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| 7             | Supplementary Information for  |
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| 9             | Plant biodiversity and the regeneration of soil fertility                        |
| 10            | 5 6 5  |
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| 16            |  |
| 17            | This PDF file includes:  |
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Figure S1: Soil chemistry (area density g m<sup>-2</sup>) of each plot vs. plant diversity in 2017, the 23<sup>rd</sup> 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 linear regressions  $\pm$  1 S.E. (n = 154 plots).



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Figure S2: Soil chemistry (area density g m<sup>-2</sup>) of each plot vs. plant diversity before planting 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 versus number of planted species (1, 2, 4, 8, or 16). Lines are linear regressions  $\pm$  1 S.E. (n = 154 plots).



Figure S3: Mean soil chemistry (concentration) vs. plant diversity. Mean ± 1 S.E. of soil
chemistry (0-20 cm depth; before planting in 1994 in green (diamond) and in 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; log scale). Lines are
linear regressions ± 1 S.E. (n = 154 plots).



Figure S4: Soil chemistry (concentration) of each plot vs. plant diversity in 2017, the 23<sup>rd</sup> 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 linear regressions  $\pm$  1 S.E. (n = 154 plots).



Figure S5: Soil chemistry (concentration) of each plot vs. plant diversity before planting in
1994. Mean ± 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 linear regressions ± 1 S.E. (n =
154 plots).



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Figure S6: **a-d** Tissue nutrient content in aboveground biomass of each plot (concentration of element \* biomass g m<sup>-2</sup>) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** 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** Total aboveground dry plant biomass in each plot (g m<sup>-2</sup>) versus plant diversity. Regression lines show dependence of each variable on the natural log of the number of species  $\pm 1$  S.E. (n = 154).



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Figure S7: **a-d** Tissue nutrient content in belowground biomass of each plot (concentration of element \* biomass g m<sup>-2</sup>) vs. plant diversity in 2017. Nutrient content of **a** nitrogen, **b** potassium, **c** calcium and **d** magnesium contained in belowground plant biomass of each plot (red; diamond), showing the total mass of each element in biomass (g m<sup>-2</sup>). **e** Total belowground dry plant biomass in each plot (g m<sup>-2</sup>) versus plant diversity. Regression lines show dependence of each variable on the natural log of the number of species  $\pm 1$  S.E. (n =

104 154).



109 functional group composition. These panels display the mean  $\pm 1$  S.E. for each functional

| 110 | group composition for aboveground and belowground biomass (2017, g m <sup>-2</sup> ; roots 0-30 cm),                    |
|-----|---|
| 111 | the quantity of nitrogen, potassium, calcium, and magnesium in those plant tissues (2017, g                             |
| 112 | m <sup>-2</sup> ), and the change in soil carbon, nitrogen, potassium, calcium and magnesium (g m <sup>-2</sup> , 0-20  |
| 113 | cm, 2017 - 1994). a aboveground biomass, b belowground biomass (0-30 cm), c change in                                   |
| 114 | soil carbon, <b>d</b> quantity of nitrogen in aboveground biomass, <b>e</b> quantity of nitrogen in                     |
| 115 | belowground biomass, $\mathbf{f}$ change in soil nitrogen, $\mathbf{g}$ quantity of potassium in aboveground            |
| 116 | biomass, <b>h</b> quantity of potassium in root biomass, <b>i</b> change in soil potassium, <b>j</b> quantity of        |
| 117 | calcium in above<br>ground biomass, $\mathbf{k}$ quantity of calcium in below<br>ground biomass, $\mathbf{l}$ change in |
| 118 | soil calcium, $\mathbf{m}$ quantity of magnesium in above ground biomass, $\mathbf{n}$ quantity of magnesium in         |
| 119 | belowground biomass, o change in soil magnesium. Functional group compositions: G =                                     |
| 120 | grasses only, $n = 22$ ; F = forb only, $n = 10$ ; L = legumes only, $n = 11$ ; FL = at least 1 forb and                |
| 121 | 1 legume, $n = 5$ ; GL = at least 1 grass and 1 legume, $n = 23$ ; GF = at least 1 grass and 1 forb,                    |
| 122 | n = 14; GFL = at least 1 grass, 1 legume and 1 forb, $n = 69$ . Letters indicate if means differ                        |
| 123 | (P < 0.05) following a Tukey correction.  |
| 124 |   |
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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 = forb143 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 legume and 1 forb n = 69. Means did not differ (all P > 0.05). 145



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





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

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

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

164 regression (1994: R<sup>2</sup>=0.20, p<0.001, 2017: R<sup>2</sup>=0.52 p<0.001)

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## **2. Supplemental Tables:**

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

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

173 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

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

177 have *P*<0.0001.

| Soil Variable | Year | F-Statistic [1,152] | $\mathbb{R}^2$ | P-value  |
|---------------|------|---------------------|----------------|----------|
| Calcium       | 1994 | 0.051               | 0.00           | 0.82111  |
| Carbon        | 1994 | 0.646               | 0.00           | 0.42268  |
| CEC           | 1994 | 0.017               | 0.00           | 0.89523  |
| Magnesium     | 1994 | 0.079               | 0.00           | 0.77967  |
| Nitrogen      | 1994 | 0.126               | 0.00           | 0.72318  |
| pН            | 1994 | 0.004               | 0.00           | 0.95127  |
| Phosphorus    | 1994 | 0.077               | 0.00           | 0.78137  |
| Potassium     | 1994 | 1.666               | 0.01           | 0.19873  |
| Calcium       | 2017 | 40.496              | 0.21           | < 0.0001 |
| Carbon        | 2017 | 26.690              | 0.15           | < 0.0001 |
| CEC           | 2017 | 43.372              | 0.22           | < 0.0001 |
| Magnesium     | 2017 | 35.440              | 0.19           | < 0.0001 |
| Nitrogen      | 2017 | 23.999              | 0.14           | < 0.0001 |
| pН            | 2017 | 30.938              | 0.17           | < 0.0001 |
| Phosphorus    | 2017 | 0.020               | 0.00           | 0.88636  |
| Potassium     | 2017 | 136.373             | 0.47           | < 0.0001 |

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 each variable in 1994 (pre-treatment) and in 2017 (n=154). Note that all 1994 regressions P 

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

| Soil Variable | Year | F-Statistic [1,152] | $\mathbb{R}^2$ | P-value  |
|---------------|------|---------------------|----------------|----------|
| Calcium       | 1994 | 0.003               | 0.00           | 0.95768  |
| Carbon        | 1994 | 0.760               | 0.00           | 0.38484  |
| Magnesium     | 1994 | 0.036               | 0.00           | 0.85036  |
| Nitrogen      | 1994 | 0.220               | 0.00           | 0.64002  |
| Phosphorus    | 1994 | 0.156               | 0.00           | 0.69372  |
| Potassium     | 1994 | 1.266               | 0.01           | 0.26229  |
| Calcium       | 2017 | 38.836              | 0.20           | < 0.0001 |
| Carbon        | 2017 | 24.691              | 0.14           | < 0.0001 |
| Magnesium     | 2017 | 37.007              | 0.20           | <0.0001  |
| Nitrogen      | 2017 | 22.574              | 0.13           | <0.0001  |
| Phosphorus    | 2017 | 0.000               | 0.00           | 0.99463  |
| Potassium     | 2017 | 126.390             | 0.45           | < 0.0001 |

201 Table S4: Linear regressions testing the dependence on log<sub>e</sub>(number of plant species) of the

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

belowground (roots) biomass (0-30 cm) and the quantity of nitrogen, calcium, magnesium,
and potassium within those tissues. See Figure 2 for graphs of regressions for all variables

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

| Nutrient  | Shoots   Roots | F-Statistic [1,152] | $\mathbb{R}^2$ | P-value  |
|-----------|----------------|---------------------|----------------|----------|
| Biomass   | Shoots         | 185.605             | 0.55           | < 0.0001 |
| Calcium   | Shoots         | 107.760             | 0.41           | < 0.0001 |
| Magnesium | n Shoots       | 89.289              | 0.37           | < 0.0001 |
| Nitrogen  | Shoots         | 92.032              | 0.38           | < 0.0001 |
| Potassium | Shoots         | 180.298             | 0.54           | < 0.0001 |
| Biomass   | Roots          | 114.936             | 0.43           | < 0.0001 |
| Calcium   | Roots          | 60.450              | 0.28           | < 0.0001 |
| Magnesium | n Roots        | 61.385              | 0.29           | < 0.0001 |
| Nitrogen  | Roots          | 90.581              | 0.37           | < 0.0001 |
| Potassium | Roots          | 79.612              | 0.34           | < 0.0001 |

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

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

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, 1 legume and 1 forb n = 69. Group letters

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

| Nutrient   | Functional<br>Group | Mean  | SE   | degrees of<br>freedom | Lower<br>confidence<br>interval<br>(95%) | Upper<br>confidence<br>interval<br>(95%) | Group |
|------------|---------------------|-------|------|-----------------------|--|--|-------|
| Calcium    | G                   | 10.19 | 2.19 | 21                    | 5.64                                     | 14.74                                    | С     |
| Calcium    | F                   | 18.98 | 4.24 | 9                     | 9.39                                     | 28.57                                    | С     |
| Calcium    | L                   | 26.02 | 6.53 | 12                    | 11.83                                    | 40.20                                    | BC    |
| Calcium    | GF                  | 9.44  | 3.63 | 13                    | 1.61                                     | 17.28                                    | С     |
| Calcium    | FL                  | 42.02 | 9.69 | 12                    | 20.98                                    | 63.06                                    | ABC   |
| Calcium    | GL                  | 50.90 | 4.97 | 22                    | 40.57                                    | 61.22                                    | AB    |
| Calcium    | GFL                 | 57.93 | 2.66 | 65                    | 52.61                                    | 63.24                                    | А     |
| Magnesium  | G                   | 2.11  | 0.89 | 21                    | 0.24                                     | 3.97                                     | С     |
| Magnesium  | F                   | 4.07  | 1.21 | 9                     | 1.32                                     | 6.82                                     | BC    |
| Magnesium  | L                   | 3.80  | 1.38 | 9                     | 0.69                                     | 6.91                                     | BC    |
| Magnesium  | GF                  | 1.64  | 1.16 | 14                    | -0.85                                    | 4.12                                     | С     |
| Magnesium  | FL                  | 5.53  | 2.04 | 9                     | 0.92                                     | 10.14                                    | ABC   |
| Magnesium  | GL                  | 8.06  | 1.11 | 22                    | 5.76                                     | 10.36                                    | AB    |
| Magnesium  | GFL                 | 9.91  | 0.59 | 68                    | 8.73                                     | 11.09                                    | А     |
| Nitrogen   | G                   | 6.10  | 3.77 | 21                    | -1.75                                    | 13.95                                    | В     |
| Nitrogen   | F                   | -0.63 | 8.44 | 9                     | -19.76                                   | 18.49                                    | В     |
| Nitrogen   | L                   | 21.67 | 6.01 | 15                    | 8.86                                     | 34.48                                    | В     |
| Nitrogen   | GF                  | 2.85  | 6.77 | 13                    | -11.78                                   | 17.47                                    | В     |
| Nitrogen   | FL                  | 30.88 | 8.92 | 15                    | 11.88                                    | 49.87                                    | AB    |
| Nitrogen   | GL                  | 51.80 | 7.38 | 22                    | 36.50                                    | 67.11                                    | А     |
| Nitrogen   | GFL                 | 47.93 | 2.72 | 68                    | 42.50                                    | 53.35                                    | А     |
| Phosphorus | G                   | -1.95 | 0.42 | 21                    | -2.83                                    | -1.07                                    | А     |
| Phosphorus | F                   | -1.79 | 0.41 | 9                     | -2.71                                    | -0.86                                    | А     |
| Phosphorus | L                   | -2.50 | 0.54 | 16                    | -3.65                                    | -1.34                                    | А     |
| Phosphorus | GF                  | -2.73 | 0.85 | 13                    | -4.57                                    | -0.89                                    | А     |
| Phosphorus | FL                  | -1.65 | 0.81 | 16                    | -3.37                                    | 0.06                                     | А     |
| Phosphorus | GL                  | -1.69 | 0.40 | 22                    | -2.52                                    | -0.85                                    | А     |
| Phosphorus | GFL                 | -1.11 | 0.23 | 68                    | -1.58                                    | -0.65                                    | А     |
| Potassium  | G                   | 0.96  | 0.47 | 21                    | -0.03                                    | 1.94                                     | С     |
| Potassium  | F                   | 0.81  | 1.65 | 9                     | -2.93                                    | 4.54                                     | С     |
| Potassium  | L                   | -0.55 | 1.31 | 12                    | -3.40                                    | 2.30                                     | С     |
| Potassium  | GF                  | 1.82  | 0.87 | 13                    | -0.06                                    | 3.70                                     | С     |
| Potassium  | FL                  | 6.70  | 1.94 | 12                    | 2.47                                     | 10.92                                    | ABC   |
| Potassium  | GL                  | 7.89  | 0.85 | 21                    | 6.11                                     | 9.66                                     | В     |
| Potassium  | GFL                 | 11.80 | 0.52 | 66                    | 10.76                                    | 12.85                                    | Α     |

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

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

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

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

| Nutrient    | Coefficient | Std. Error | Adjusted SE | z value | Pr(> z ) |
|-------------|-------------|------------|-------------|---------|----------|
| K           | 51.254      | 8.790      | 8.860       | 5.785   | < 0.001  |
| logNumSp    | 156.232     | 25.602     | 25.810      | 6.053   | < 0.001  |
| С           | 0.227       | 0.050      | 0.050       | 4.536   | < 0.001  |
| Ν           | 2.871       | 0.667      | 0.672       | 4.273   | < 0.001  |
| Р           | -7.980      | 6.400      | 6.453       | 1.237   | 0.216    |
| Mg          | -4.929      | 4.612      | 4.649       | 1.060   | 0.289    |
| (Intercept) | -65.922     | 91.138     | 91.759      | 0.718   | 0.472    |

| 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.   |

| Species                 | Functional Group | Family      |
|-------------------------|------------------|-------------|
| Achillea millefolium    | Forb             | Asteraceae  |
| Amorpha canescens       | Legume           | Fabaceae    |
| Andropogon gerardii     | C4 Grass         | Poaceae     |
| Asclepias tuberosa      | Forb             | Apocynaceae |
| Koeleria macrantha      | C3 Grass         | Poaceae     |
| Lespedeza capitata      | Legume           | Fabaceae    |
| Liatris aspera          | Forb             | Asteraceae  |
| Lupinus perennis        | Legume           | Fabaceae    |
| Monarda fistulosa       | Forb             | Lamiaceae   |
| Panicum virgatum        | C4 Grass         | Poaceae     |
| Dalea purpureum         | Legume           | Fabaceae    |
| Poa pratensis           | C3 Grass         | Poaceae     |
| Schizachyrium scoparium | C4 Grass         | Poaceae     |
| Solidago rigida         | Forb             | Asteraceae  |
| Sorghastrum nutans      | C4 Grass         | Poaceae     |

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 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 monoculture plots, the resulting tradeoff surface was similar, with  $F_{2,30} = 8.26$ ,  $R^2 = 0.36$ , p =0.00139. Removing *M. fistulosa* and *P. pratensis* increased the fit to  $F_{2,27} = 13.87$ ,  $R^2 = 0.51$ , p < 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  $x \left(\frac{\text{mg}_x}{\text{Kg}_{\text{soil}}}\right)$   $\rho =$  bulk density  $\left(\frac{\text{g}_{\text{soil}}}{\text{cm}^3}\right)$ T = depth or thickness of soil layer (cm)

Area density quantity of nutrient  $x_{\frac{g}{m^2}} = C_{\frac{mg_x}{Kg_{soil}}} * \frac{1_{g_x}}{1000_{mg_x}} * \rho_{\frac{g_{soil}}{cm^3}} * \frac{1_{Kg_{soil}}}{1000_{g_{soil}}} * T_{cm} * \frac{100_{cm}}{1_m} * \frac{100_{cm}}{1_m}$ 

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

Where:

 $M_{\rm soil}$  is the mass of soil

$$M_{\text{soil}} = \rho_{\frac{g_{\text{soil}}}{\text{cm}^3}} * T_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}$$
$$M_{\text{soil}} = \frac{g}{m^2}$$

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:

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

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

 $T_{\rm add}$  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.

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

 $T_{\text{subtract}}$  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 ~ 1.45  $\frac{g}{\text{cm}^3}$  which aligns with 1.4  $\frac{g}{\text{cm}^3}$  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):

 $\begin{array}{l} \text{Area density quantity of nutrient } x_{\frac{g}{m^2}} = [\rho_{\frac{g_{\text{soil}}(0-20\text{cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{g_x}{g_{\text{soil}}}}(0-20\text{cm}) + \\ [\rho_{\frac{g_{\text{soil}}(20-40\text{cm})}{\text{cm}^3}} * T_{\text{add}_{\text{cm}}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}] * X_{\frac{g_x}{g_{\text{soil}}}}(20-40\text{cm}) \end{array}$ 

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  $x_{\frac{g}{m^2}} = \left[\rho_{\frac{g_{\text{soil}}(0-20\text{cm})}{\text{cm}^3}} * 20_{\text{cm}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}\right] * X_{\frac{g_x}{g_{\text{soil}}}}(0-20\text{cm}) + \left[\rho_{\frac{g_{\text{soil}}(0-20\text{cm})}{\text{cm}^3}} * T_{\text{subtract}_{\text{cm}}} * \frac{100_{\text{cm}}}{1_{\text{m}}} * \frac{100_{\text{cm}}}{1_{\text{m}}}\right] * X_{\frac{g_x}{g_{\text{soil}}}}(0-20\text{cm})$ 



## 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.

| nd S11   |
|--|
| density quantities measured in each plot.            |
| Aetadata   |
| Experimental plot number                             |
| Number of planted species                            |
| Five letter abbreviation of the name of each         |
| planted species in the experiment within             |
| each plot. The field represents the first three      |
| etters of the genus and the first two letters of     |
| pecies   |
| The functional group set of the planted              |
| pecies including a separate field for C3             |
| grasses, C4 grasses, Forbs and Legumes.              |
| The functional group set of the planted              |
| pecies where all grasses are classified as           |
| being in the same functional group, G                |
| The difference from 1994 to 2017 in                  |
| exchangeable calcium in a plot, using soil           |
| collected from 0-20 cm; expressed as grams           |
| of Ca per meter squared                              |
| The difference from 1994 to 2017 in grams            |
| of C per meter squared for total carbon <sup>1</sup> |
| ising soil collected from 0-20 cm.                   |
| The difference from 1994 to 2017 in                  |
| nilliequivalents per 100 gram soil of cation         |
| exchange capacity for soil collected from 0-         |
| 20 cm  |
| he difference from 1994 to 2017 in                   |
| exchangeable magnesium in a plot, using soil         |
| collected from 0-20 cm; expressed as grams           |
| of Mig per meter squared                             |
| ne difference from 1994 to 2017 in grams             |
| of N per meter squared for total nitrogen            |
| Ising soil collected from 0-20 cm.                   |
| a f dried soil all 1.1 dryssilwater                  |
| using soil collected from 0.20 cm                    |
| The difference from 1004 to 2017 in grams            |
| of P per meter squared for Bray phosphorus           |
| ising soil collected from 0-20 cm                    |
| The difference from 1994 to 2017 in                  |
| exchangeable potassium in a plot using with          |
|  |

|                                 | soil collected from 0-20 cm; expressed as  |
|---------------------------------|--|
|                                 | grams of K per meter squared   |
|                                 | The amount of Ca in grams per meter  |
|                                 | squared in 1994 of exchangeable calcium  |
| gm2_1994_0.20cm_Calcium_aa      | using soil collected from 0-20 cm  |
|                                 | The amount of C in grams per meter squared   |
|                                 | in 1994 of total carbon using soil collected   |
| gm2_1994_0.20cm_Carbon_ea       | from 0-20 cm   |
|                                 | The amount in milliequivalents per 100 gram  |
|                                 | soil in 1994 of cation exchange capacity   |
| gm2_1994_0.20cm_CEC_aa          | using soil collected from 0-20 cm  |
|                                 | The amount of Mg in grams per meter  |
|                                 | squared in 1994 of exchangeable magnesium  |
| gm2 1994 0.20cm Magnesium aa    | using soil collected from 0-20 cm  |
|                                 | The amount of N in grams per meter squared   |
|                                 | in 1994 of total nitrogen using soil collected   |
| gm2_1994_0.20cm_Nitrogen_ea     | from 0-20 cm   |
|                                 | The amount in -log[H+] in 1994 of dried soil   |
|                                 | pH 1:1 drysoil:water using soil collected  |
| _gm2_1994_0.20cm_pH_1.1w        | from 0-20 cm   |
|                                 | The amount of P in 1994 in grams per meter   |
|                                 | squared of Bray phosphorus using soil  |
| gm2_1994_0.20cm_Phosphorus_bray | collected from 0-20 cm   |
|                                 | The amount of K in grams per meter squared   |
| 2 1004 0 20 D                   | in 1994 of exchangeable potassium using  |
| gm2_1994_0.20cm_Potassium_aa    | soil collected from 0-20 cm  |
|                                 | The amount of Ca in grams per meter  |
| 2 2017 0 20 m Galainen an       | squared in 2017 of exchangeable calcium  |
| gm2_2017_0.20cm_Calcium_aa      | Using soil collected from 0-20 cm  |
|                                 | in 2017 of total and and arrive will allocated   |
| 2 2017 0 20 m Garlan as         | in 2017 of total carbon <sup>2</sup> using soil collected  |
| gm2_2017_0.20cm_Carbon_ea       | The second time illippoint of the second sec |
|                                 | The amount in millequivalents per 100 gram   |
| $m^{2}$ 2017 0.20 $m$ CEC as    | soil in 2017 of cation exchange capacity   |
| gm2_2017_0.20cm_CEC_aa          | The emount of Mg in groups per motor   |
|                                 | squared in 2017 of exchangeable magnesium  |
| am2 2017 0.20cm Magnesium aa    | squared in 2017 of exchangeable magnesium  |
|                                 | The amount of N in grams per meter squared   |
|                                 | in 2017 of total nitrogen <sup>1</sup> using soil collected  |
| am2 2017 0 20cm Nitrogen es     | from 0-20 cm   |
|                                 | The amount in -log[H+] in 2017 of dried soil   |
|                                 | nH 1.1 drysoil water using soil collected  |
| gm2 2017 0 20cm nH 1 1w         | from 0-20 cm   |
| <u></u>                         |  |

|                                 | The amount of P in 2017 in grams per meter |
|---------------------------------|--|
|                                 | squared of Bray phosphorus using soil      |
| gm2_2017_0.20cm_Phosphorus_bray | collected from 0-20 cm                     |
|                                 | The amount of K in grams per meter squared |
|                                 | in 2017 of exchangeable potassium using    |
| gm2_2017_0.20cm_Potassium_aa    | soil collected from 0-20 cm                |
|                                 | The amount in grams per meter squared of   |
|                                 | aboveground biomass as the average of 2015 |
| AbvBioAnnProd.2015.2017         | and 2017                                   |
|                                 | The amount in grams per meter squared of   |
|                                 | root biomass collected 0-30 cm deep as the |
| Root030cm.2015.2017             | average of 2015 and 2017                   |
|                                 | The sum of aboveground and belowground     |
|                                 | biomass in grams per meter squared as the  |
| TotalProd.2015.2017             | average of 2015 and 2017                   |
|                                 | The amount of nitrogen as grams per meter  |
|                                 | squared of N in aboveground biomass. N     |
|                                 | was measured in 2017 and multiplied by the |
|                                 | average aboveground biomass measured in    |
| Nitrogen_gm2_Abv                | 2015 and 2017                              |
|                                 | The amount of phosphorus as grams per      |
|                                 | meter squared of P in aboveground biomass. |
|                                 | P was measured in 2017 and multiplied by   |
|                                 | the average aboveground biomass measured   |
| Phosphorus_gm2_Abv              | in 2015 and 2017                           |
|                                 | The amount of potassium as grams per meter |
|                                 | squared of K in aboveground biomass. K     |
|                                 | was measured in 2017 and multiplied by the |
|                                 | average aboveground biomass measured in    |
| Potassium_gm2_Abv               | 2015 and 2017                              |
|                                 | The amount of magnesium as grams per       |
|                                 | meter squared of Mg in aboveground         |
|                                 | biomass. Mg was measured in 2017 and       |
|                                 | multiplied by the average aboveground      |
| Magnesium_gm2_Abv               | biomass measured in 2015 and 2017          |
|                                 | The amount of calcium as grams per meter   |
|                                 | squared of Ca in aboveground biomass. Ca   |
|                                 | was measured in 2017 and multiplied by the |
| Calaine and Alex                | average aboveground blomass measured in    |
| Calcium_gm2_Abv                 | 2015 and 2017                              |
|                                 | The amount of nitrogen as grams per meter  |
|                                 | squared of N in belowground blomass. N     |
|                                 | was measured in 2017 and multiplied by the |
| Nitrogen am? Beet 0.20am        | average belowground biomass (0-30 cm)      |
| initiogen_ginz_kool.0.30cm      | measured in 2015 and 2017                  |

|  | The amount of phosphorus as grams per   |
|--|---|
|  | meter squared of P in belowground biomass.  |
|  | P was measured in 2017 and multiplied by  |
|  | the average belowground biomass (0-30 cm)   |
| Phosphorus gm2 Root.0.30cm   | measured in 2015 and 2017   |
|  | The amount of potassium as grams per meter  |
|  | squared of K in belowground biomass. K  |
|  | was measured in 2017 and multiplied by the  |
|  | average belowground biomass (0-30 cm)   |
| Potassium gm2 Root.0.30cm  | measured in 2015 and 2017   |
|  | The amount of magnesium as grams per  |
|  | meter squared of Mg in belowground  |
|  | biomass. Mg was measured in 2017 and  |
|  | multiplied by the average belowground   |
|  | biomass (0-30 cm) measured in 2015 and  |
| Magnesium gm2 Root 0.30cm  | 2017  |
|  | The amount of calcium as grams per meter  |
|  | squared of Ca in belowground biomass. Ca  |
|  | was measured in 2017 and multiplied by the  |
|  | average belowground biomass (0-30 cm)   |
| Calcium gm2 Root.0.30cm  | measured in 2015 and 2017   |
|  | The sum of the difference in soil calcium in  |
|  | grams of Ca per meter squared at 0-20 cm  |
|  | deep from 1994-2017, the amount of calcium  |
|  | in aboveground biomass in grams per meter   |
|  | squared and the amount of calcium in  |
|  | belowground biomass in grams per meter  |
| Calcium total.soil.plant.g.m2                                      | squared.  |
|  | The sum of the difference in soil magnesium   |
|  | in grams of Mg per meter squared at 0-20 cm   |
|  | deep from 1994-2017, the amount of  |
|  | magnesium in aboveground biomass in   |
|  | grams per meter squared and the amount of   |
|  | magnesium in belowground biomass in   |
| Magnesium total.soil.plant.g.m2                                    | grams per meter squared.  |
|  | The sum of the difference in soil nitrogen in   |
|  | grams of N per meter squared at 0-20 cm   |
|  | deep from 1994-2017, the amount of  |
|  | nitrogen in aboveground biomass in grams  |
|  | per meter squared and the amount of   |
|  | nitrogen in belowground biomass in grams  |
| Nitrogen total soil plant g.m2                                     | per meter squared.  |
|  | The sum of the difference in soil phosphorus  |
|  | in grams of P per meter squared at 0-20 cm  |
|  | deep from 1994-2017, the amount of  |
| Phosphorus total soil plant o m?                                   | phosphorus in aboveground biomass in  |
| Nitrogen total.soil.plant.g.m2<br>Phosphorus_total.soil.plant.g.m2 | <ul> <li>nitrogen in aboveground biomass in grams</li> <li>per meter squared and the amount of</li> <li>nitrogen in belowground biomass in grams</li> <li>per meter squared.</li> <li>The sum of the difference in soil phosphorus</li> <li>in grams of P per meter squared at 0-20 cm</li> <li>deep from 1994-2017, the amount of</li> <li>phosphorus in aboveground biomass in</li> </ul> |

|                                 | grams per meter squared and the amount of phosphorus in belowground biomass in |
|---------------------------------|--|
|                                 | grams per meter squared.   |
|                                 | The sum of the difference in soil potassium                                    |
|                                 | in grams of K per meter squared at 0-20 cm                                     |
|                                 | deep from 1994-2017, the amount of   |
|                                 | potassium in aboveground biomass in grams                                      |
|                                 | per meter squared and the amount of  |
|                                 | potassium in belowground biomass in grams                                      |
|                                 | per meter squared.   |
| Potassium total.soil.plant.g.m2 |  |

<sup>1</sup> 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.

| Variable    | Metadata   |
|-------------|--|
| Species     | The plant species  |
| func        | The functional group of that plant species. $F = \text{forb}$ ; $L = \text{legume}$ ; $C3 = C3$ grass; $C4 = C4$ grass   |
| Calcium     | The mean aboveground tissue calcium in %   |
| Magnesium   | The mean aboveground tissue magnesium in %   |
| Nitrogen    | The mean aboveground tissue nitrogen in %  |
| Phosphorus  | The mean aboveground tissue phosphorus in %  |
| Potassium   | The mean aboveground tissue potassium in %   |
| RootBiomass | The mean belowground root biomass (0-30 cm) across all monoculture plots for each species measured in grams per meter squared from all available data across all years |

| Dataset_S3  |
|---|
| Figures: Figure S3, S4 and S5   |
| Comment: This dataset contains soil nutrients measured in dry soil from each plot |

| Variable | Metadata   |
|----------|--|
| Plot     | The experimental plot  |
| NumSp    | The number of species planted in a plot  |
| Depth    | The depth of the soil sample in centimeters  |
| Year     | The year the soil samples were collected: 1994 or 2017. Total C and total N represent the average of 2015 and 2017 measured values.  |
| nut      | The nutrient measured  |
| val      | The measured value of the soil sample  |
| unit     | The corresponding unit of each sample where:<br>mgX.kgSoil = milligrams of nutrient x per kilogram of soil;<br>meq.100gSoil = milliequivalents per 100 g soil; log.H = -log[H+]. |

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<sup>2</sup> and N<sup>2</sup>).

| Variable                        | Metadata  |
|---------------------------------|---|
| Nutrient                        | The nutrient measured in the soil   |
| Plot                            | The experimental plot   |
| NumSp                           | The number of species planted in a plot   |
| unit                            | The corresponding unit of each sample where:<br>mgX.kgSoil = milligrams of nutrient x per killigram of<br>soil;<br>meq.100gSoil = milliequivalents per 100 g soil;<br>log.H = -log[H+].   |
| X0.20cm_1994                    | The value of a given nutrient x measured in the soil from 0-20 cm depth in 1994   |
| X0.20cm_2017                    | The value of a given nutrient x measured in the soil from 0-20 cm depth in 2017   |
| X20.40cm_2017                   | The value of a given nutrient x measured in the soil from 20-40 cm depth in 2017  |
| Bulk.Density.g.cm3_0.20cm_1994  | Predicted soil bulk density values in grams per centimeter<br>cubed derived from a linear regression of the dependency<br>of bulk density <sup>1</sup> in 2018 (0-20 cm depth) on total soil<br>carbon <sup>2</sup> 2017 (0-20 cm depth) using measured values of<br>total soil carbon in 1994.   |
| Bulk.Density.g.cm3_0.20cm_2017  | A combination of measured soil bulk density in 2018 in<br>grams per centimeter cubed (0-20 cm depth) and predicted<br>soil bulk density values (0-20 cm depth) in grams per<br>centimeter cubed derived from a linear regression of the<br>dependency of bulk density <sup>1</sup> in 2018 (0-20 cm depth) on<br>total soil carbon <sup>2</sup> 2017 (0-20 cm depth) using measured<br>values of total soil carbon in 2017. |
| Bulk.Density.g.cm3_20.40cm_2017 | A combination of measured soil bulk density in 2018 in grams per centimeter cubed (20-40 cm depth) and predicted soil bulk density values (20-40 cm depth) in grams per centimeter cubed derived from a linear regression of the dependency of bulk density <sup>1</sup> in 2018 (20-40 cm depth) on total soil carbon <sup>2</sup> 2017 (0-20 cm depth) using measured values of total soil carbon in 2017.                |
| bd.pred                         | A variable denoting whether the value for bulk density was<br>one of 87 plots measured in 2018 or a predicted value from<br>a linear regression with total soil carbon <sup>2</sup> . "measured" =  |

|                 | measured plot; "predicted" = derived from a linear regression.  |
|-----------------|---|
| Depth_add_cm    | The quantity of added or subtracted soil depth in centimeters in 2017 to give equivalent mass based on the difference in soil bulk density at 0-20 cm depth from 1994 |
| gm2_1994_0.20cm | The quantity in grams per meter squared of a given element x in the soil 0-20 cm depth in 1994  |
| gm2_2017_u      | The quantity in grams per meter squared of a given element x in the soil 0-20 cm depth in 2017 uncorrected for changes in bulk density                                |
| gm2_2017_add    | The quantity in grams per meter squared of a given element x in the soil 0-20 cm depth in 2017 to add or subtract to provide equivalent mass                          |
| gm2_2017_0.20cm | The quantity in grams per meter squared of a given element x in the soil 0-20 cm depth in 2017 corrected using the equivalent soil mass method.                       |

<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.

| Variable | Metadata  |
|----------|---|
| Plot     | The experimental plot   |
| Nutrient | The nutrient element measured in biomass  |
| Extract  | Denoting that an acid digest was used   |
| Туре     | A variable coding indicating whether the<br>measured concentration of each element<br>represents either aboveground or belowground<br>biomass (0-30 cm depth) measured in 2017.<br>"p.abv" = aboveground; "p.Root0.30cm" =<br>belowground root biomass (0-30 cm depth). |
| Year     | The year the sample was collected   |
| val      | The concentration of each nutrient in g of element x per g of dry biomass   |
| Biomass  | Depending on type: The amount in grams per<br>meter squared of dry aboveground biomass as<br>the average of 2015 and 2017; The amount in<br>grams per meter squared of dry root biomass<br>collected 0-30 cm deep as the average of 2015<br>and 2017                    |
| val.g    | The amount in grams per meter squared of each element calculated by multiplying "val" and "Biomass".  |

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.

| Variable | Metadata   |
|----------|--|
| Plot     | The experimental plot  |
| NumSp    | The number of species planted in a plot, which is its diversity treatment  |
| nut      | The nutrient measured in above or belowground biomass  |
| type     | A variable coding indicating whether the measured percent of each element represents<br>either dry aboveground or dry belowground biomass (0-30 cm) measured in 2017.<br>"p.abv" = aboveground; "p.Root0.30cm" = belowground root biomass (0-30 cm). |
| val.g    | The amount in grams per meter squared of each element in dry aboveground or dry belowground biomass (0-30 cm)  |
| mono     | The monoculture mean of "val.g" for each nutrient  |
| diff     | For each plot and nutrient, "diff" is the percent change in the "val.g" of a nutrient in a plot from the 2017 mean "val.g" of that nutrient across all monoculture plots. Formula used = $((val.g - mono)/(mono))*100$                               |

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.

| Variable | Metadata  |
|----------|---|
| Plot     | The experimental plot   |
| func     | The functional group of that plant species. F = forb; L = legume; C3 = C3 grass; C4 = C4 grass  |
| NumSp    | The number of species of the plot where the plant trait measurement was taken   |
| Species  | The plant species measured  |
| nut      | The trait variable measured of either the total aboveground percent nitrogen, phosphorus, potassium, calcium or magnesium for each species or the mean monoculture root biomass (0-30 cm) of each species' monoculture. |
| val      | The measured quantity of each trait. Either the percent nutrient in dry aboveground biomass or the dry root biomass in grams per meter squared at 0-30 cm deep.   |