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44 Figure S1: Soil chemistry (area density  $g$  m<sup>-2</sup>) of each plot vs. plant diversity in 2017, the 23<sup>rd</sup> 45 year of the experiment. Mean  $\pm$  1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h**  extractable Bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are 49 linear regressions  $\pm$  1 S.E. (n = 154 plots). 





52 Figure S2: Soil chemistry (area density  $g m^{-2}$ ) of each plot vs. plant diversity before planting 53 in 1994. Mean  $\pm$  1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable Bray phosphorus 56 versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions  $\pm$  1 S.E. (n = 154 plots).



61 **Figure S3:** Mean soil chemistry (concentration) vs. plant diversity. Mean ± 1 S.E. of soil 62 chemistry (0-20 cm depth; before planting in 1994 in green (diamond) and in 2017 in orange 63 (circle) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, 64 **e** exchangeable magnesium, **f** CEC is cation exchange capacity **g** soil pH and **h** extractable 65 bray phosphorus versus number of planted species (1, 2, 4, 8, or 16; log scale). Lines are 66 linear regressions  $\pm$  1 S.E. (n = 154 plots). 67

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**Figure S4:** Soil chemistry (concentration) of each plot vs. plant diversity in 2017, the 23<sup>rd</sup> 75 year of the experiment. Mean  $\pm$  1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange 76 (circle)) of **a** total carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable 77 calcium, **e** exchangeable magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h**  78 extractable bray phosphorus versus number of planted species (1, 2, 4, 8, or 16). Lines are 79 linear regressions  $\pm$  1 S.E. (n = 154 plots). 80



82 **Figure S5:** Soil chemistry (concentration) of each plot vs. plant diversity before planting in 83 1994. Mean ± 1 S.E. of soil chemistry (0-20 cm depth; 2017 in orange (circle)) of **a** total 84 carbon, **b** total nitrogen, **c** exchangeable potassium, **d** exchangeable calcium, **e** exchangeable 85 magnesium, **f** CEC is cation exchange capacity, **g** soil pH and **h** extractable bray phosphorus 86 versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions  $\pm$  1 S.E. (n = 87 154 plots).



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89 Figure S6: **a-d** Tissue nutrient content in aboveground biomass of each plot (concentration of 90 element \* biomass g m<sup>-2</sup>) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** 91 potassium, **c** calcium and **d** magnesium contained in aboveground plant biomass of each plot (blue; circle), showing the total mass of each element in biomass measured in 2017  $(g m<sup>-2</sup>)$ .  $e$ 93 Total aboveground dry plant biomass in each plot  $(g m<sup>-2</sup>)$  versus plant diversity. Regression 94 lines show dependence of each variable on the natural log of the number of species  $\pm$  1 S.E. (n  $95 = 154$ .



98 Figure S7: **a-d** Tissue nutrient content in belowground biomass of each plot (concentration of 99 element \* biomass g m<sup>-2</sup>) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** 

100 potassium, **c** calcium and **d** magnesium contained in belowground plant biomass of each plot

101 (red; diamond), showing the total mass of each element in biomass  $(g m<sup>-2</sup>)$ . **e** Total

- 102 belowground dry plant biomass in each plot  $(g m<sup>-2</sup>)$  versus plant diversity. Regression lines
- 103 show dependence of each variable on the natural log of the number of species  $\pm$  1 S.E. (n =
- 104 154).
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108 Figure S8: Biomass, nutrient pools and changes in soil nutrient pools from 1994 to 2017 by 109 functional group composition. These panels display the mean  $\pm$  1 S.E. for each functional





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137 Figure S9: Change in ecosystem nutrient pools for each functional group composition for 138 phosphorus. Pools defined as change from 1994 to 2017 in soil levels (0-20 cm depth 139 increment) plus amounts in aboveground biomass and in roots (0-30 cm) in 2017; sum 140 expressed as g of nutrient m<sup>-2</sup>. Each point shows the mean  $\pm$  1 SE. Bars show the relative 141 value for phosphorus in aboveground biomass (grey), phosphorus in belowground biomass 142 (yellow) and soil (blue). Functional group compositions:  $G =$  grasses only  $n = 22$ ;  $F =$  forb 143 only n =10; L = legumes only n = 11; FL = at least 1 forb and 1 legume n= 5; GL = at least 1 144 grass and 1 legume  $n = 23$ ; GF = at least 1 grass and 1 forb  $n = 14$ ; GFL = at least 1 grass, 1 145 legume and 1 forb  $n = 69$ . Means did not differ (all  $P > 0.05$ ).



148 Figure S10: Tissue trait values and root mass by functional group composition. Each bar 149 represents the mean  $\pm$  1 SE of each trait for each functional group type. Functional group 150 composition is defined as C4 grass (4 species), C3 grass (2 species), forb (5 species) and 151 legume (4 species) (See Table S7). Shoot chemistry represents the whole plant percentage of 152 each element from samples taken from monocultures and 16-species plots (C3 n = 13; C4 n = 153 30; forb  $n = 35$ ; legume  $n = 30$ ). Root biomass represents the measured values from 154 monoculture plots to a depth of 30 cm (C3 n = 3; C4 n = 10; forb n = 9; legume n = 10). 155 156 157

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Figure S11: Bivariate relationship between soil organic carbon and soil cation exchange

capacity. Baseline values from 1994 **a** are shown as green diamonds and values from 2017 **b**

are shown as orange circles (n=154). The black line represents a fit from a major axis

164 regression (1994:  $R^2$ =0.20, p < 0.001, 2017:  $R^2$ =0.52 p < 0.001)

## 168 **2. Supplemental Tables:**

169 Table S1: Soil characteristics measured in 1994 and classification of relative levels based on 170 guidelines of the University of Minnesota Agricultural Extension Service

Nutrient	Test	Value	<b>Relative Levels</b>
Organic matter	Loss on Ignition (400 C)	1.04%	Very Low
Total nitrogen	Combustion	0.046%	Very Low
Available phosphorus	Bray-1 P	$47 \text{ (mg/kg)}$	Very High
Available potassium	Ammonium Acetate pH 7	$31 \text{ (mg/kg)}$	Very Low

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Table S2: Linear regressions testing the dependence of each soil variable (area density  $g m<sup>-2</sup>$ ; except for CEC and  $pH$ ) on the natural log of the number of planted species. A separate

except for CEC and  $pH$ ) on the natural log of the number of planted species. A separate

175 regression is shown for each variable in 1994 (pre-treatment) and in 2017 (n=154). Note that

176 all 1994 regressions P values have *P*>0.25, and all 2017 regressions except for phosphorus have *P*<0.0001.

have *P*<0.0001.



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194 Table S3: Linear regressions testing the dependence of each soil variable (concentration mg kg<sup>-1</sup>) on the natural log of the number of plant species. A separate regression is shown for

 $\text{kg}^{-1}$ ) on the natural log of the number of plant species. A separate regression is shown for

196 each variable in 1994 (pre-treatment) and in 2017 (n=154). Note that all 1994 regressions P 197 values have *P*>0.25, and all 2017 regressions except for phosphorus have *P*<0.0001.



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201 Table S4: Linear regressions testing the dependence on  $log_e$  (number of plant species) of the

202 2017 % change relative to the 2017 monoculture mean for aboveground (shoots) and

203 belowground (roots) biomass (0-30 cm) and the quantity of nitrogen, calcium, magnesium, 204 and potassium within those tissues. See Figure 2 for graphs of regressions for all variables<br>205 except biomass. A separate regression is shown for each variable  $(n=154)$ .

except biomass. A separate regression is shown for each variable (n=154).



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210 Table S5: Ecosystem nutrient pools by functional group composition. Pools defined as change

211 from 1994 to 2017 in soil levels (0-20 cm depth increment) plus amounts in aboveground

212 biomass and in roots (0-30 cm) in 2017; sum expressed as g of nutrient  $m<sup>2</sup>$ . Functional group

213 compositions: G = grasses only  $n = 22$ ; F = forb only  $n = 10$ ; L = legumes only  $n = 11$ ; FL = at

214 least 1 forb and 1 legume n = 5; GL = at least 1 grass and 1 legume n = 23; GF = at least 1

215 grass and 1 forb n = 14; GFL = at least 1 grass,  $\overrightarrow{1}$  legume and 1 forb n = 69. Group letters

216 indicate if means differ  $(P<0.05)$  following a Tukey correction.



218 Table S6: Summary table displaying model-averaged coefficients for linear regressions testing

219 the dependency of the sum of aboveground plus belowground biomass (Root 0-30 cm) (2015

220 and 2017) on the natural log of the number of planted species and on soil variables (total N,

221 total C, Bray-P, exchangeable Ca, Mg, K and soil pH). The conditional average is presented<br>222 for each coefficient. for each coefficient.



225 Table S7: List of the fifteen herbaceous perennial plant species that persisted in monocultures

226 and mixtures. For each species, its functional group and plant family is shown. Each species is 227 represented by one point in Figure 4.



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 **3. Supplemental Text:** Supplemental discussion of empirical tradeoff surface shown in Fig. 4.

 We conducted additional analyses to test the robustness of the tradeoff surface shown in Fig 4. Because of poor establishment or poor survival in monoculture or the presence of a second species in a monoculture, there were difficulties in accurately determining root mass for *Poa pratensis* and *Monarda fistulosa.* We therefore tested two subsets of the available data and found that all had a fitted planar surface defining tradeoffs just like those of Fig. 4. Removing monoculture data for *P. pratensis,* which survived in only one monoculture and 249 was rare in it, improved the fit  $(F_{2,11} = 11.3, R^2 = 0.67, p = 0.0021)$ . Removing both *M*. *fistulosa and P. pratensis gave a similar fit*  $(F_{2,10} = 10.28, R^2 = 0.67, p = 0.0038)$ . To better estimate aboveground tissue chemistry for each species, Fig. 4 uses the species-specific average of tissue chemistry measurements in monoculture and in five 16- species plots. When instead we use only species-specific chemistry measured values from 254 monoculture plots, the resulting tradeoff surface was similar, with  $F_{2,30} = 8.26$ ,  $R^2 = 0.36$ ,  $p =$ 255  $0.00139$ . Removing *M. fistulosa* and *P. pratensis* increased the fit to  $F_{2,27} = 13.87$ ,  $R^2 = 0.51$ , p  $256 \le 0.0001$ . 

## **4. Supplemental Methods:** Estimation of area density quantities of soil nutrients using equivalent soil mass method

Formula to calculate the amount in  $\frac{g}{m^2}$  of a nutrient in a soil core of length *T*.

- where:  $x =$  element in soil  $C =$  concentration of element  $x \left( \frac{mg_x}{Kg} \right)$  $\frac{\text{mg}_x}{\text{Kg}_{\text{soil}}}\right)$  $\rho =$  bulk density  $\left(\frac{g_{\text{solid}}}{\text{cm}^3}\right)$
- $T =$  depth or thickness of soil layer (cm)

Area density quantity of nutrient  $x_{\frac{g}{m^2}} = C_{\frac{mg_x}{Kg_{\text{soil}}}} * \frac{1_{g_x}}{1000_r}$ 1000mg*<sup>x</sup>*  $*\rho_{\frac{\text{g}_\text{sol}}{\text{cm}^3}}*\frac{1_{\text{Kg}_\text{sol}}}{1000_\text{g}}$  $1000_{\rm g_{soil}}$  $*T_{\text{cm}}*\frac{100_{\text{cm}}}{1}$  $1<sub>m</sub>$  $*\frac{100_{cm}}{1}$  $1<sub>m</sub>$ 

Formula to determine the mass of soil per m<sup>−</sup><sup>2</sup> of surface area in a block of soil with a thickness of *T*. Reference (1).

Where:

*M*soil is the mass of soil

$$
M_{\text{soil}} = \rho_{\frac{\text{g}_{\text{soil}}}{\text{cm}^3}} * T_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}
$$

$$
M_{\text{soil}} = \frac{\text{g}}{\text{m}^2}
$$

If a soil is sampled a second time and its bulk density has changed, we must calculate the added or subtracted thickness required to sample the same dry mass of soil.

Formula to calculate added or subtracted thickness:

 $T_{\text{add}}$  is the amount of extra thickness to add from deeper depths for equivalent mass. If  $T_{\text{add}}$ is negative, the soil became more dense and it is the amount to subtract to give equivalent mass, called  $T_{\text{subtract}}$  below.

If the soil bulk density decreased through time i.e. it has expanded and is less dense, then we must add soil mass from the subsoil to give a mass equivalent to the original.

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T_{\text{add}} = \frac{(M_{(0\text{-}20\text{cm}),1994\frac{\text{g}}{m^2}} - M_{(0\text{-}20\text{cm}),2017\frac{\text{g}}{m^2}}) * \frac{1_{\text{m}}}{100_{\text{cm}}} * \frac{1_{\text{m}}}{100_{\text{cm}}}}{\rho_{(20\text{-}40\text{cm}),2017\frac{\text{g}}{\text{cm}^3}}}
$$

$$
T_{\text{add}} = \text{cm}
$$

 $T_{\text{add}}$  is the depth of added soil required to keep the total soil mass the same as the original.

If the soil bulk density increased through time i.e. it has contracted and is now more dense. We must subtract soil mass from the target soil to give equivalent mass.

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T_{\text{subtract}} = \frac{(M_{(0\text{-}20\text{cm}),1994\frac{\text{g}}{\text{m}^2}} - M_{(0\text{-}20\text{cm}),2017\frac{\text{g}}{\text{m}^2}}) * \frac{1_{\text{m}}}{100_{\text{cm}}} * \frac{1_{\text{m}}}{100_{\text{cm}}}}{\rho_{(0\text{-}20\text{cm}),2017\frac{\text{g}}{\text{cm}^3}}
$$

$$
T_{\text{subtract}} = \text{cm}
$$

*T*subtract is the depth of soil removed required to keep the total soil mass the same as the original.

The soil bulk density was estimated in 1994 (0-20 cm) based on a regression of the dependence of soil bulk density in 2018 on  $\%$  soil C in 2017 (0-20 cm). The regression was used with measured values of % soil C in 1994 (0-20 cm) to estimate soil bulk density in 1994 (0-20 cm). Estimated values in 1994 had a mean of  $\sim 1.45 \frac{g}{cm^3}$  which aligns with 1.4  $\frac{g}{cm^3}$  from a soil survey of this Nymore series 0-23 cm (2).

To estimate the area density quantity  $\frac{g}{m^2}$  of a given soil nutrient (0-20 cm) in 2017 when the soil bulk density has decreased from the value in 1994 (0-20 cm):

Area density quantity of nutrient  $x_{\frac{g}{m^2}} = [\rho_{\frac{g_{\text{sol}}(0-20\text{cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}}$  $1<sub>m</sub>$  $*\frac{100_{cm}}{1}$  $1<sub>m</sub>$  $] * X_{\frac{g_x}{g_{\text{soil}}}}(0\n-20cm) +$  $[\rho_{\frac{\rm{g}_{\rm{soil}}(20-40cm)}{cm^3}} * T_{\rm{add_{cm}}} * \frac{100_{\rm{cm}}}{1_{\rm{m}}}$  $1<sub>m</sub>$  $*\frac{100_{\text{cm}}}{1}$  $1<sub>m</sub>$  $] * X_{\frac{g_x}{g_{\text{soil}}}}(20\n-40cm)$ 

To estimate the area density quantity  $\frac{g}{m^2}$  of a given soil nutrient (0-20 cm) in 2017 when the soil bulk density has increased from the value in 1994 (0-20 cm):

Area density quantity of nutrient  $x_{\frac{g}{m^2}} = [\rho_{\frac{g_{\text{sol}}(0-20\text{cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}}$  $1<sub>m</sub>$  $*\frac{100_{\text{cm}}}{1}$  $1<sub>m</sub>$  $] * X_{\frac{g_x}{g_{\text{soil}}}}(0{\text -}20\text{cm}) +$  $\left[\rho_{\frac{g_{\text{soll}}(0-20\text{cm})}{\text{cm}^3}} * T_{\text{subtract}_{\text{cm}}} * \frac{100_{\text{cm}}}{1_{\text{m}}} \right]$  $1<sub>m</sub>$  $*\frac{100_{\text{cm}}}{1}$  $1_{\rm m}$  $\frac{1}{4} * X_{\frac{g_x}{g_{\text{sol}}}}(0\n-20cm)$ 



## References

- 1. B. H. Ellert, J. R. Bettany, Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can. J. Soil. Sci. 75, 529–538 (1995).
- 2. D. F. Grigal, Soils of the Cedar Creek Natural History Area (Agricultural Experiment Station, University of Minnesota, 1974).

**5. Supplemental Metadata:** Metadata for datasets S1-S7.











 $1$  Total C and total N represent the average of 2015 and 2017 measured values.

Dataset S2

Figures: Fig. 4

Comment: This dataset contains mean plant tissue chemical traits, using dried biomass, measured at the species level in plots of the biodiversity experiment (E120). Root biomass was measured as the mean of all monoculture plots for each species across all years. Root biomass has been sampled in years 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2006, 2010, 2015 and 2017.







Dataset S4

Comment: This dataset contains soil nutrient content and soil bulk density. It can be used to reproduce the conversion of the soil concentration data to the grams per meter squared values used in Dataset S1. Note that in several cases 2017 is the "year" denoted as the reference year when either 2018<sup>1</sup> (bulk density) or the average 2015 and 2017 were used (total  $C^2$  and  $N^2$ ).





<sup>1</sup> Bulk density was measured in 2018

<sup>2</sup> Total C and total N represent the average of 2015 and 2017 measured values.

Dataset\_S5

Comment: This dataset contains plant tissue concentrations measured in each plot. It can be used to reproduce the conversion of tissue concentrations to area density quantities.



Dataset\_S6

Figures: Fig. 2

Comment: This dataset contains plant tissue area density quantities in each plot. It can be used to reproduce the derived response variables in Fig. 2 as the percent change from the monoculture mean.



Dataset S7

Figures: Figure S10

Comment: This dataset contains species-specific plant tissue chemical traits and root mass, as measured in plots of the biodiversity experiment (E120). Root biomass is the species-specific mean across all monoculture plots and all measured years for each species. Root biomass was measured in years 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2006, 2010, 2015 and 2017.

