Oikos 122: 84–94, 2013
doi: 10.1111/j.1600-0706.2012.20546.x

Subject Editor: Benta Graae. Accepted 9 March 2012

Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function

Paul Kardol, Lara Souza and Aimée T. Classen

P. Kardol (paul.kardol@slu.se), L. Souza and A. T. Classen, Dept of Ecology and Evolutionary Biology, Univ. of Tennessee, 569 Dabney Hall, 1416 Circle Drive, Knoxville, TN 37996, USA. PK also at: Dept of Forest Ecology and Management, Swedish Univ. of Agricultural Sciences, SE-90183, Umeå, Sweden.

Assembly history, including the order in which species arrive into a community, can influence long-term community structure; however we know less about how timing of species arrival may alter assembly especially under varying resource conditions. To explore how the timing of species arrival interacts with resource availability to alter community assembly, we constructed experimental plant communities and manipulated the interval between plantings of groups of seedlings (0, 5, 10, 15 or 20 days) at low and high levels of soil nutrient supply. To see if community changes influenced ecosystem-scale processes, we measured parameters across the plant–soil continuum (e.g. plant biomass and net ecosystem carbon dioxide exchange).

We found that the timing of species arrival had a large impact on community assembly, but the size of the effect depended on soil fertility. As planting interval increased, plant communities diverged further from the control, but the divergence was stronger at high than at low nutrient supply. Our data suggest that at high nutrient supply, early-planted species preempted light resources more quickly, thus preventing the successful establishment of later arriving species even at short planting intervals. Finally, we found that assembly related divergence in plant communities scaled to impact ecosystem-level characteristics such as green leaf chemistry, but had little effect on total community biomass and net ecosystem exchange of CO_2 and water vapor. Our data indicate that the effect of a stochastic factor, here the timing of species arrival on community composition, depends on the resource level under which the community assembles.

The stochastic concept of community assembly suggests alternate pathways and end points depending on the probabilistic outcome of interactions among and between species and their abiotic environment (Gleason 1939). If the stochastic concept holds true, species interactions, more than environmental conditions, would explain the structure of emerging plant communities. Community assembly theory posits that due to colonization history, multiple community states (both transient and stable) can result from a single species pool (Drake 1991, Chase 2003, Fukami and Nakajima 2011). Specifically, the order of species arrival – often a rather stochastic process – can be an important driver of community structure (Fukami et al. 2005). The importance of the timing of species arrival, however, is less well understood as are the consequences of assembly-related shifts in plant community structure for ecosystem functioning (Belyea and Lancaster 1999, Ejrnæs et al. 2006, Körner et al. 2008).

Priority effects, where early-arriving species influence the establishment and growth of later-arriving species, may be the main mechanism underlying the impact of colonization history on plant community structure (Young et al. 2001, Fukami et al. 2005). Niche preemption, where early-arriving species gain precedence to limiting resources, such as nutrients and light, is one of the principle priority effects associated with historical colonization patterns (Kardol et al. 2008). Thus, early establishment may promote species dominance in the community and potentially exclude coexistence with later arriving species (Connell and Slatyer 1977, Cole 1983).

'Being first' however does not guarantee success; there must be sufficient time for the early colonizer to reach dominance to preclude the establishment of later arriving species (Robinson and Edgemon 1988). Longer time intervals between arrival events should result in stronger priority effects than short intervals because the early arriving species have ample time to use available resources more completely, thereby preempting resources for later-arriving species. In the 1950s and 1960s several studies showed that subtle variation in the time interval between species arrivals (days, weeks), and not arrival order, affected the relative abundance of species in the community (Sagar and Harper 1960, Harper 1961, Ross and Harper 1972). More recently, studies have tested delayed arrival order effects by sequentially introducing selected plant functional types (Körner et al. 2008), or specialist versus opportunist plant species (Ejrnæs et al. 2006). But these studies used relatively long-time intervals between introductions (weeks to years). Thus the stochastic and the short-term nature of intervals between species arrivals that happen in natural plant community assembly (Pakeman and Small 2005) have been overlooked.

Historical contingency in community assembly is often resource-related; thus the role of assembly history may be more important in productive environments because priority effects intensify (Chase 2003, 2010). Theoretical models and empirical studies indicate that both assembly history and nutrient availability can influence plant species productivity to control compositional dissimilarity among communities (Steiner and Leibold 2004, Ejrnæs et al. 2006). Ejrnæs et al. (2006) demonstrated that the impact of plant species arrival order on grassland community structure was contingent on soil nutrient availability. Thus, plant species establishment within a community depends on the resources available at the time of arrival, resource preemption, as well as the community of plants already established. Previous studies have focused on resource preemption resulting from differences in composition of previously colonized species, i.e. the degree of niche overlap (Mwangi et al. 2007), however they have ignored timing of arrival effects on community structure.

Through effects on community structure, historical contingency in community assembly may also lead to shifts in ecosystem functions (Fukami and Morin 2003, Fukami et al. 2010, Dickie et al. 2012). For example, colonization history of wood-decaying fungal communities can alter carbon and nitrogen dynamics under both laboratory and field settings (Fukami et al. 2010, Dickie et al. 2012). For plant communities, shifts in plant functional trait composition can alter biogeochemical cycling rates as well as physiological plant community attributes (Lavorel and Garnier 2002). Linking plant community dynamics to ecosystem functions is a rapidly developing area in ecology (Foster et al. 2004); however, how assembly history (i.e. timing of species arrival) and resource availability interact to shape ecosystem functioning has yet to be addressed.

Here, we constructed microcosm ecosystems with the objective to test how timing of plant species arrival and soil nutrient availability interact to shape plant community structure and ecosystem function. We expected that sequentially assembled communities would become more dissimilar from control communities with increasing intervals between species arrivals and that ecosystem functions would shift to reflect changes in the plant community. We also expected that the establishment of late-arriving species will be limited by light and/or space more than by nutrient availability. We tested the following hypotheses: 1) in communities assembled under low-nutrient conditions where plant growth is slow and light and space may still be available, later-arriving species will have a better chance of establishing than in communities assembled under high nutrient supply where increases in production limit space and/or light. These interactions will lead to stronger effects of timing of species arrival on plant community divergence, i.e. becoming more dissimilar from control communities, under high than under low nutrient supply. 2) Nutrient availability will have a greater influence on ecosystem functioning in our experiment (e.g. through influencing plant community productivity) than community assembly; but, the timing of plant species arrival will modify ecosystem responses to nutrient availability via shifts in plant community composition.

Methods

Soils and plants

In December 2007, we collected soil from the upper 15 cm of an old field at the Oak Ridge National Environmental Research Park (35°54′12″N, 84°20′22″W), located in Oak Ridge, Tennessee, USA. The site was abandoned from agricultural use in 1943 and left fallow until 1964 when a managed fescue field was established. Management ended in 2002, and thereafter, a diverse old-field plant community developed. The soil is classified as Captina silt loam with moderate-to-medium granular structure and medium internal drainage. The soil was sieved and homogenized using a 10 mm mesh and large stones and roots were removed.

We selected plant species that were among the fifty most common species (cover and/or frequency) found in old fields near our site (Souza et al. 2011a). Sixteen species were chosen to represent three functional groups: six grasses, six non-legume forbs, and four legumes (Table 1). Seeds were collected from several local wild populations or provided by specialized suppliers. Seeds were sown in glass beads, moistened with demineralized (DI) water and placed in a germination cabinet (16/8 light/dark photo regime, 18/22°C). Because not all species germinated at the same time, one week after germination seedlings were placed in a climate chamber at 4°C with light according to day/night regime, until transplanting to ensure that all species were of comparable ontogenetic state at the start of the experiment.

Table 1. The planting order for the plant species groups. Species were randomly allocated to the groups. G = grass, F = non-legume forb, L = legume.

1st group	Code	2nd group	Code
Andropogon virginicus [G]	And vir	*Desmodium paniculatum* [L]	Des pan
Symphyotrichum pilosum [F] var. *pilosum*	Sym pil	*Festuca elatior* ssp. *arundinacea* [G]	Fes aru
Phleum pratense [G]	Phl pra	*Sorghastrum nutans* [G]	Sor nut
Plantago lanceolata [F]	Pla lan	*Trifolium campestre* [L]	Tri cam
3rd group		4th group	
Ambrosia artemisiifolia [F]	Amb art	*Desmanthus illinoensis* [L]	Des ill
Dactylis glomerata [G]	Dac glo	*Lespedeza cuneata* [L]	Les cun
Dichanthelium clandestinum [G]	Dic cla	*Rumex acetosella* [F]	Rum ace
Rumex crispes [F]	Rum cri	*Solidago altissima* [F]	Sol alt

Experimental design

Experimental units consisted of microcosms (12.5 cm long, 12.5 cm wide and 17 cm deep) filled with a mixture of old-field soil and sterilized sand (1:1 volumetric ratio). Sand was added to increase water filtration and permeability in our high clay content soils. Prior to the start of the experiment five subsamples of the 1:1 soil-sand mixture were analyzed for physicochemical properties (Supplementary material Appendix A1 Table A1). Each microcosm was planted with one individual of each of the sixteen plant species (Supplementary material Appendix A1 Fig. A1). Seedlings of all sixteen species were allocated to fixed positions, i.e. each plant community had the same spatial configuration that was randomly defined prior to planting (Supplementary material Appendix A1 Fig. A2). Plant species were randomly assigned to four groups that determined the planting order of each species (Table 1). We assembled plant communities using five different planting treatments that varied in the interval between planting each group in the following ways (Table 2): 1) planting all groups at the same time (control), 2) planting groups at 5-day intervals, (3) planting groups at 10-day intervals, 4) planting groups at 15-day intervals, and 5) planting groups at 20-day intervals. The groups were always planted in the same order. Hereafter, treatments will be referred to as C, t5, t10, t15 and t20. Seedlings that died within the first two days of planting were replaced. Keeping the order of species plantings constant allowed us to explicitly test for temporal aspects of species arrival without confounding effects of species identity.

For each treatment, half of the microcosms received 'high' nutrients, whereas the other half of the microcosms received 'low' nutrients. Plants were fertilized with 20-20-20 water-soluble fertilizer mixed with DI water 100 ppm. Nutrient supply treatments began immediately. Every five days, microcosms with the high nutrient supply received 100 ml of fertilizer, whereas microcosms with low nutrient supply received 25 ml of fertilizer (plus 75 ml di water). Over the period of the experiment, for the high nutrient supply treatments, fertilizer addition corresponded with 35.7 kg ha^{-1} NH_4-N, 189.0 kg ha^{-1} NO_3-N, 151.1 kg ha^{-1} urea-N, 61.2 kg ha^{-1} P, 117.3 kg ha^{-1} K, 0.42 kg ha^{-1} Mg, 0.047 kg ha^{-1} B, 0.025 kg ha^{-1} Cu, 0.35 kg ha^{-1} Fe, 0.18 kg ha^{-1} Mn, 0.0070 kg ha^{-1} Mo, and 0.018 kg ha^{-1} Zn. To ensure that water was non-limiting, plants were watered daily with deionized water.

Table 2. Planting schedules used to assemble the plant communities. Groups were planted at the following time intervals: C = all groups planting at day 1, t5 = groups planted at 5-day intervals, t10 = groups planted at 10-day intervals, t15 = groups planted at 15-day intervals, t20 = groups introduced at 20-day intervals.

	Treatment				
Day	Control	t5	t10	t15	t20
1	Group 1–4	Group 1	Group 1	Group 1	Group 1
6		Group 2			
11		Group 3	Group 2		
16		Group 4		Group 2	
21			Group 3		Group 2
31			Group 4	Group 3	
41					Group 3
46				Group 4	
61					Group 4

We established 50 microcosms (five planting treatments × two nutrient supply levels × five replicates) randomly distributed on a greenhouse bench. Microcosm positions were shifted every two days to account for microclimate variation within the greenhouse. Throughout the experiment, light regime was minimally 16 h of light per day and natural daylight was supplemented with metal halide lamps (225 μmol m^{-2} photosynthetically active radiation, PAR) to ensure minimum light supply and a temperature regime of 24/16°C. Seedlings recruiting from propagules present in the field soil were removed.

Plant growth and foliar chemistry

Plants were grown for 111 days after which the shoots were clipped at soil surface, and sorted to species. Roots were washed from the soil using a hydropneumatic elutriator with a 530 μm filter. Roots could not be separated into species because they were too difficult to distinguish visually and were growing intermixed with one another (so difficult to tease apart with the shoot intact). Shoot and root dry weights were determined after drying for at least 72 h at 70°C. To examine temporal changes in plant growth and community composition during and after the assembly period, every five days, shoot length of each seedling was measured to the nearest mm, and shoot biomass was estimated using allometric equations (Supplementary material Appendix A1).

To quantify how planting interval and nutrient supply affected green leaf nutrients, we measured the concentration of leaf nitrogen (N) and phosphorus (P) in two dominant species: *Plantago lanceolata* and *Phleum pratense*. Both of these species were in the first planting group, and survived until the final harvest across all microcosms. Dried leaf samples were ground on a ball mill to a fine powder. Total N and P were determined using a modified micro-Kjeldahl digestion (Parkinson and Allen 1975) and a flow-injection analyzer.

Net ecosystem exchange

As an indicator of shifts in whole-ecosystem function, we measured net ecosystem exchange (NEE) of [CO_2] and [H_2O] vapor, using a gas-exchange chamber system (Vourlitis et al. 1993). The system was made up of a floorboard, a portable gas exchange chamber, and an infra-red gas analyzer. The chamber measured 70 × 70 × 70 cm, and consisted of a metal frame covered with semi-transparent, woven ripstop polyethylene (PE) skin. The PE skin allowed 82% of PAR to pass into the chamber while permitting thermal regulation to leave the chamber. The base of the chamber was provided with closed-cell, CO_2-impermeable, foam tape; when the chamber was placed on the floorboard the system was closed for gas-exchange. The [CO_2] and [H_2O] vapor was measured using an open-path infrared gas analyzer (IRGA). The sensor head was mounted on a tripod placed in the chamber. The sensor head was connected to the IRGA through a sealed outlet in the wall of the chamber. The chamber was affixed with two small window air circulation fans to ensure thorough air mixing.

At day 110 of the experiment (one day before the harvest), midday NEE of [CO_2] and [H_2O] vapor were measured for each microcosm. Measurements were taken in the greenhouse between 11.30 and 14.30 h on a clear day. To ensure equal light levels throughout the measurement time period, we used halide lamps to provide additional light. In random order, microcosms were placed in the NEE chamber. For each measurement, we placed the chamber over a microcosm and allowed the air to mix well by fans (ca 30 s). NEE was monitored for 180 s to measure the slope of change of [CO_2] and [H_2O] over time. To estimate NEE (µmol CO_2 microcosm^{-1} s^{-1}) we corrected CO_2 slope values with total chamber volume and microcosm area. The chamber was well ventilated between each measurement. The temperature in the chamber (± 25°C) did not vary significantly during the measurement period.

Data analysis

We explored the temporal development of plant community composition in our microcosms using principal response curves (PRCs) according to Lepš and Šmilauer (2003). PRC is an extension of redundancy analysis (RDA) and expresses treatments as deviations from a reference treatment. PRC first accounts for variation in species composition due to time and then attributes the remaining variation to the experimental treatments (i.e. planting interval). We tested the effects of planting interval on plant community composition by including all treatments (t5, t10, t15, t20) × the points in time that plant biomass was estimated (1 day, 5 days, 11 days, 16 days, etc.) as explanatory variables in RDA. Next we generated PRC diagrams comparing the treatments to the control by plotting the first principal component of the treatment effects against time. PRC diagrams were generated separately for low and high nutrient supply treatments. We tested the significance of the first and of higher order PRCs by Monte Carlo permutations tests. Both for low and high nutrient supply, only the first order PRC was significant (low N supply: F-ratio = 508.40, $p = 0.01$; high N supply: F-ratio = 439.19, $p < 0.01$); higher order PRCs are no further considered. We interpreted the directional changes in plant community composition by integrating the response of the individual plant species in the PRC diagrams, using a species weight diagram showing the affinity of the plant species with the treatment responses. Multivariate analyses were run using CANOCO, ver. 4.5 (Ter Braak and Šmilauer 2002).

Divergence of sequentially assembled plant communities (t5, t10, t15, t20) from simultaneously assembled communities (i.e. the control) was measured in terms of community dissimilarity. Using data on total biomass at the final harvest, we calculated dissimilarity as the Bray–Curtis index $BC_{ij} = (S_i + S_j - 2C_{ij})/(S_i + S_j)$, where C_{ij} is the sum of the lower of the two biomass values for plant species that occur in both communities, and S_i and S_j are the number of species occurring in community i and community j. Hence, BC_{ij} incorporates both species presence and species dominance. As a reference, we calculated mean community dissimilarity among control treatments (separately for high and low nutrient supply), and for each treatment combination we tested whether community dissimilarity to the control differed from mean dissimilarity among the respective control treatments using one-way analysis of variance (ANOVA) with Tukey contrasts.

All other univariate data were analyzed using two-way ANOVA with treatment (i.e. planting interval: C, t5, t10, t15 and t20), nutrient supply (low vs high) and their interaction as fixed factors and their interaction. The proportion of explained variance (expl. var.) in ANOVAs was calculated as $SS_{\text{between-groups}}/SS_{\text{total}}$. Normality and homogeneity of variances in ANOVA were checked with Shapiro–Wilk tests and Levene's tests, respectively. If the assumptions were not met, data were log- or square-root transformed. Univariate analyses were run using R 2.13.1.

Results

Divergence in plant community composition

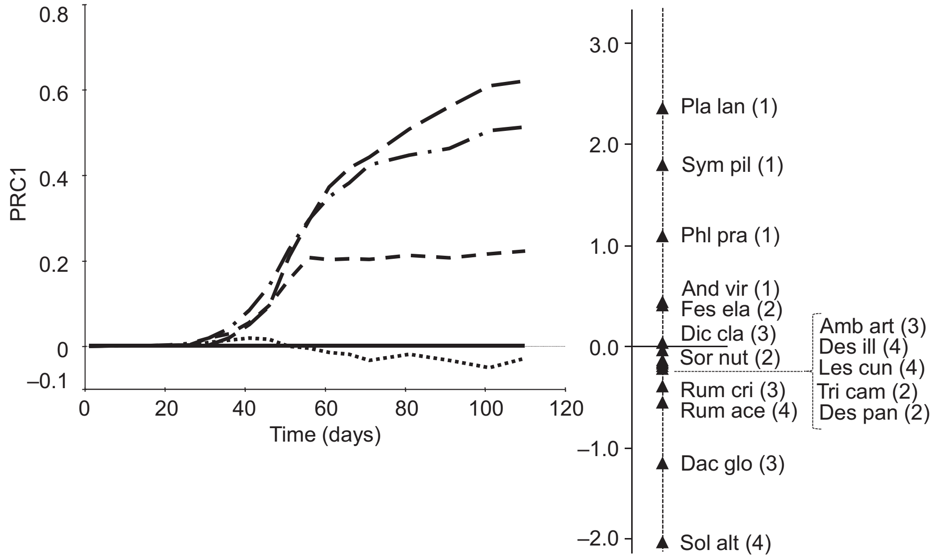
Plant community composition diverged in response to the assembly treatments, but the effect of timing depended on soil nutrient availability (Fig. 1). Generally, communities showed stronger divergence from the control at high nutrient supply than at low nutrient supply, as indicated by the higher scores on the first PRC axis (Fig. 1). For example, at low nutrient supply, 5-day intervals between plantings had no effect on temporal community dynamics (Fig. 1a). In contrast, at high nutrient supply, 5-day intervals between plantings resulted in community divergence from the control (Fig. 1b). Divergence from the control was mainly due to enhanced performance of the species from the first planting group (see species weight diagrams in Fig. 1; Supplementary material Appendix A1 Fig. A3). At high nutrient supply, several plant species in the fourth planting group were not able to establish in the community, particularly at longer time intervals (15 and 20 days) (Supplementary material Appendix A1 Fig. A3); this contributed to enhanced divergence with increased arrival time from the control under high nutrient supply as illustrated by the low species weight value for *Solidago altissima* (Fig. 1b).

Dissimilarity to the control treatment was significantly higher than mean dissimilarity among control treatments, except for communities assembled at 5-day intervals (one-way ANOVA with Tukey contrasts, $p < 0.010$; Fig. 2). Dissimilarity in community composition between simultaneously assembled and sequentially assembled communities increased with planting interval, and was higher at high nutrient supply than at low nutrient supply (Fig. 2); hence, communities diverged further from the control with increasing planting interval, and with increasing nutrient supply. There was no interaction between planting interval and nutrient supply on community dissimilarity relative to the control.

Plant biomass

Both total biomass and shoot biomass were significantly altered by nutrient supply, but there were no significant effects of planting interval and no significant interactions between nutrient supply and planting interval on total biomass and shoot biomass (Table 3a). Elevated nutrients increased total

(a) Low nutrient supply



(b) High nutrient supply

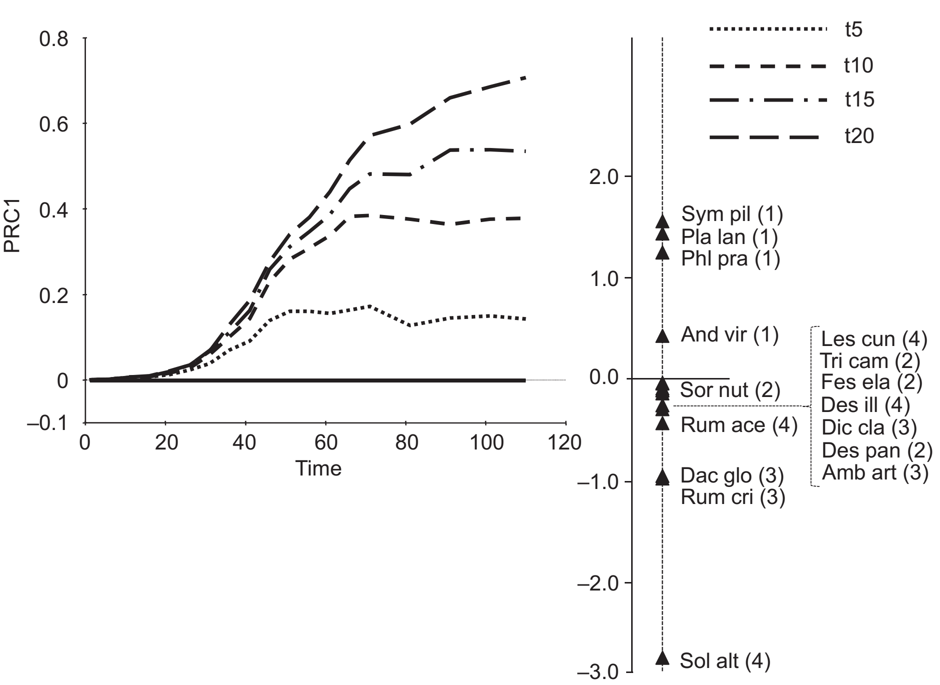


Figure 1. Principal response curves (PRCs) for the first axes from redundancy analysis showing the response over time of plant community composition to planting interval treatments relative to the control treatments, which are presented as a horizontal line along the time axis. (a) Divergence in plant community composition at low nutrient supply. (b) Divergence in plant community composition at high nutrient supply. The first principal response curve explained 21.1% and 21.3% of the variance at high and low nutrient supply, respectively. The vertical one-dimensional plots at the right side of the diagrams are species weight diagrams showing the relative abundance of each species compared to the control treatment. A positive score indicates an increase in abundance and a negative score indicates a decline. Species weight values can be combined with the scores from a PRC curve to predict the relative change (compared to the control) of the species abundance for a particular treatment and time (Lepš and Šmilauer 2003). Numbers in brackets refer to the order in which the plant species were introduced, i.e. the four planting groups. For species codes, see Table 1. C = control treatment, t5 = 5-day intervals between planting; t10 = 10-day intervals, etc.

community biomass and shoot biomass by 31% and 58%, respectively. Interestingly, there was no significant main or interactive effect of planting interval or nutrient supply on root biomass.

For each individual planting group (1–4), there was a significant interaction between planting interval and nutrient supply on shoot biomass (Fig. 3, Table 3b). Shoot biomass of the first planting group increased with high nutrient supply,

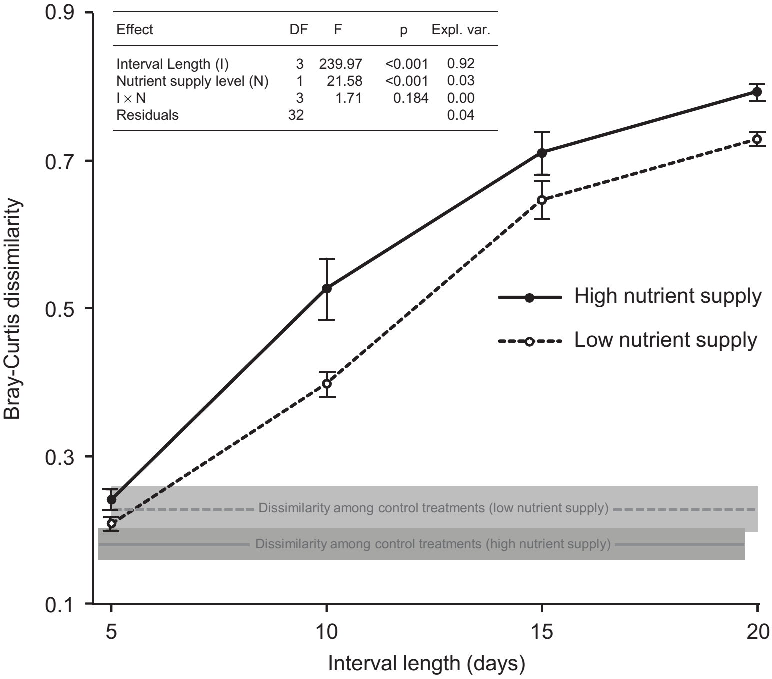


Figure 2. The change in community dissimilarity to the control treatment with planting interval of plant species groups at both low and high nutrient supply. Data are mean ± SD (n = 5). For each microcosm, the mean Bray–Curtis dissimilarity value of five pair-wise comparisons with control communities was used. Inset shows results from two-way ANOVA. Horizontal lines show mean dissimilarity among control treatments at low nutrient supply (dotted) and at high nutrient supply (solid); shaded areas are SD (n = 5).

and this difference was greater with planting interval. Shoot biomass of the first planting group increased with planting interval, but more so under high relative to low nutrient supply (Fig. 3). Shoot biomass of the second planting group was greater under low relative to high nutrient supply, but only when the groups were planted at intervals of 10 days or longer. Shoot biomass of the second planting group was affected by the assembly treatments, but biomass patterns did not respond linearly to planting interval (Fig. 3). Shoot biomass of the third planting group was greater at high nutrient supply than at low nutrient supply, but only when all groups were planted simultaneously, or at 5-day intervals. Shoot biomass of the third planting group decreased with planting interval, such that at long intervals, biomass was not affected by nutrient supply (Fig. 3). Shoot biomass of the fourth planting group was greater at low nutrient supply than at high nutrient supply; however, when all groups were introduced simultaneously nutrient supply had no effect on shoot biomass. Shoot biomass of the fourth planting group decreased with planting interval. At long planting intervals, shoot biomass of the fourth group was very low, with close to zero biomass at high nutrient levels (Fig. 3). Together, these

Table 3. Results from 2-way ANOVA testing effects of planting interval and nutrient supply on shoot, root, and total community biomass (A), and on shoot biomass of planting groups (B). Expl. var. = explained variance.

A

		Plant community								
		Shoot biomass			Root biomass			Total biomass		
Effect	DF	F	p	Expl. var.	F	p	Expl. var.	F	p	Expl. var.
Planting interval (I)	4	1.89	0.131	0.02	1.18	0.328	0.10	0.45	0.775	0.01
Nutrient supply (N)	1	287.49	< 0.001	0.85	0.01	0.946	0.00	129.54	< 0.001	0.74
I × N	4	0.92	0.462	0.01	1.08	0.379	0.08	1.18	0.335	0.03
Residuals	40			0.12			0.81			0.23

B

		Planting groups (shoot biomass)											
		1st group			2nd group			3rd group			4th group		
Effect	DF	F	p	Expl. var	F	p	Expl. var.	F	p	Expl. var.	F	p	Expl. var.
Planting interval (I)	4	109.82	< 0.001	0.72	6.77	< 0.001	0.23	145.68	< 0.001	0.76	136.74	< 0.001	0.86
Nutrient supply (N)	1	97.34	< 0.001	0.16	25.46	< 0.001	0.21	76.50	< 0.001	0.10	7.13	0.011	0.01
I × N	4	7.48	< 0.001	0.05	6.80	< 0.001	0.23	16.42	< 0.001	0.09	10.18	< 0.001	0.06
Residuals	40			0.07			0.33			0.05			0.06

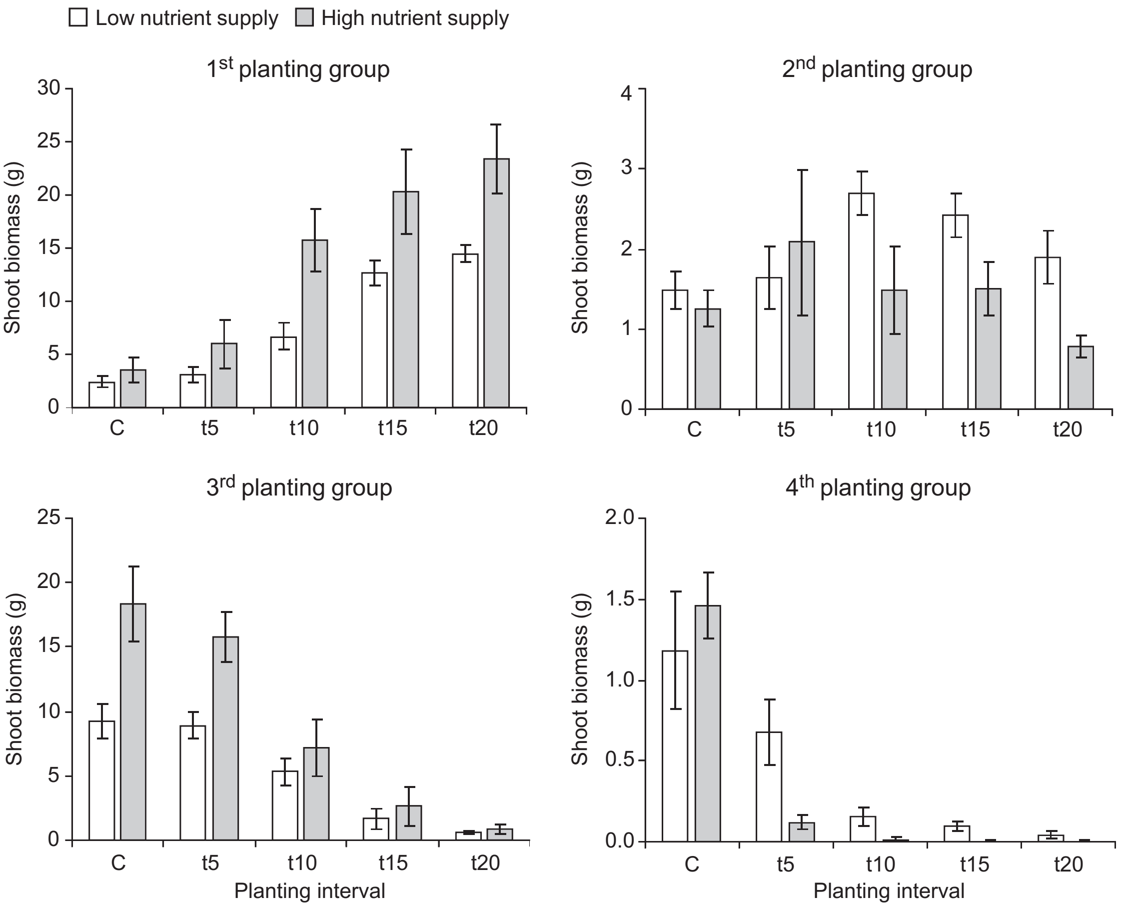


Figure 3. The response of the community shoot biomass (mean ± SE) for sequentially planted groups of plant species to assembly treatments at low and high nutrient supply. C = control treatment; t5 = 5-day intervals between planting of groups; t10 = 10-day intervals, etc. Note the difference in scale of the y-axes.

patterns show that at high nutrient supply, but not necessarily at low nutrient supply, early-planted species inhibited establishment and growth of later arriving species even at short planting intervals.

The general pattern of our results for the four planting groups was supported by the performance of the individual plant species within groups. However, some distinctive species-specific effects were observed (Supplementary material Appendix A1 Fig. A4).

Plant community richness

There was a significant interaction between planting interval and nutrient supply on species richness at the time of harvest (Fig. 4; $F_{4,40} = 14.20$, $p < 0.001$, expl. var. = 0.11). Species richness was up to about 40% lower when groups were planted sequentially than when they were planted simultaneously, but only under high nutrient supply. When groups were planted sequentially, at high nutrient supply some species from later planting groups did not establish; at low nutrient levels all species generally established regardless of planting interval. There were also significant main effects of planting interval ($F_{4,40} = 24.11$, $p < 0.001$, expl. var. = 0.19) and nutrient supply ($F_{1,40} = 322.51$, $p < 0.001$, expl. var. = 0.63) on species richness.

Foliar nutrient concentrations

Both for *Phleum pratense* and *Plantago lanceolata* – two species from the first planting group – there were (marginally) significant interactions between the planting interval

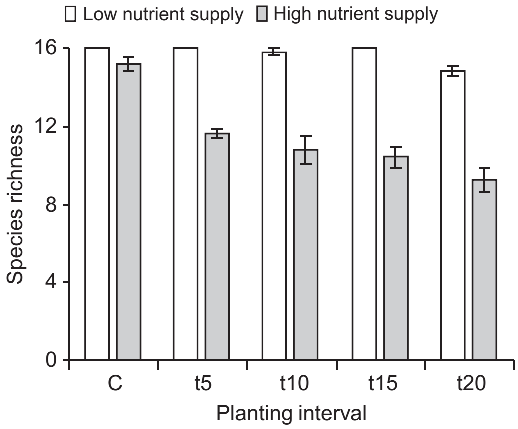


Figure 4. Response of plant species richness to assembly treatments at low and high nutrient supply. Data are mean ± SE. C = control treatment; t5 = 5-day intervals between planting of groups; t10 = 10-day intervals, etc.

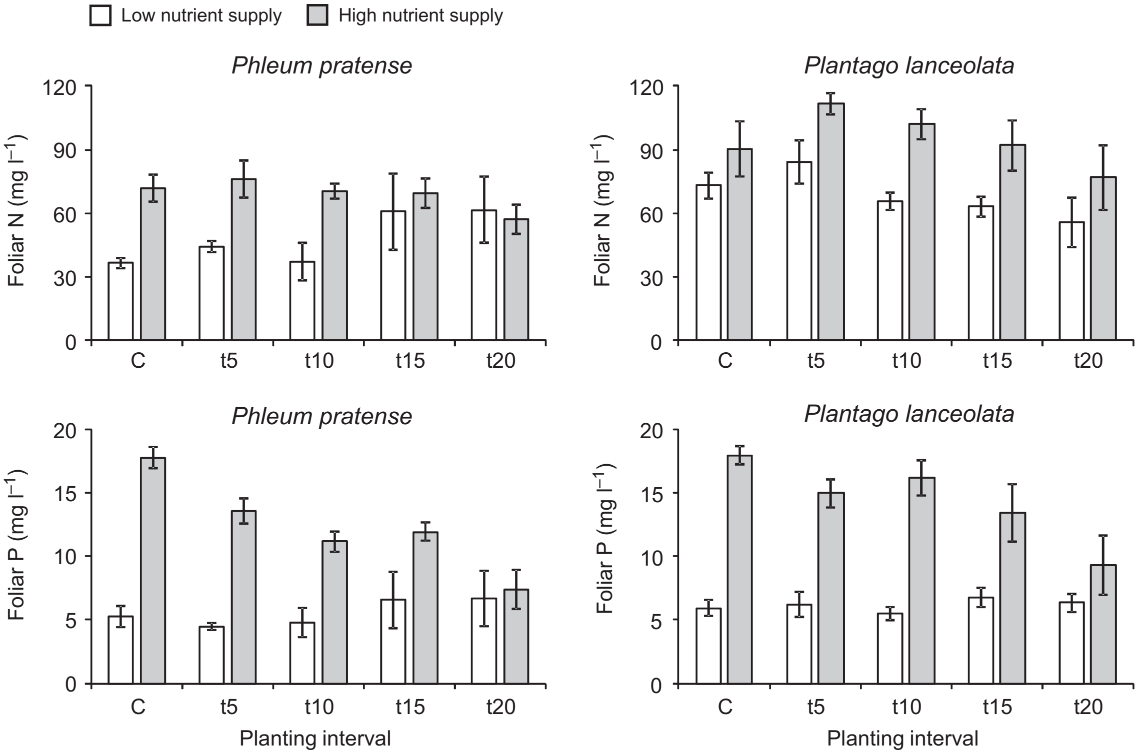


Figure 5. The response of foliar N and P of *Phleum pratense* and *Plantago lanceolata* to assembly treatments at low and high nutrient supply. Data are mean ± SE. C = control treatment; t5 = 5-day intervals between planting of groups; t10 = 10-day intervals, etc.

and nutrient supply on foliar nutrient concentrations (Fig. 5, Supplementary material Appendix A1 Table A3). For *P. pratense*, green leaf N and P concentrations were greater under high nutrient supply than under low nutrient supply, but only when groups of species were planted simultaneously or at short planting intervals (t5, t10). For *P. pratense* and *P. lanceolata*, green leaf P concentrations tended to decrease with increasing intervals (Fig. 5). For *P. lanceolata*, green leaf N concentration was greater under high nutrient supply than under low nutrient supply, but it tended to decrease with increasing intervals.

Net ecosystem exchange

Net ecosystem CO_2-exchange tended to be greater under high nutrient supply than under low nutrient supply (Fig. 6a), except for t15 treatments; but within-treatment variation in net CO_2-exchange was high relative to across-treatment variation and there were no significant effects of nutrient supply ($F_{1,40} = 2.41$, $p = 0.13$, expl. var. = 0.05), planting interval ($F_{4,40} = 0.28$, $p = 0.89$, expl. var. = 0.02), or a significant interaction between interval and nutrient supply ($F_{4,40} = 1.83$, $p = 0.14$, expl. var. = 0.14) on NEE. Net ecosystem H_2O-exchange (ecosystem evapotranspiration) was, on average, greater under high nutrient supply than under low nutrient supply (Fig. 6b; $F_{1,40} = 4.38$, $p = 0.04$, expl. var. = 0.09). Net ecosystem H_2O-exchange was not affected by planting interval ($F_{4,40} = 0.38$, $p = 0.82$, expl. var. = 0.03), and there was no significant interaction between interval and nutrient supply ($F_{4,40} = 0.65$, $p = 0.63$, expl. var. = 0.05).

Discussion

Our study demonstrates that the influence of plant species arrival timing on plant community divergence is strongly dependent on resource availability. Community divergence increased with planting interval, and divergence was stronger under high than low nutrient supply. Further, our results suggest that assembly-related divergence in plant communities can affect key ecosystem parameters such as individual biomass, and green leaf chemistry, but has less of an impact on total community biomass and net ecosystem exchange (NEE) of [CO_2] and [H_2O] vapor. These results suggest that the ecosystem impact of plant assembly history decreases with increasing ecological scale (from community to ecosystems).

Our results support the presumption that under harsh conditions – in our case, low nutrient supply – strong niche selection decreases the importance of stochastic factors in affecting community composition (Chase 2007). As we predicted, nutrient-driven variation in plant community biomass production altered the state of preemption of other growth limiting factors such as light and/or space. Plant species that arrived early in the system had first access to the nutrient supply, were not constrained by light or space, and thus were able to grow quickly and exclude later-arriving species. However, when nutrients constrained the productivity of early arriving species, later-arriving species were able to compete for nutrients, light, and space. Three lines of evidence support this finding. First, increased dissimilarity from the control treatment under high relative to low nutrient supply indicates that establishment of the later-arriving

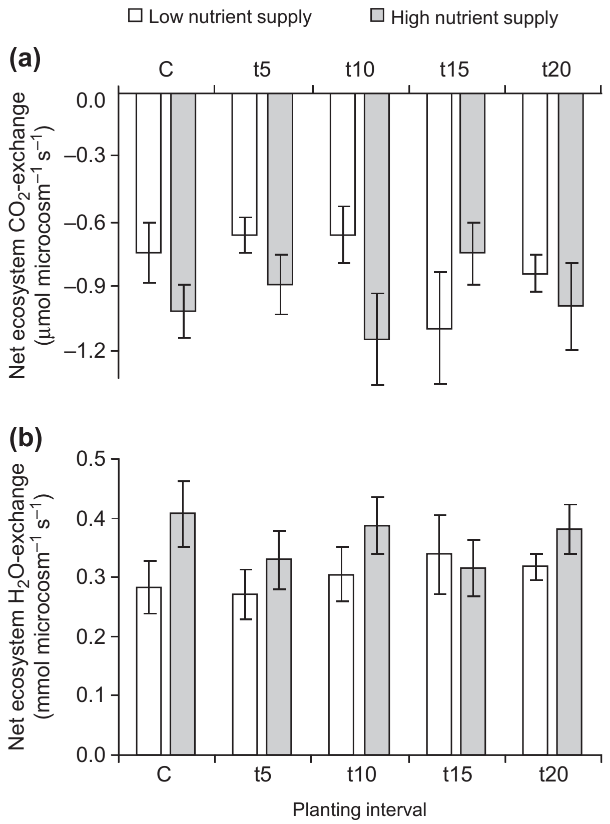


Figure 6. The response of (a) net ecosystem CO_2-exchange and (b) net ecosystem H_2O-exchange (ecosystem evapotranspiration) to assembly treatments at low and high nutrient supply. If net ecosystem CO_2-exchange values are negative, this indicates ecosystem CO_2-uptake outweighs CO_2-loss to ecosystem respiration. On the other hand, positive CO_2-exchange values indicate that CO_2-loss to ecosystem respiration outweighs ecosystem CO_2-uptake. Data are mean ± SE. C = control treatment; t5 = 5-day intervals between planting of groups; t10 = 10-day intervals, etc.

species was limited by light and/or space to a greater extent than by nutrient availability. Second, green leaf nitrogen and phosphorus data indicate that the biomass production of early arriving species in low-nutrient conditions was constrained by nutrient availability. Finally, later-arriving species (i.e. the fourth planting group) established more successfully and produced more biomass under low nutrient supply than under high nutrient supply. In our study, effects of nutrient availability on the rate of preempting light and space are inferred indirectly; future studies should test these effects directly by factorially manipulating multiple resources (sensu Hautier et al. 2009).

A priority-driven competitive advantage of early arriving species over later arriving species affected the probability of species coexistence. At high nutrient supply, early arriving species grew quickly and reduced the establishment of later arriving species, which led to reduced species richness in sequentially assembled communities compared to controls. The rate of competitive exclusion was thus higher at high than at low nutrient supply, probably because of increased light competition at high soil fertility (Hautier et al. 2009). Thus, at low nutrient supply species richness was not affected by the assembly treatments. Priority effects on species coexistence are expected to be strongest at high nutrient supply where interspecific competitive asymmetry is predicted to also be high (Keddy et al. 1997). Interestingly, competitive exclusion of late arriving species at high nutrient supply did not affect total community biomass indicating that early arriving species were able to fully exploit the available resources. Our results are, of course, experimental. In natural communities, larger species pools will likely include plants with more diverse nutrient acquisition traits, and thus the impact of species arrival time on productivity may be stronger (Foster and Dickson 2004, Foster et al. 2004).

While arrival time can play a role in community assembly, the ability of a species to become established and dominate in a community can also depend on its functional attributes and the degree of niche overlap with the already established species (Firn et al. 2010, Peay et al. 2011). For example, the much larger cumulative shoot biomass for species from the third planting group than for species of the second planting group – not to be predicted based on assembly rules alone – was largely attributable to one fast-growing species (i.e. *Ambrosia artemisiifolia*). Nevertheless, the patterns of community divergence in response to assembly treatments and nutrient supply demonstrated here are robust for the following reasons. First, planting groups were comprised by random selection of species from the species pool; thus, resource acquisition traits were randomly (though not necessarily evenly) distributed across the groups. Second, each group consisted of four species, somewhat leveling out species-specific effects within our treatments. Third, within planting groups, despite species-specific variation, individual species generally showed similar responses to the treatments, suggesting that shuffling species among groups would not qualitatively affect our results.

Species traits can influence their ability to establish in a community and local competitive conditions can filter out or suppress unsuitable species, while allowing others to successfully establish (Mwangi et al. 2007, Petermann et al. 2010). To illustrate, we showed that within planting groups, some species were more responsive to the assembly treatments, or responded differently, than others. For example, while *Lespedeza cuneata* (4th planting group) failed to establish in the community when introduced long after the first groups, other species were more successful at establishing when introduced at later time intervals. *Lespedeza cuneata*, a slow growing nitrogen fixer, performed poorly under high nutrient treatments. As nutrients increase and the biomass of other species increase, light limitation would make a nitrogen-fixing legume a poor competitor with earlier-established species (Ritchie and Tilman 1995, Souza et al. 2011b). In the second planting group, species generally produced more biomass at low than at high nutrient supply (presumably because of alleviated light competition at low nutrient supply). However, the fast-growing C_3 grass *Festuca elatior* produced equal or more biomass at high than at low nutrient supply. This pattern could emerge because *F. elatior* was able to exploit the high nutrient levels and can tolerate lower light availability relative to the other C_4 grasses and nitrogen fixers in the second planting group.

If community dynamics are slow, assembly history can have long-lasting effects on plant community composition (Hubbell 2001). However, sooner or later 'late' species will get new opportunities to colonize areas via disturbance or the death of currently established species. First arriving and established species have profound impacts on the subsequent stages of colonization and longer-term plant community dynamics via ecological legacies. For example, the early-established species can induce changes in soil physiochemical and biological properties that differentially affect performance of later-arriving species (Kardol et al. 2007, Grman and Suding 2009). So, even though on the long-term (decades, centuries), environmentally-driven processes may move plant communities toward a common state (Clements 1928), at shorter times scales – relevant to policy makers, conservationist and nature managers – recognition of 'alternative transient states' (Fukami and Nakajima 2011) resulting from stochastic variation in colonization history is meaningful.

Assembly-related shifts in plant communities have the potential to exert strong effects on the functioning of ecosystems because individual plant species might differ in key functional traits (e.g. the ability to fix nitrogen may alter nutrient inputs) (Lavorel and Garnier 2002, Körner et al. 2008). However, while our treatments did alter plant chemistry, plant composition, and plant species-specific biomass, we did not see our assembly treatments 'scale-up' to alter integrated ecosystem functions such as total community biomass and NEE, a pattern that is not uncommon in ecosystem studies (Purdy et al. 2010). Because NEE is an integrator of the whole ecosystem response and our experiment ran for a relatively short period (less than four months), it is possible that if we ran the experiment for a longer period of time, and the plants had more time to influence their soil environment, plant community assembly history could alter NEE.

Not surprisingly, and in concert with the plant biomass data, increased nutrient supply increased both NEE of [CO_2] and evapotranspiration in our study. Plant nutrient status regulates leaf photosynthesis and respiratory carbon losses (Reich et al. 1998, Aeschlimann et al. 2005), and hence, could have driven our observed NEE responses to the nutrient treatment. These findings suggest that, at least in the short-term, whole-system functioning is largely resistant to shifts in plant community composition, especially in systems with plants who may have overlapping traits. However, larger-scale, long-term studies are needed to further explore how assembly-related shifts in plant communities may impact ecosystem functions over time. Also, understanding ecosystem consequences of natural assembly scenarios involving sheer unlimited numbers of combinations of species (or trait) arrival order, time, and location, and resulting larger-scale ecosystem implications, may benefit from ecological modeling; 'simple' empirical experiments like ours could be the foundation.

In conclusion, our study indicates that effects of stochasticity on controlling community assembly are contingent on the environment in which the community is establishing. Hence, when resource input or uptake changes, either along natural environmental gradients or in response to human-induced global changes, arrival time of a plant into a community could become more or less important. In this context, future research should address the relative importance of order and timing of species arrivals while simultaneously testing the role of resident and colonizing plant resource acquisition traits in addressing the effects of assembly history on plant community and ecosystem dynamics.

Acknowledgements – We thank Courtney Campany, Gloria Jimenez, Jana Phillips and Haley Smith for technical assistance. Ken McFarland assisted with the greenhouse work. We thank Emily Austin, Sara Kuebbing and Jean-Philippe Lessard for valuable comments on an earlier version of the manuscript. Research was sponsored by the US Dept of Energy, Office of Science, Biological and Environmental Research Program, grant no. DE-FG02-02ER63366.

References

Aeschlimann, U. et al. 2005. Responses of net ecosystem CO_2 exchange in managed grassland to long-term CO_2 enrichment, N fertilization and plant species. – Plant Cell Environ. 28: 823–833.

Belyea, L. R. and Lancaster, J. 1999. Assembly rules within a contingent ecology. – Oikos 86: 402–416.

Chase, J. M. 2003. Community assembly: when should history matter? – Oecologia 136: 489–498.

Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. – Proc. Natl Acad. Sci. USA 104: 17430–17434.

Chase, J. M. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. – Science 328: 1388–1391.

Clements, F. E. 1928. Plant succession and indicators. – H. W. Wilson, New York.

Cole, B. J. 1983. Assembly of mangrove ant communities – patterns of geographical distribution. – J. Anim. Ecol. 52: 339–347.

Connell, J. H. and Slatyer, R. O. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. – Am. Nat. 111: 1119–1144.

Dickie, I. A. et al. 2012. Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. – Ecol. Lett. 15: 133–141.

Drake, J. A. 1991. Community-assembly mechanics and the structure of an experimental species ensemble. – Am. Nat. 137: 1–26.

Ejrnæs, R. et al. 2006. Community assembly in experimental grasslands: suitable environment or timely arrival? – Ecology 87: 1225–1233.

Firn, J. et al. 2010. Early emergence and resource availability can competitively favour natives over a functionally similar invader. – Oecologia 163: 775–784.

Foster, B. L. and Dickson, T. L. 2004. Grassland diversity and productivity: the interplay of resource availability and propagule pools. – Ecology 85: 1541–1547.

Foster, B. L. et al. 2004. Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. – J. Ecol. 92: 435–449.

Fukami, T. and Morin, P. 2003. Productivity–biodiversity relationships depend on the history of community assembly. – Nature 424: 423–426.

Fukami, T. and Nakajima, M. 2011. Community assembly, alternative stable states or alternative transient states. – Ecol. Lett. doi: 10.1111/j.1461-0248.2011.01663.x.

Fukami, T. et al. 2005. Species divergence and trait convergence in experimental plant community assembly. – Ecol. Lett. 8: 1283–1290.

Fukami, T. et al. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. – Ecol. Lett. 13: 675–684.

Gleason, H. A. 1939. The individualistic concept of the plant association. – Am. Midl. Nat. 21: 92–110.

Grman, E. and Suding, K. N. 2009. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. – Restor. Ecol. 18: 664–670.

Harper, J. L. 1961. Approaches to the study of plant competition. – Soc. Exp. Biol. Symp. 15: 1–39.

Hautier, Y. et al. 2009. Competition for light causes plant biodiversity loss after eutrophication. – Science 324: 636–638.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. – Princeton Univ. Press.

Kardol, P. et al. 2007. Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. – Ecol. Monograph. 77: 147–162.

Kardol, P. et al. 2008. Restoration of species-rich grasslands on ex-arable land: seed addition outweighs soil fertility reduction. – Biol. Conserv. 141: 2208–2217.

Keddy, P. et al. 1997. Experimental evidence that interspecific competitive asymmetry increases with soil productivity. – Oikos 80: 253–256.

Körner, C. et al. 2008. Small differences in arrival time influence composition and productivity of plant communities. – New Phytol. 177: 698–705.

Lavorel, S. and Garnier, E. 2002. Predicting change in community composition and ecosystem functioning from plant traits: revising the Holy Grail. – Funct. Ecol. 16: 545–556.

Lepš, J. and Šmilauer, P. 2003. Multivariate analysis of ecological data using CANOCO. – Cambridge Univ. Press.

Mwangi, P. N. et al. 2007. Niche preemption increases with species richness in experimental plant communities. – J. Ecol. 95: 65–78.

Pakeman, R. J. and Small, J. L. 2005. The role of the seed bank, seed rain and the timing of disturbance in gap regeneration. – J. Veg. Sci. 16: 121–130.

Parkinson, J. A. and Allen, S. E. 1975. Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological material. – Commun. Soil Sci. Plan. 6: 1–11.

Peay, K. G. et al. 2011. Phylogenetic relatedness predicts priority effects in nectar yeast communities. – Proc. R. Soc. B doi: 10.1098/rspb.2011.1230.

Petermann, J. S. et al. 2010. Biology, chance or history. The predictable reassembly of temperate grassland communities. – Ecology 91: 408–421.

Purdy, K. J. et al. 2010. Systems biology for ecology: from molecules to ecosystems. – Adv. Ecol. Res. 43: 87–149.

Reich, P. B. et al. 1998. Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. – Funct. Ecol. 12: 395–405.

Ritchie, M. E. and Tilman, D. 1995. Responses of legumes to herbivores and nutrients during succession on a nitrogen-poor soil. – Ecology 76: 2648–2655.

Robinson, J. F. and Edgemon, M. A. 1988. An experimental evaluation of the effect of invasion history on community structure. – Ecology 69: 1410–1417.

Ross, M. A. and Harper, J. L. 1972. Occupation of biological space during seedling establishement. – J. Ecol. 60: 77–88.

Sagar, G. R. and Harper, J. L. 1960. Factors affecting the germination and early establishment of plantains. – In: Harper, J. L. (ed.), The biology of weeds. Blackwell, pp. 236–244.

Souza, L. et al. 2011a. Biotic and abiotic influences on native and exotic richness relationship across spatial scales: favourable environments for native species are highly invasible. – Funct. Ecol. 25: 1106–1112.

Souza, L. et al. 2011b. Similar biotic factors affect early establishment and abundance of an invasive plant species across spatial scales. – Biol. Invas. 13: 255–267.

Steiner, C. F. and Leibold, M. A. 2004. Cyclic assembly trajectories and scale-dependent productivity–diversity relationships. – Ecology 85: 107–111.

Ter Braak, C. and Šmilauer, P. 2002. CANOCO Reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (ver. 4.5). – Microcomputer Power.

Vourlitis, G. L. et al. 1993. A system for measuring in situ CO_2 and CH_4 flux in unmanaged ecosystems: an arctic example. – Funct. Ecol. 7: 369–379.

Young, T. P. et al. 2001. Community succession and assembly. – Ecol. Restor. 19: 5–18.

Supplementary material (available online as Appendix O20546 at <www.oikosoffice.lu.se/appendix>). Appendix A1, Table A1–A3, Fig. A1–A4.