
Cotton vs. rice		0.25	0.04
Cotton vs. sorghum		0.40	0.046
Cotton vs. wheat		0.13	0.043
Rice vs. wheat		0.40	0.017
Soybean vs. rice		0.20	0.04
Soil texture	12	0.15	< 0.0001
Irrigation	2	0.12	< 0.0001
Cropping system[a]	3	0.04	0.004
1 vs. 2	0.08	0.08	0.003
Crop rotation[b]	2	0.05	0.007
Glyphosate trait rotation[c]	2	0.02	0.13

[a] Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops.

[b] Crop rotation: (0) = continuous cropping; (1) = crop rotation.

[c] Glyphosate-resistant (GR) trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait.

Table 3. Procrustes analysis of ordinations of aboveground weed flora (Young et al. 2013) from four sample dates (t1 = prior to crop planting, t2 = prior to postemergence herbicide applications, t3 = two weeks following the postemergence herbicide application, and t4 = at crop harvest) and pooled (tPool) as test ordinations scaled to fit the target ordination of the soil seedbank. Ordinations were 2-dimensional NMDS solutions based upon species presence/absence.

	Test ordination				
	tPool	t1	t2	t3	t4
Number of samples with missing coordinates omitted from analysis[a]	11	76	16	33	42
Procrustes RMS residual	0.48	0.60	0.53	0.64	0.70
Scaled RMS residual (% of target axis 1 length)	19.29	23.94	21.24	25.62	27.95
% of overall squared residuals attributed to axis 1	52.66	40.14	55.28	56.05	62.08
% of overall squared residuals attributed to axis 2	47.34	59.86	44.72	43.95	37.92

[a] Disjunct sites, i.e., those with unique species, were omitted from the analysis. The large number of omitted sites for test ordination t1 reflects the large number of disjunct sites from that date (18 disjunct sites out of 30 sites for IA, 8 of 27 for IL, 21 of 22 for IN, 12 of 19 for MS, 4 of 28 for NC, 6 of 24 for NE, in addition to 11 sites missing from all time period comparisons).

(timing of management) variation in weed seedbank-field emergence similarity (Kelton et al. 2011).

Weeds are persistent problems in crop production and have been known to decrease the biodiversity of a cropping system. A reduction in agroecosystem biodiversity has been inferred in previous studies to be associated with the use of GR crops (Owen 2008). In this study, we show that diversification of the weed community, both in the weed seedbank and aboveground, is reflective of geographic region cropping system being implemented and crop rotation, but not frequency of the use for the GR crop trait. The weed community diversity under specific crops, i.e., cotton, corn, and soybean, where at least two-thirds of which include the GR crop trait (Shaw et al. 2011; Young et al. 2013), are not determined solely by GR trait status. Rather, the method in which a grower integrates the GR technology (varying cultural and mechanical weed management tactics; diversity of herbicide modes of action, application timings, and frequency of glyphosate use; rotation of crops or the GR crop trait) into a cropping system and manages

principally due to a relatively good fit between the first axis of the seedbank and aboveground weed flora ordinations at crop harvest (t4). Procrustes RMS residuals were unrelated to cropping system, glyphosate trait rotation, or crop rotation (all tests, P > 0.05). The best fit between the target (weed seedbank) and test (aboveground weed flora) ordinations occurred in region 6 compared with the other regions (data not shown), especially two weeks following postemergence herbicide applications and at crop harvest reflecting geographic and temporal

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weeds with other tactics determines the diversity of agricultural weeds and their prevalence in the soil seedbank.

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