Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity

Valentín D. Picasso[1]*, E. Charles Brummer[2], Matt Liebman[3], Philip M. Dixon[4] and Brian J. Wilsey[5]

[1]Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Garzón 780, Montevideo 12900, Uruguay.
[2]The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA.
[3]Department of Agronomy, Iowa State University, Ames, IA 50011, USA.
[4]Department of Statistics, Iowa State University, Ames, IA 50011, USA.
[5]Department of Ecology, Organismal and Evolutionary Biology, Iowa State University, Ames, IA 50011, USA.
*Corresponding author: vpicasso@gmail.com

Abstract

Cropping systems that rely on renewable energy and resources and are based on ecological principles could be more stable and productive into the future than current monoculture systems with serious unintended environmental consequences such as soil erosion and water pollution. In nonagricultural systems, communities with higher species diversity have higher productivity and provide other ecosystem services. However, communities of well-adapted crop species selected for biomass production may respond differently to increasing diversity. Diversity effects may be due to complementarity among species (complementary resource use and facilitative interactions) or positive selection effects (e.g., species with higher productivity dominate the mixture), and these effects may change over time or across environments. Our goal was to identify the ecological mechanisms causing diversity effects in a biodiversity experiment using agriculturally relevant species, and evaluate the implications for the design of sustainable cropping systems. We seeded seven perennial forage species in a replicated field experiment at two locations in Iowa, USA, and evaluated biomass productivity of monocultures and two- to six-species mixtures over 3 years after the establishment year under management systems of contrasting intensity: one or three harvests per year. Productivity increased with seeded species richness in all environments, and the positive relationship did not change over time. Polyculture overyielding was due to complementarity among species in the community rather than to selection effects of individual species. Complementarity increased as a log-linear function of species richness in all environments, and this trend was consistent across years. Legume–grass facilitation may explain much of this complementarity effect. Although individual species with high biomass production had a major effect on productivity of mixtures, the species producing the highest biomass in monoculture changed over the years in most environments. Furthermore, transgressive overyielding was observed and was more prevalent in later years, in some environments. We conclude that choosing a single well-adapted species for maximizing productivity may not be the best alternative over the long term and that high levels of species diversity should be included in the design of productive and ecologically sound agricultural systems.

Key words: monoculture, polyculture, richness, overyielding, transgressive overyielding, legume, grass

Introduction

A growing sector of the agriculture community believes that research should focus on developing systems that 'depend largely on contemporary energy, current sunlight rather than scarce fossil fuels (...) and recognize the overriding importance of ecology and uniqueness of place'[1]. Polyculture systems, in which multiple species are grown in mixture, may reduce dependence on external inputs, economic risk and food insecurity, while protecting natural resources[2]. Perennial herbaceous polycultures are mixtures of perennial grass and forb species grown for

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agricultural purposes (grain, forage or biomass production) that can increase diversity and productivity in agricultural landscapes while reducing soil erosion, especially in lands suitable for low-input systems[3–6]. Because these plant communities are composed of perennial species, understanding their long-term dynamics is critically important for management recommendations.

Ecological studies in non-agricultural systems have shown that plant species and functional diversity can increase biomass productivity compared to monocultures, a phenomenon termed as overyielding, but the mechanisms responsible for these effects are still debated[7,8]. Species-rich communities have a greater diversity of traits (e.g., various rooting depths) and may use resources more completely than monocultures. Positive interactions among species can enhance productivity in diverse communities (e.g., legumes can improve nitrogen availability to other species[8,9]). These mechanisms, collectively referred to as complementarity, provided the initial hypothesis for the positive relationship between diversity and productivity[10]. Overyielding may also occur when highly productive species dominate the community, that is, there is a covariance between the species yield in monoculture and its relative yield in the mixture, an example of the selection effect. Because complementarity and selection have different theoretical and practical implications and both may operate at the same time, it is relevant to distinguish between these effects in biodiversity experiments[10].

A second relevant issue is whether overyielding effects observed in biodiversity experiments are consistent across time and environments. Long-term studies in grasslands have shown a consistent and increasingly positive diversity effect on productivity and stability[11], and experiments conducted at multiple locations have shown, in general, a consistent increase in productivity with diversity across locations[12]. However, experiments may operate at spatial and temporal scales smaller than relevant to fully capture agroecosystem dynamics[13,14].

Individual species may perform differently in alternative environments and/or years, and therefore diversity–productivity relationships may change with species composition in agricultural contexts[15]. Also, because agricultural systems require species and cultivars that involve important breeding efforts, understanding the role of individual species on polyculture productivity is relevant to design future breeding programs. Furthermore, identifying the amount of species diversity required to maximize productivity and desirable ecosystem functions is a timely issue[16]. Of special interest in an agricultural context is the identification of situations promoting transgressive overyielding, that is, when a polyculture yields more than the highest yielding component species in monoculture, a phenomenon not usually found in biodiversity experiments[17].

In the first 2 years of a biodiversity experiment, we found a positive biomass productivity–species richness relationship, but the presence of a single high-yielding species in each environment explained most of this diversity effect[15].

In the first paper we did not attempt to identify the ecological mechanisms causing this relationship. However, with an additional year of data, we attempted to evaluate the productivity–species richness relationship in more detail. Because perennial forage species expend energy during establishment developing roots below ground, a complex community may be expected to be in an establishment phase in the first 1–2 years post-seeding. Thus, the third year of the experiment would represent the first year that all species, especially the warm-season grasses, were fully established, and with the addition of that year's data, changes in community dynamics over time could be detected. Therefore, in this paper, we extend the results from our previous paper by analyzing the results of 3 years of our experiment, with the goal of identifying in detail the ecological mechanisms that sustained higher biomass yields over a longer term and evaluating the implications for the design of sustainable cropping systems. We specifically test the following hypotheses: (1) that a positive productivity–species richness relationship was consistent across environments over 3 years; (2) that the identity of the highest yielding species changed over time in 3 years; (3) that a positive overyielding–species richness relationship was due to complementarity among species and not to a selection effect; (4) that complementarity increased as a function of species richness across environments and years; and (5) transgressive overyielding increased over time across environments.

Materials and Methods

Experimental design

Forty-eight plant community entries (i.e., treatments) were assembled using seven perennial species from three taxonomic groups: legumes [alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacM. ex B.L. Robins. & Fern.)]; C_3 grasses [orchardgrass (*Dactylis glomerata* L.) and intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey)]; and C_4 grasses [switchgrass (*Panicum virgatum* L.) and eastern gamagrass (*Tripsacum dactyloides* (L.) L.)]. Monocultures of each species were included (7 entries), as well as selected polycultures of two (19 entries), three (13 entries), four (6 entries) and six (3 entries) species (for the list of entries see Picasso et al.[15]). The suite of treatments was not random but systematic so that for most levels of species richness some entries included each individual species and other entries did not. This design allowed us to analyze the effect of individual species on the richness–productivity relationship. Because legumes have been shown to have a major impact on the results of biodiversity experiments[18,19], we included at least one legume in all entries except for monocultures and two-species combinations of grasses, and the same grass species and mixtures were included with each of the three legumes. Monoculture seed

identity and seed density were: alfalfa (cv. 54H51) 838 pure live seeds (PLS) m^{-2}, white clover (cv. Alice) 795 PLS m^{-2}, bundleflower (University of Minnesota accessions) 199 PLS m^{-2}, orchardgrass (cv. Duke) 1625 PLS m^{-2}, inter-mediate wheatgrass (cv. Oahe) 239 PLS m^{-2}. switchgrass (cv. Cave-in-Rock), 621 PLS m^{-2}, and eastern gamagrass (cv. PMK-24) 15 PLS m^{-2} based on standard agronomic recommendations for Iowa[20,21]. A replacement design was used[22], such that seed density for species in polycultures was reduced proportionally according to the number of species (e.g., a two-species mix included half of the seeds of each monoculture). Thus, the expected proportion of each species in the polyculture was 0.50, 0.33, 0.25 and 0.17 for two, three, four and six species, respectively, and expected evenness was 1 for all polycultures.

Each entry was replicated three times in an alpha lattice design at two locations: the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone, Boone Co., IA, USA, with a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and the ISU Hinds Research Farm in Ames, Story Co., IA, USA with a Spillville loam soil (fine-loamy, mixed, super-active, mesic Cumulic Hapludolls). In spring 2003, plots were tilled before drilling seeds into 4-m × 3-m plots con-sisting of 20 rows spaced 0.15 m apart. Plots were mowed in June and September 2003 to a 0.15-m height to control weeds. In October 2003, the number of plants of each species present along a 1-m transect per plot was counted. Eastern gamagrass was reseeded by hand in April 2004, because no plants were found in 2003. More details on site characteristics and management practices can be found elsewhere[15].

In 2004, each plot was split into half to form two 2 m × 3 m subplots that were allocated to either a multiple harvest system with removal of all biomass, or to a single-harvest management simulating a perennial grain system with only seed biomass of selected species being removed. The same allocations were used in 2005 and 2006 as well. Plots were machine clipped with a flail-type harvester (Carter Manufacturing, Brookston, IN, USA) equipped with an electronic weigh system. A single 1-m-wide × 3-m-long strip was harvested for biomass through the center of each subplot. The adjacent 50-cm strips on either side of the measured area were cut immediately after data col-lection so that all biomass was clipped to ground level. Dry matter percentage of samples taken from each subplot immediately before each harvest was used to calculate dry biomass weight per subplot (hereafter referred as total biomass).

In the multiple harvest system, subplots were harvested three times per year in 2004 and 2005, in late-May, mid-July and mid-September; and twice in 2006, in late-June and mid-August. At Ames in 2004 the third harvest was incomplete because of field labor constrains, and so these harvest data were dropped from the analysis. In the single-harvest management, total accumulated biomass of the subplots was harvested in late-October to early November depending on the year. Before that, reproductive structures of four species (Illinois bundleflower, orchardgrass, inter-mediate wheatgrass and switchgrass) were hand harvested from the entire subplot area as each species matured, weighed and added to the total subplot biomass in 2004 and 2005, but no seed harvest was conducted in 2006.

Two different methods were used to estimate relative abundance of each species (both seeded and weeds). In 2004 and 2005, individual species biomass was measured by clipping two 0.09-m^2 quadrats per plot, sorting species in the laboratory, and weighing them. In 2005 and 2006, individual species ground cover percentage was recorded from visual observations on each plot by the same observer, using a 1 m^2 quadrat located in the center of the plot. Linear regressions for the relative abundance of each species based on biomass and ground cover for 2005 revealed a strong association between the two variables (all $P < 0.0001$ and average $R^2 = 0.78$) for most seeded species with the excep-tion of species with low yields in certain environments (eastern gamagrass in B1 and B3, white clover in A1, wheat-grass in A3), although linear coefficients were not equal to one for several species. Therefore, relative abundance in 2006 was estimated using relative cover data for 2006 and regression coefficients from 2005. We estimated the biomass using the regression coefficients, rather than using the cover estimates, because the linear coefficients differed among species due to density differences, that is, biomass per unit of cover varied among species.

Data analyses

We found location and management system × entry interactions (with crossover), and so all analyses were conducted separately for location and management combi-nations, hereafter referred to as environments: Boone-one harvest (B1), Boone-three harvests (B3), Ames-one harvest (A1) and Ames-three harvests (A3). The variable of interest was biomass productivity of seeded species, and so biomass of weeds (either spontaneous species or seeded species not intended in a plot) was excluded from the analyses (similar results were obtained when all species were considered, see[15]). For each environment a heterogeneous regression lines mixed model was fit, including richness of seeded species (natural log transformed, continuous variable), year (repeated measures on each plot), richness × year interac-tion, replications (large complete blocks) and blocks within replication (smaller incomplete blocks of the alpha lattice design). All factors were considered fixed except for replication and block(rep), which were considered random. An autoregressive covariance structure for the residuals was used. In order to fit the correct model, we first tested the hypothesis that the effect of richness was zero for all years by fitting a model with only year and year × richness effects, which we rejected if the year × richness effect was significant. If the first hypothesis was rejected, we tested a second hypothesis of no year × richness interaction by fitting a full model with year, richness and year × richness,

and rejecting it if year × richness was significant[23]. Least-squares means for each entry were calculated, and means were separated using Tukey's multiple comparisons test. Because the presence of individual species was shown to affect the relationship richness–productivity[15], the effect of the single species with higher 3-year average monoculture yields at each environment was tested by conducting regressions for the subsets of plots including and excluding each of these single species for all environments and years.

In order to identify the ecological mechanisms behind the relationships richness–productivity, we partitioned the net richness effect into complementarity and selection effects using the method of Loreau and Hector[10]:

$$\Delta Y = Y_O - Y_E = N \cdot \overline{\Delta RY} \cdot \overline{M} + N \cdot \mathrm{cov}(\Delta RY, M)$$

where the net richness effect ΔY is the difference between the observed yield of the polyculture (Y_O) and the expected yield (Y_E) based on the sum of the component species' monoculture yields weighted by their proportion in the mixture. A positive net richness effect means that the polyculture yields were greater than the average of the monocultures, a condition we define as 'polyculture overyielding'.

The complementary effect is denoted by $N \cdot \overline{\Delta RY} \cdot \overline{M}$, where N is the number of species in the mixture, $\overline{M}$ is the mean monoculture yield of the component species and $\overline{\Delta RY}$ is the mean relative yield deviation across the i species in the polyculture. The relative yield deviation of a given species, ΔRY_i is the difference of that species' observed relative yield (RY_i)—its polyculture yield divided by its monoculture yield—and its expected relative yield (ERY_i) based on the proportion seeded in the polyculture. A positive value of ΔRY_i means that the species in polyculture yielded more than expected, a condition defined as 'species overyielding'. The selection effect is defined as $N \cdot \mathrm{cov}(\Delta RY, M)$, i.e., the covariance between relative yield deviations and monoculture yields. Positive complementarity effect occurs when the species in the polyculture, on average, yield more than expected based on their monoculture yield and their proportion in the mixture. The selection effect is present if the relative yield deviation is related to monoculture performance. A selection effect is positive when as monocultures yields increase, relative yield deviations in polycultures also increase, and it is negative when as monoculture yields increase, relative yield deviations decrease. Polyculture overyielding, complementarity and selection effects can each be positive or negative independently, and so this method provides a practical framework to understand the mechanisms involved in the richness–productivity relationship. In our calculations, we used the monoculture yields averaged over the three replications in the experiment, and so the mean monoculture yield by environment and year was the basis for comparisons to polyculture yield and relative yield as is recommended for intercropping experiments by Federer[24]. Monoculture yield of eastern gamagrass was zero in 2004

in Boone, and so we excluded it from the analyses for that environment and year to avoid the problem of relative yields approaching infinity when monoculture values are too small[8].

A mixed model similar to the one used for productivity was fit for each of the three effects (i.e., polyculture overyielding, complementarity, and selection) and least squares means by the richness level were calculated across years by environment. The effect of year on the relationship richness–overyielding, richness–complementarity and richness–selection was tested using the heterogeneous regression lines mixed-modeling approach described above.

In order to identify transgressive overyielding we subtracted from each polyculture yield the highest monoculture yield of the species seeded in each mixture. Least-square means for this variable by entry were calculated by environment and year, using a mixed model fitted as described for productivity. Mixtures with means significantly different from zero (at the 10% probability level) were identified as showing transgressive overyielding. The proportion of mixtures showing transgressive overyielding was calculated by environment and year, and differences in this proportion between first and last years were assessed with a Z-test assuming a normal approximation of the binomial distribution.

All analyses of variance and contrasts were performed using PROC MIXED and regression coefficients were calculated using PROC REG, in the SAS statistical software package[25]. Statistical significance was assessed at the 5% level unless otherwise indicated.

Results

Productivity–richness relationship over time

There was a strong linear association between the observed richness of seeded species and the original richness of seeded species at the time of sowing for each combination of environment and year ($P < 0.0001$; data not shown). Analyses of effects of richness on productivity gave similar results for both measures of richness. For clarity and consistency with the complementarity-selection analyses, we show the results here using seeded species richness.

We found an overall effect of species richness on productivity for all environments ($P < 0.0001$). Environments B1 and B3 had no richness × year interaction; therefore, a model without interaction was fit, where productivity was a log-linear function of richness (for B1, $b_1 = 280$; for B3, $b_1 = 478$, all $P < 0.0001$). A richness × year interaction was found in A1 ($P = 0.02$) and A3 ($P = 0.03$), but slopes were positive for all years (for A1: $b_1 = 275$ in 2004, $b_1 = 192$ in 2005 and $b_1 = 331$ in 2006; for A3: $b_1 = 261$ in 2004, $b_1 = 395$ in 2005 and $b_1 = 405$ in 2006, all $P < 0.0001$). There was no consistent trend of either strengthening or weakening of the richness–productivity relationship with years, except for environment A3, where the slope increased over time.

Individual species effects on productivity over time

Biomass yield of monoculture plots differed among species within environments for all years (Fig. 1) except for Ames in 2006. In B1, the highest monoculture yields were from intermediate wheatgrass in 2004, but no significant differences were found among the three highest yielding species in 2005 and 2006. In B3, alfalfa had the highest yields in monoculture every year, and no other species had equivalent yield in any year. In A1 intermediate wheatgrass had the highest monoculture yields in 2004, but no differences were observed among the three highest yielding monocultures in 2005 and 2006. In A3, no significant differences were found among species in any year. Therefore, maximum monoculture yields were consistently observed from a single species in only one environment every year (alfalfa in B3); no single species show a similar pattern in the other environments.

Alfalfa in the multiple harvest management and intermediate wheatgrass in the single-harvest management were the species with highest yields in monoculture in both locations averaged over the 3 years. In A3 and B3, productivity of plots with alfalfa was consistently higher than that in plots without alfalfa across richness levels. In A1 and B1, productivity of plots with intermediate wheatgrass was usually higher than that in plots without intermediate wheatgrass across richness levels.

For the subset of plots excluding the highest-yielding species in each environment, in general productivity increased log-linearly with seeded species richness (Table 1). The only exception was year 2005 for the one harvest management environments (plots without intermediate wheatgrass), where no increase in productivity was found with richness. For the subset of plots including the highest-yielding species in each environment there was a more diverse response of productivity to richness (Table 1 and Fig. 2) across environments. In B3, plots including alfalfa in the seeded mix had constant and high productivity regardless of seeded species richness. In A1 and B1, in 2004 and 2005 productivity of plots including intermediate wheatgrass did not change with richness, but in 2006, productivity did increase with richness. Finally, in A3, the first 2 years productivity increased with species richness and in 2006 there was no change.

Polyculture overyielding, complementarity and selection effects

A positive polyculture overyielding effect was observed across all environments (Figs. 3 and 4). Therefore, polyculture yields were greater than the average of the component species in monoculture. For all environments polyculture overyielding increased as a log-linear function of species richness ($b_1 = 255.3$ in B1, $b_1 = 461.4$ in B3, $b_1 = 219.8$ in A1, $b_1 = 333.1$ in A3, $P < 0.0001$ for all environments).
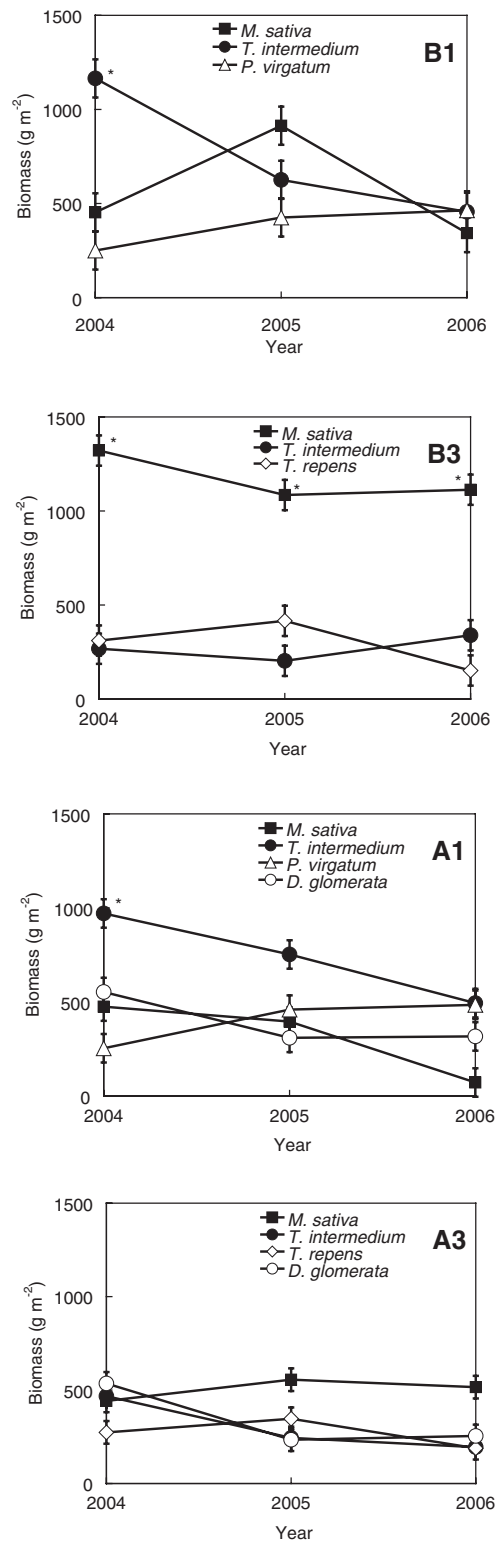

Figure 1. Means of biomass productivity ($g\,m^{-2}$) of the highest-yielding monocultures over the 3 years in each environment (B1: Boone-one harvest; A1: Ames-one harvest; B3: Boone-three harvests; and A3: Ames-three harvests). Species included had either the highest or second to high yield in at least 1 year in each environment. Standard error bars for the mean in each environment are shown. Means marked with * were significantly different from the other means shown below in that year in a Tukey multiple comparisons test at 5%.

Table 1. Linear regression parameters (b_0 and b_1), P-values and adjusted R^2 for models of biomass productivity of seeded species ($g\,m^{-2}$) as a function of natural logarithm of seeded species richness in each environment and year, for subsets of plots including (with) and excluding (without) the species with highest monoculture yields averaged over the 3 years in each combination of location and harvest management, referred as environments (B1: Boone-one harvest, A1: Ames-one harvest, B3: Boone-three harvests and A3: Ames-three harvests). The one harvest management includes seed and vegetative biomass harvested at the end of growing season, the three harvest management is the sum of three vegetative biomass cuts throughout the growing season. In the cases that linear regressions are not significant at 5%, the null model is shown (mean).

Environment	Subset of plots	Year	b_0	b_1	P-value	Adj. R^2	Mean
Boone-one harvest (B1)	With *T. intermedium*	2004					706
		2005					832
		2006	333	135	0.0311	0.07	
	Without *T. intermedium*	2004	−32	365	<0.0001	0.17	
		2005					535
		2006	165	144	0.0274	0.04	
Boone-three harvests (B3)	With *M. sativa*	2004					1040
		2005					1110
		2006					1052
	Without *M. sativa*	2004	−67	329	<0.0001	0.24	
		2005	−11	241	<0.0001	0.19	
		2006	10	251	<0.0001	0.21	
Ames-one harvest (A1)	With *T. intermedium*	2004					792
		2005					672
		2006	299	197	0.0005	0.20	
	Without *T. intermedium*	2004	149	211	0.0110	0.06	
		2005					299
		2006	42	272	0.0015	0.10	
Ames-three harvests (A3)	With *M. sativa*	2004	361	148	0.0458	0.06	
		2005	475	136	0.0244	0.08	
		2006					640
	Without *M. sativa*	2004	115	196	0.0031	0.08	
		2005	−84	307	<0.0001	0.22	
		2006	−91	277	<0.0001	0.31	

A positive complementarity effect was observed across all environments (Fig. 3), and complementarity increased as a log-linear function of species richness ($b_1 = 447.8$ in B1, $b_1 = 331.9$ in B3, $b_1 = 208.2$ in A1, $b_1 = 223.8$ in A3, $P < 0.0001$ for all environments). This indicated that on average the species in polycultures had relative yields greater than expected based on their seeded proportion (i.e., overyielding of individual species was observed) and that this complementarity effect was greater at higher richness levels.

Selection effects differed among environments (Fig. 3). In B1 selection effects were negative overall, and decreased as negative log-linear function of richness ($b_1 = −215.1$, $P = 0.0011$). In B3 and A1, there were no trends in selection effects with richness ($P = 0.1651$ in B3 and $P = 0.8639$ in A1). In A3, selection effects were mainly zero or positive across species richness levels, and the selection effect increased as a log-linear function of richness ($b_1 = 118.2$, $P = 0.0083$).

The relative yield deviation ($\Delta RY_{i,}$) of orchardgrass ranged from 0.4 to 1.8 and was the largest of all species in polycultures across all richness levels in all environments, indicating that orchardgrass overyielding was highest of all

species. Relative yield deviation for alfalfa ranged between 0 and 0.3 across richness levels in all environments. The relative yield deviations for all other species were usually zero across richness levels in all environments.

Although polyculture overyielding was observed throughout the experiment in all environments and years, transgressive overyielding was detected mainly in Ames environments (Fig. 4). The frequency of transgressive overyielding (percent of mixtures sown showing this effect) increased over time, reaching 20% in A1 and 30% in A3 in the last year. Transgressive overyielding was observed in mixtures with all levels of species richness, but was more common in the mixtures with six species sown (up to 67% of these plots in A3-2005 and 2006). Mixtures with 2 years of transgressive overyielding were: alfalfa–orchardgrass, alfalfa–orchardgrass–wheatgrass, clover–orchardgrass–wheatgrass, alfalfa–clover–orchardgrass–wheatgrass, alfalfa–bundleflower–orchardgrass–wheatgrass, alfalfa–bundleflower–orchardgrass–wheatgrass–switchgrass–gamagrass and clover–bundleflower–orchardgrass–wheatgrass–switchgrass–gamagrass. The absolute value of transgressive overyielding (difference between the mixture yield and maximum monoculture yield) ranged from 142 ± 81 to
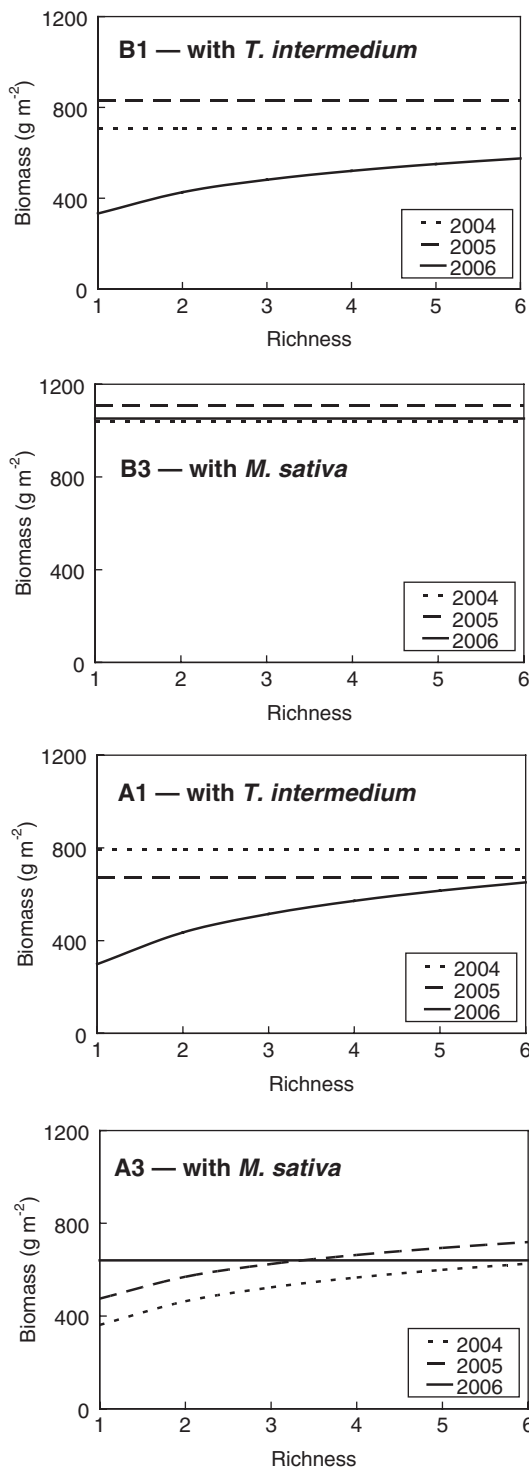
Figure 2. Regression lines for biomass productivity of seeded species ($g\,m^{-2}$) as a function of seeded species richness in each year, for plots including the species with highest 3-year average monoculture yield in each environment (B1: Boone-one harvest; A1: Ames-one harvest; B3: Boone-three harvests; and A3: Ames-three harvests). Regression parameters or means in each case are given in Table 1.

$489 \pm 147\,g\,m^{-2}$. It should be noted that in order to detect (statistically) significant differences in our experiment the required yield (agronomical) differences were relatively
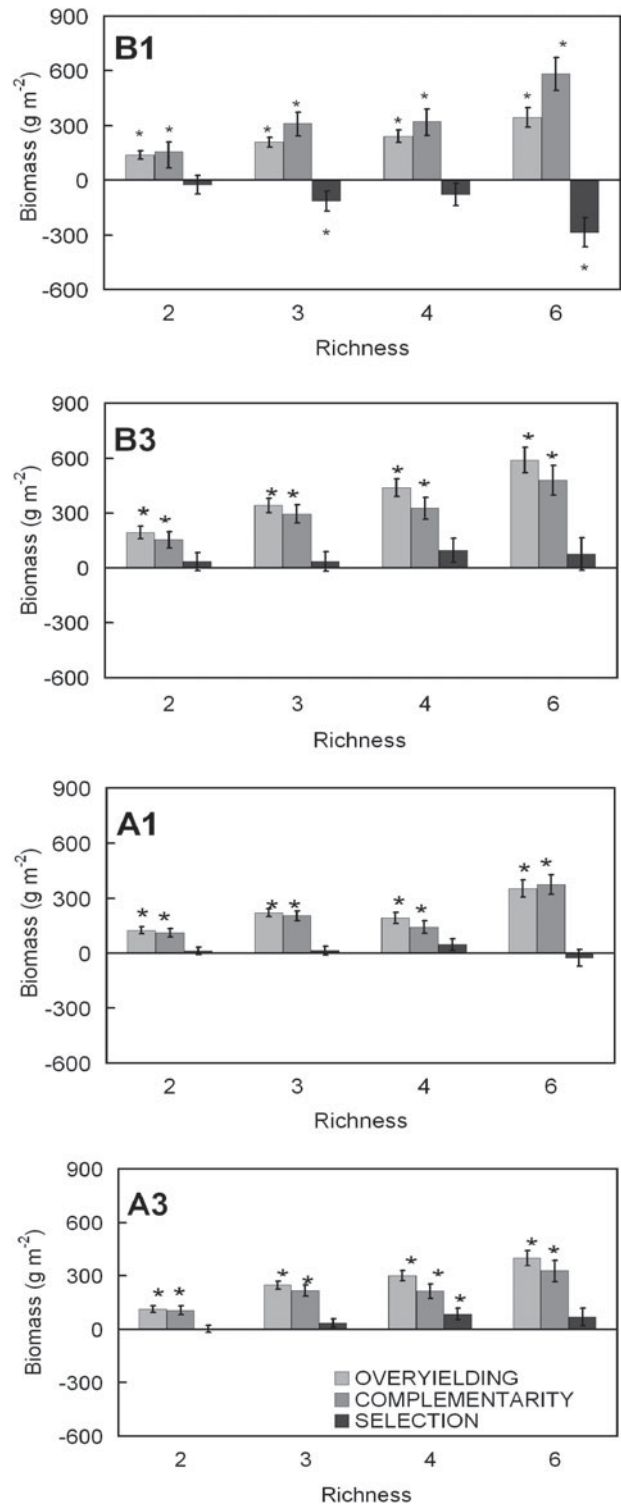
Figure 3. Polyculture overyielding (polyculture yields greater than the average of the monocultures), complementarity (species in polyculture yield more than expected based on their monoculture yield and proportion in the mixture) and selection (species relative yield deviation related to monoculture performance) effect means ($g\,m^{-2}$) by seeded species richness averaged over the 3 years for each environment (B1: Boone-one harvest; A1: Ames-one harvest; B3: Boone-three harvests; and A3: Ames-three harvests). Means marked with * were significantly different from zero at 5%.
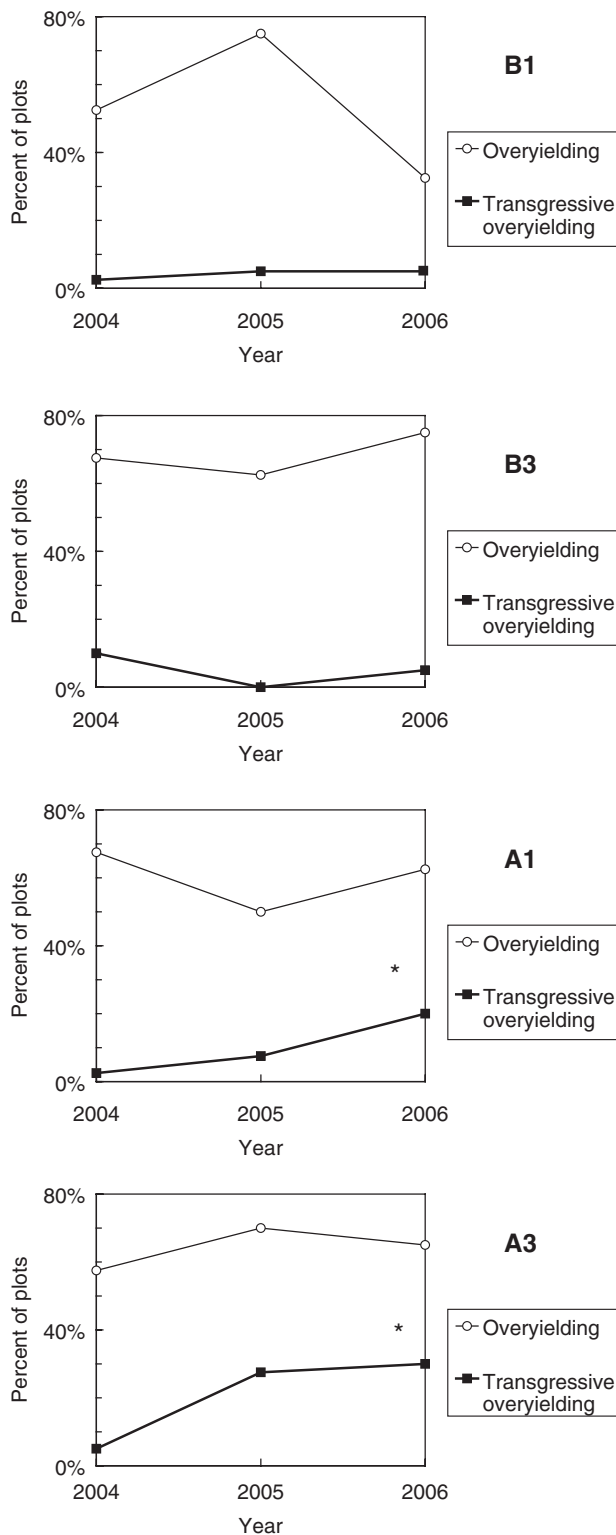
Figure 4. Percent of plots that showed significant polyculture overyielding (polyculture yields greater than the average of the monocultures in the mixture, open circles) and transgressive overyielding (polyculture yields greater than the highest monoculture in the mixture, filled squares) over the 3 years of the experiment in each environment (B1: Boone-one harvest; A1: Ames-one harvest; B3: Boone-three harvests; and A3: Ames-three harvests). Values in 2006 marked with * were significantly different from the values in 2004, at 5%.

high for productive agriculture situations (1420–4890 kg ha^{-1}). In order to find mixtures with greater yield than any monoculture in the experiment, we further identified mixtures that included the highest-yielding species in monoculture in that particular environment and year. When this environment-wise transgressive overyielding was assessed, we found only 5% of plots in A1 and 23% of plots in A3 in the last year.

Discussion

The positive relationship between productivity and seeded species richness did not change over time in our experiment with agronomically relevant species in agricultural conditions. This result provides further evidence that richness effects are not transient, in accord with the results of many ecological studies[17]. Other studies lasting longer than 5 years have found a strengthening of the positive richness–productivity effect, but over the 3 years of our study, we could not detect this effect.

Across all environments and richness levels, the net richness effect was positive; that is, polyculture overyielding was observed. Complementarity effects were positive (Figs. 5 and 6) and selection effects either negative or zero. Complementarity arises when the average relative yield deviation of individual species in the polyculture versus their performance in monoculture is greater than zero. In the absence of a selection effect, the polyculture will exhibit overyielding if complementarity is present. However, complementarity may be counteracted by a negative selection effect, resulting in a polyculture that does not exhibit overyielding. In our experiment, orchardgrass and alfalfa showed species overyielding—i.e., they consistently had ΔRY greater than zero, whereas most other species had ΔRY consistently less than zero. This suggests that orchardgrass and alfalfa were largely responsible for the positive complementarity effect. The largest complementarity effect was the result of orchardgrass overyielding in polycultures with legumes. Although we did not measure plant nitrogen, orchardgrass leaves in polycultures with legumes were visually greener than leaves in monocultures (personal observation). This suggests that nitrogen facilitation may have explained the complementarity effect, consistent with other experiments that measured nitrogen pools (see e.g., Fargione et al.[8]). Positive selection effects are observed when species with high yields in monoculture overyield in polycultures and low-yielding monocultures perform poorly in polycultures. In contrast, negative selection effects exist when species with low yields in monoculture overyield in polycultures and species with high monoculture performance underyield[26,27]. In our experiment, alfalfa generally was highly productive in monoculture and overyielded in mixtures, intermediate wheatgrass was highly productive in A1 but did not overyield in mixtures, and orchardgrass yielded poorly in monocultures but overyielded in mixtures in all environments. As a result of these species interactions, in most

Figure 5. Overview of perennial polyculture plots in Boone, IA, USA in June 2006, before biomass harvest.

Figure 6. Overview of perennial polyculture plots in Ames, IA, USA in May 2005, after the first biomass harvest.

environments no selection effects were observed because the positive effect of alfalfa was canceled out by the negative effect of orchardgrass. In B1, a negative selection effect was observed due to orchardgrass overyielding and alfalfa, with a high monoculture performance, not over-yielding substantially in mixtures.

Our results using cultivars of highly productive forage species are consistent with ecological experiments at multiple sites throughout Europe[12] where polycultures over-yielded and complementarity effects were positive, but selection effects were variable across sites. Also, long-term experiments on Minnesotan grasslands showed that the complementarity effect increased consistently over time, whereas the selection effect was positive during the first 2 years and negative later[8]. This shift occurred because monoculture yields changed with time as initially low-yielding species became the highest-yielding ones in later years[8]. A negative selection effect has been observed in communities with varying evenness and richness[26] and in polycultures containing a grass [*Arrhenatherum elatius* (L.)

P. Beauv. ex J. Presl et C. Presl] with high monoculture performance that reduced all other species in mixtures[28]. Species that increase overyielding by increasing the relative yields of other highly productive species, due to improving nitrogen availability for instance, can produce a positive selection effect, as observed for *Trifolium pratense* L. and *T. repens* L.[28]. Finally, a recent meta-analysis of plant experiments found that, on average, complementarity increased with time in experiments lasting more than 5 years, while selection effects remained constant[17]. Over-yielding and transgressive overyielding (i.e., the polyculture yields more than the highest-yielding monoculture) increased in experiments that ran for longer time frames because complementarity effects among species increased with time. Since most experiments are short term and transgressive overyielding in plant communities may take 5 years to be observed, the potential for transgressive over-yielding has likely been underestimated. This also is likely in our experiment.

Several high-diversity polycultures had yields similar to the highest-yielding species in monoculture. Furthermore, in various environments and years, transgressive over-yielding was not observed, and plots including the highest yielding species in monoculture did not change their yields with richness. Therefore, in certain environments (e.g., B3) with certain species well adapted to intense management (e.g., alfalfa), monocultures may be the best production option. Results similar to these have been used to argue that monocultures or simple mixtures of two species (bicultures) should be a better and more achievable target for sustainable agriculture systems in the short term[16]. Although we agree that levels of plant diversity comparable to natural ecosystems (>10 species) may not be needed in agricultural systems, 'reasonable' levels of diversity (four to six species) may be of better value. We support this statement based on two of our findings.

First, in most of our environments the species with highest monoculture yields changed over time. Therefore, over the long term, it is not possible to define the 'best' monoculture. Individual species may change their relative productivity because of external factors like climatic variability (rainfall and temperature) or pests and disease pressure over the years, but also because of internal factors related to their life cycle and age of the plant. In our experiment for instance, switchgrass initially had a very low productivity, which increased constantly over time (possibly related to the root growth in the establishment years), whereas intermediate wheatgrass had an opposite behavior (perhaps related to disease pressure). If we additionally consider that management (e.g., harvesting frequency) can (and usually does) change in real farming situations over the years, we find that choosing a single 'best' perennial species that could maximize productivity becomes very difficult.

Second, in several environments and years, transgressive overyielding was observed, and productivity did increase with species richness even in the plots including the highest-yielding species in monoculture. This suggests that diverse polycultures may be more stable over longer time frames than monocultures or bicultures, at least in perennial agriculture systems using low levels of inputs. Although in the short term the best-adapted monocultures may equal or exceed polyculture performance, in the long term, polycultures may be advantageous and transgressive overyielding may become more common.

Finally, in this paper, we focus only on one ecosystem function, i.e., biomass productivity. Sustainable agriculture has multiple goals and, as other authors have suggested, when other ecosystem functions are considered (e.g., pest and weed suppression, soil fertility), the minimum number of species required to maximize all functions may increase[15,29,30].

Conclusion

In summary, as our perennial polyculture communities had more time to establish and grow and a third year of data were measured and analyzed, the positive biomass productivity–species richness relationship remained consistent across time and environments, and this was due to increasing ecological complementarity over time and not selection effects. In some environments, transgressive overyielding started to be detected, and the identity of the highest-yielding species changed over time, which contrasts with our results from the first 2 years. This stresses the need for continuous funding of long-term studies of perennial polyculture communities.

In this experiment, we used commonly grown forage species to examine the ecological mechanisms that explain the relationship between productivity and species richness in mixtures. We intended to relate our results using agriculturally relevant species to previous research using grassland and non-agricultural assemblages of plants. Our results are broadly congruent with the ecological literature, in which positive productivity-richness effects are due to complementarity among species, but not selection effects. We could extrapolate our work based on the ecology literature to suggest that the value of mixtures would increase with time as complementarity effects become more common and transgressive overyielding is more frequent. Therefore, choosing a single well-adapted species for maximizing productivity in a monoculture may not be the best alternative over the long term and reasonably high levels of species diversity (four to six species) should be included in the design of productive and ecologically sound perennial agricultural systems.

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