**Supplementary files**

**Table S1.** Short summary for measured variables and their related method/technology

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Variable | Method/Techonolgy | | Reference |
| Microbes | functional gene abundance | Geochip 4.6 | | Lu et al., 2012 |
|  | microbial biomass  (based on PLFA analyses) | Single-phase Bligh and Dyer method | | White et al., 1979; Si et al., 2015 |
| Labile-fraction soil organic matter  (ρ≤2.25 g cm-3) | labile C (alkyl compounds, polysaccharides) | Py-GC-MS/MS analysis | | Chen et al., 2019; Lu et al., 2011 |
|  | recalcitrant C (lignin, phenols, chitin, N compounds, aromatics, and polyaromatics) | EGA/PY-3030D multi-shot pyrolyzer,  Frontier Lab | |
|  |  | Agilent 7890 gas chromatograph | |
|  |  | Agilent 7000B triple quadrupole  mass spectrometer | |
| Soil enzymes | hydrolases: invertase, β-glucosidase, urease, and phosphatase; | Colorimetric method and measuring wavelengths: polyphenol oxidase 490 nm, peroxidase 294 nm, β–glucosidase 400 nm, invertase 508 nm, urease 578 nm, alkaline phosphatase 430 nm. | | Eivazi and Tabatabai 1988; Gopal et al., 2007; Allison and Jastrow 2006; Kandeler and Gerber 1988; Tabatabai, 1982 |
|  | oxidases: phenol oxidase and peroxidase |  | |  |
| Plant | Species richness | Field record, include: Stipa purpurea, Kobresia pygmaea, Oxytropis glacialis, Potentillasaundersiana, Poa annua, Saussurea japonica, Androsace tapete and Arenaria pulvinata | | Liu et al., 2013 |
|  | above ground biomass | Clipped and dried at 75 °C for 48 h,  then weighed | |  |
| Soil properties | pH | Sartorius PB‐10 digital pH meter. | |  |
|  | total organic carbon (TOC) | TOC-VCPH, Shimadzu, Japan |  | |
|  | total nitrogen (TN) | CuSO4 Se powder diffusion method |  | |
|  | total phosphorous (TP) | NaOH fusion-Mo colorimetric method |  | |
|  | water-soluble organic carbon/nitrogen (WSOC/N) | TOC-TN analyser, Shimadzu, Japan |  | |
|  | NH4+,NO3-,NO2- | Auto Analyser 3, Bran Luebbe, Germany |  | |

**Table S2.** List of compounds found in the studied soil samples through Py-GC-MS/MS.

|  |  |
| --- | --- |
| Compound names | Composition |
| **Alkyl compounds** | Alkanes, C8-C32 |
|  | Alkenes, C8-C24 |
| **Aromatics** | Benzene |
|  | Toluene |
|  | Ethylbenzene |
|  | Dimethylbenzene/*p-*xylene |
|  | Styrene |
|  | C3-Alkylbenzene |
|  | C3-Alkylbenzene |
|  | C3-Alkylbenzene |
|  | C3-Alkylbenzene |
|  | C3-Alkylbenzene |
|  | C3-Alkylbenzene |
|  | Ethenylmethylbenzene |
|  | C4-Alkylbenzene |
|  | C6-Benzene to C14-Benzene |
| **Polyaromatics** | Indene |
|  | 1*H*-Indene,2,3-dihydro-4-methy 1 |
|  | 1,1-Methyl-1*H*-indene |
|  | Naphthalene,1,2-dihydro |
|  | Naphthalene |
|  | Biphenyl |
|  | C1-Alkylnaphthalene |
|  | C1-Alkylnaphthalene |
|  | 1,6-Dimethylnaphthalene |
|  | 1,7-Dimethylnaphthalene |
|  | Fluorene |
| **Lignin-derived compounds** | Guaiacol |
|  | 4-Methylguaiacol |
|  | 4-Ethylguaiacol |
|  | 4-Vinylguaiacol |
|  | Syringol |
|  | 4-Formylguaicol |
|  | 4-(1-Propenyl) guaiacol |
|  | 4-Methylsyringol |
|  | 4-(Propan-2-one) syringol |
|  | Guaiacol-COOH vanillic acid |
|  | 4-Acetylguaiacol |
|  | 4-(prop-1-enyl) syringol |
|  | 4-Vinlsyringol |
|  | 4-Acetylsyringol |
| **Phenols** | Acetophenone |
|  | Phenol |
|  | 2-Methylphenol |
|  | 3-Methylphenol |
|  | 3-Ethylphenol |
|  | Methoxytrimethylphenol |
|  | Ph6 |
| **Polysaccharide-derived compounds** | 2-Propan-2-one tetrahydrofuran |
|  | (2*H*)-Furan-3-one |
|  | 2-Methylfuran |
|  | 2,3-Dihydro-5-methylfuran-2-one |
|  | 2-Furaldehyde |
|  | 3-Furaldehyde |
|  | 2-Acetylfuran |
|  | Levoglucosenone |
|  | Methylbenzofuran |
|  | Methylbenzofuran |
|  | Levoglucosan |
|  | 1,4-Dideoxy-D-glycero-hex-1-enopyranos-3-ulose |
|  | Levogalactosan |
|  | Levomannosan |
| **N-compounds** | Pyridine |
|  | (1*H*)-Pyrrole, dimethyl |
|  | Pyridine, x,x-dimethyl- |
|  | Indole |
|  | 1*H*-Indole-3-ethanamide |
|  | (Iso)quinoline |
|  | Diketodipyrrole |
| **Chitin** | Acetamide |
|  | Acetamidofuran |
|  | Acetoxypyridine |
|  | 3-Acetomido-5-methylfuran |
|  | 3-Acetomido-2/4-pyrone |
|  | 3-Acetamido-6-methyl-n-pyrone |

**Table S3.** Gene names and the full names of corresponding enzymes

|  |  |  |
| --- | --- | --- |
| Function | Gene | encoding enzymes |
| C fixation | PCC/ACC | propionyl-CoA/acetyl-CoA carboxylase |
|  | Rubisco | ribulose-1,5-bisphosphate carboxylase/oxygenase |
|  | CODH | carbon monoxide dehydrogenase |
|  | *aclB* | ATP citrate lyase |
| Methane cycle | *mcrA* | methyl coenzyme M reductase |
|  | *pmoA* | methane monooxygenase |
|  | *mmoX* | particulate methane monooxygenase |
| Starch degradation | *amyA* | amylase |
|  | *nplT* | neopullulanase |
|  | *pulA* | pullulanase |
|  | *glucoamylase* | glucoamylase |
| Hemicellulose | *ara* | arabinofuranosidase |
| degradation | *mannanase* | mannanase |
|  | *xylanase* | 1,4-beta-xylanase |
|  | *xylA* | alpha-ketoglutaric semialdehyde dehydrogenase |
| Cellulose | *endoglucanase* | beta-glucosidase |
| degradation | CDH | cellobiose dehydrogenase |
|  | *exoglucanase* | exoglucanase |
| Chitin degradation | *acetylglucosaminid* | acetylglucosaminidase |
|  | *endochitinase* | endochitinase |
| Lignin degradation | *phenol oxidase* | phenol oxidase |
|  | *mnp* | manganese peroxidase |
|  | *glx* | glucosidase |
|  | *lip* | lignin peroxidase |
| Ammonification | *gdh* | glutamate dehydrogenase |
|  | *ureC* | urease |
| Anammox | *hzo* | nitrate reductase |
| Nitrification | *hao* | hydroxylamine oxidoreductase |
| Denitrification | *norB* | nitric oxide reductase |
|  | *nirS* | nitrite reductase |
|  | *nirK* | nitrite reductase |
|  | *narG* | nitrite reductase |
|  | *nosZ* | nitrous oxide reductase |
| Assimilatory N | *nasA* | nitrate reductase |
| reduction | *nirA* | ferredoxin-nitrite reductase |
|  | *NirB* | nitrite reductase |
|  | *NiR* | nitrite reductase |
| Dissimilatory N | *napA* | nitrate reductase |
| reduction | *nrfA* | c-type cytochrome nitrite reductase |
| N fixation | *nifH* | nitrogenase |
| P utilization | *ppx* | exopolyphosphatase |
|  | *ppk* | polyphosphate kinase |
|  | *phytase* | catalyses the hydrolysis of polyphosphate |

**Table S4.** Soil physical and chemical properties

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| N addition rate | 0 kg N ha-1 yr-1 | 10 kg N ha-1 yr-1 | 20 kg N ha-1 yr-1 | 40 kg N ha-1 yr-1 | 80 kg N ha-1 yr-1 | 160 kg N ha-1 yr-1 |
| pH | 7.82±0.04a | 7.86±0.03a | 7.85±0.03a | 7.82±0.02a | 7.8±0.01a | 7.67±0.05b |
| TOC (g·kg-1) | 27.89±1.04a | 29.52±1.64a | 25.66±1.43a | 27.25±2.31a | 26.06±2.35a | 29.08±1.85a |
| TN (g·kg-1) | 2.28±0.1a | 2.57±0.17a | 2.23±0.09a | 2.38±0.13a | 2.44±0.25a | 2.68±0.12a |
| TP (g·kg-1) | 0.66±0.02a | 0.71±0.03a | 0.69±0.04a | 0.67±0.04a | 0.71±0.04a | 0.68±0.02a |
| WSOC(mg·kg-1) | 73.34±9.47ab | 83.95±7.15ab | 96.73±11.2a | 69.89±3.09b | 61.05±6.68b | 68.31±8.85b |
| WSON(mg·kg-1) | 15.56±2.2b | 18.06±1.44b | 20.05±2.74b | 19.68±2.45b | 24.67±3.23b | 39.48±7.79a |
| NH4+ (mg·kg-1) | 4.22±1.43a | 5.17±0.72a | 6.93±1.87a | 5.8±0.27a | 3.28±1.01a | 4.7±1.92a |
| NO3- (mg·kg-1) | 17.78±1.63b | 18.07±1.33b | 17.62±6.98b | 35.58±9.32b | 63.77±19.8b | 123.3±30.31a |
| NO2- (mg·kg-1) | 1.81±0.43b | 1.59±0.5b | 2.16±0.53b | 1.87±0.51b | 2.75±0.72b | 6.39±1.32a |
| NO2-/NH4+ | 0.47±0.18b | 0.30±0.13b | 0.33±0.12b | 0.33±0.14b | 0.94±0.29b | 1.82±0.8a |
| TOC/TN | 12.23±0.11a | 11.48±0.14ab | 11.49±0.38ab | 11.41±0.35b | 10.73±0.26b | 10.82±0.2b |
| TN/TP | 3.46±0.03b | 3.61±0.08ab | 3.22±0.04b | 3.56±0.01ab | 3.41±0.28b | 3.98±0.29a |
| WSOC/WSON | 4.73±0.13a | 4.65±0.06a | 5.07±1.09a | 3.62±0.27ab | 2.6±0.51bc | 1.79±0.18c |
| AGB (g·m-2) | 29.85±13.94b | 53.38±17.86ab | 47.06±6.97ab | 45.32±13.29ab | 59.91±9.15ab | 75.38±12.64a |
| PR | 10.99±1.74b | 11.66±1.74b | 12.01±1.19b | 13.99±1.15b | 15.36±-0.91ab | 16.99±-0.55a |
| MB (nmol·g-1) | 51.73±4.08a | 47.42±3.95ab | 42.86±12.71b | 43.58±9.95b | 44.06±6.23b | 39.51±8.27b |
| MD | 10.08±0.10a | 10.18±0.06a | 10.16±10.07a | 10.09±0.04ab | 10.04±0.02ab | 9.74±0.12b |

Note: pH: 1:2.5 mixture of soil and deionized water; TOC: soil total organic carbon; TN: soil total nitrogen; TP: soil total phosphorous; WSOC: water-soluble organic carbon in soil; WSON: water-soluble organic nitrogen in soil; PR: plant species richness; AGB: above-ground biomass; MB: microbial biomass; MD: microbial diversity. All data are presented as mean ± s.e (n=3). Significant (*p*<0.05) differences among N gradients are indicated by alphabetic letters above the bars.

**Table S5.** Diversity indices of all functional genes, carbon cycling genes and nitrogen cycling genes in soil samples

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N addition rate  (kg N ha-1 yr-1) | Whole Chip | | | Carbon cycling | | | Nitrogen cycling | | |
| Richnessa | Shannonb | Simpsonc | Richness | Shannon | Simpson | Richness | Shannon | Simpson |
| 0 | 22448±389ad | 10.02±0.02ab | 1615±38ab | 2174±41ab | 7.85±0.02a | 2542±48ab | 1615±38ab | 7.38±0.02ab | 1605±37ab |
| 10 | 22930±250a | 10.04±0.01a | 1664±19a | 2254±36a | 7.87±0.02a | 2622±40a | 1664±19a | 7.41±0.01a | 1653±18a |
| 20 | 22600±277a | 10.02±0.01ab | 1619±20a | 2197±25a | 7.86±0.01a | 2576±29a | 1619±20a | 7.39±0.01ab | 1610±19a |
| 40 | 20792±473b | 9.94±0.02b | 1492±33b | 2021±51b | 7.77±0.02a | 2372±54b | 1492±33b | 7.31±0.02b | 1486±33b |
| 80 | 17656±351c | 9.78±0.02c | 1255±27c | 1730±41c | 7.62±0.02b | 2024±44c | 1255±27c | 7.13±0.02c | 1250±27c |
| 160 | 15696±1005d | 9.66±0.07d | 1111±76d | 1504±107d | 7.48±0.07c | 1772±120d | 1111±76d | 7.01±0.07d | 1107±75d |

Note: All data are presented as mean ± s.e (n=3). Significant (p<0.05) differences among N gradients are indicated by alphabetic letters above the bars.

a Detected gene number.

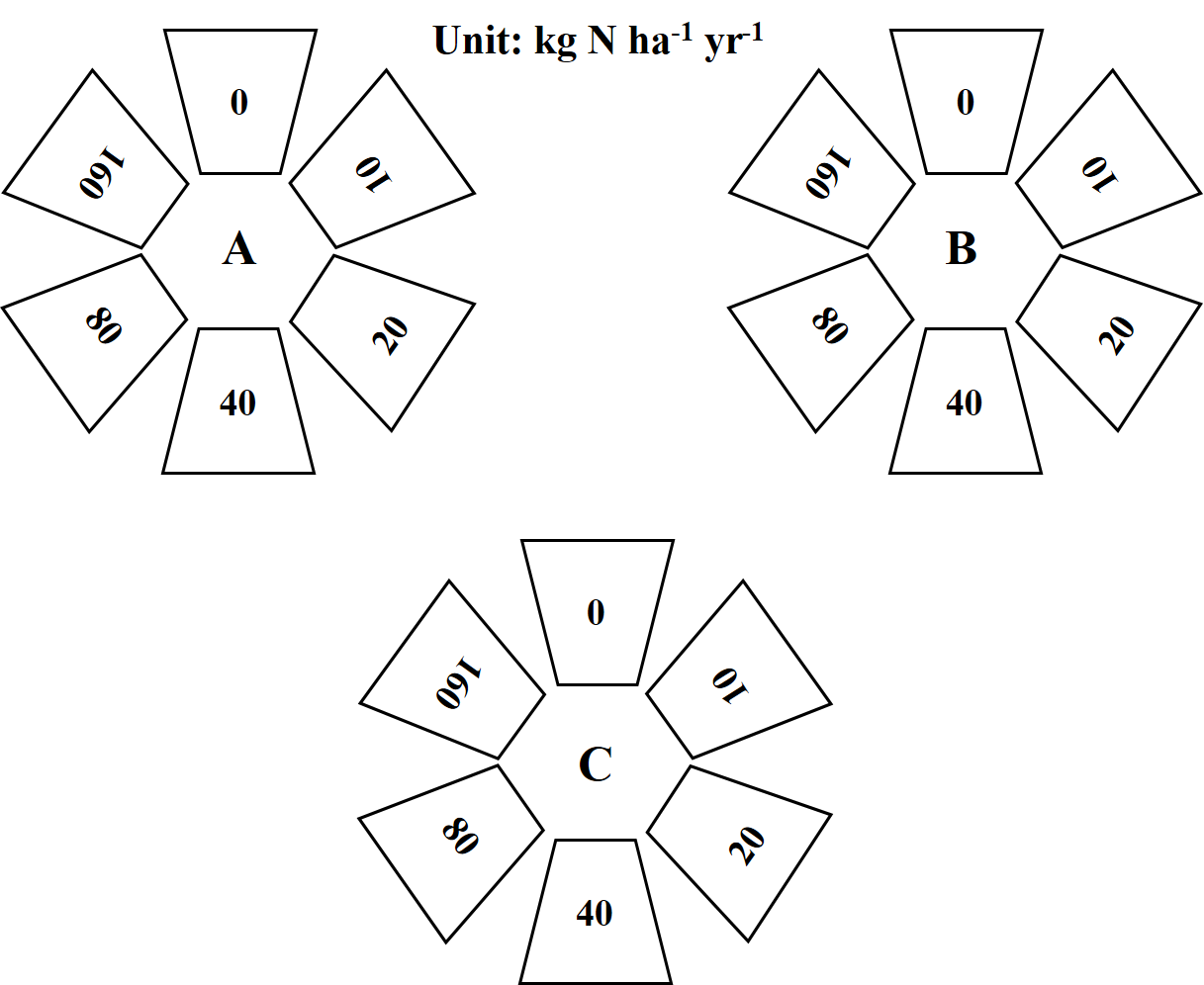
b Shannon-Weiner index. Higher number represents higher diversity.

c Simpson’s index. Higher number represents higher diversity.

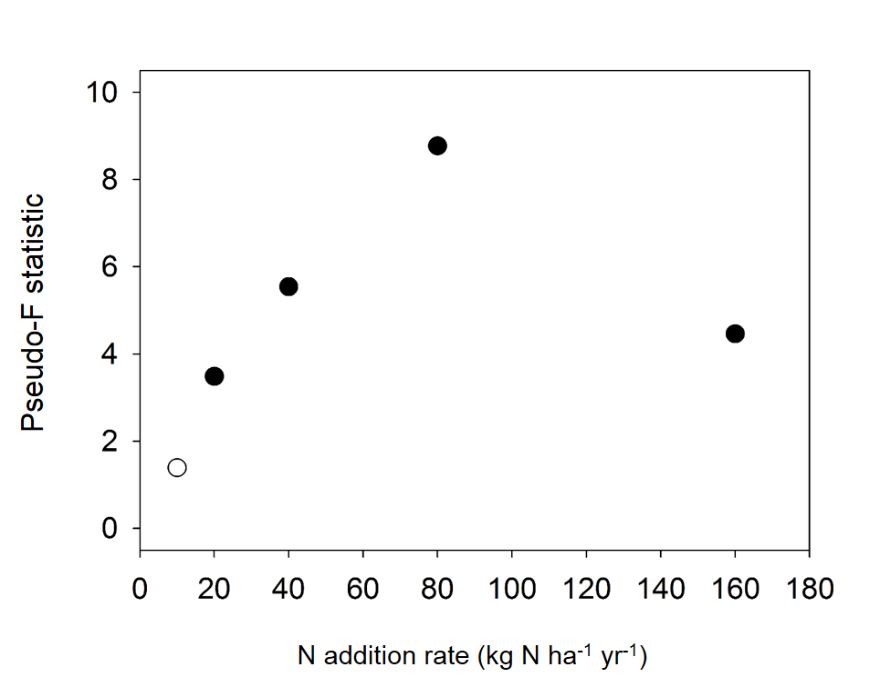
d Significant differences among N gradients are indicated by alphabetic letters.

**Table S6.** Permutation test results of canonical correlation analysis (CCA).

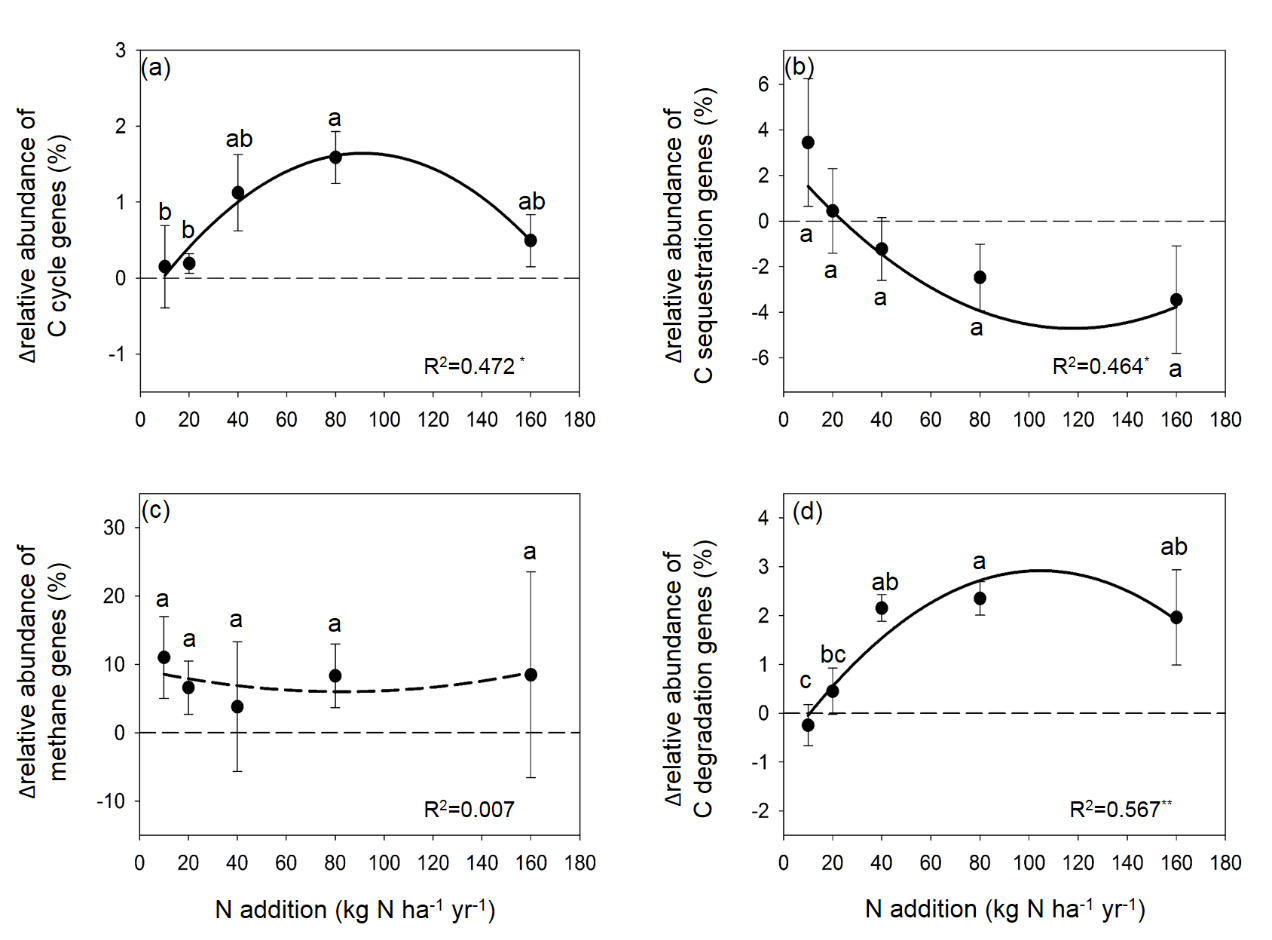
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Df | Chi-square | F | *p* |
| pH | 1 | 0.00066 | 1.497 | 0.145 |
| TP | 1 | 0.00044 | 0.989 | 0.393 |
| NO3- | 1 | 0.00054 | 1.222 | 0.245 |
| NO2-/NH4+ | 1 | 0.00070 | 1.583 | 0.121 |
| Plant richness | 1 | 0.00273 | 6.214 | **0.001** |
| TOC/TN | 1 | 0.00035 | 0.800 | 0.569 |
| TN/TP | 1 | 0.00061 | 1.389 | 0.154 |
| WSOC/WSON | 1 | 0.00048 | 1.096 | 0.270 |
| Constrained |  | 0.00651 |  | **0.003** |
| Residual | 9 | 0.00396 |  |  |



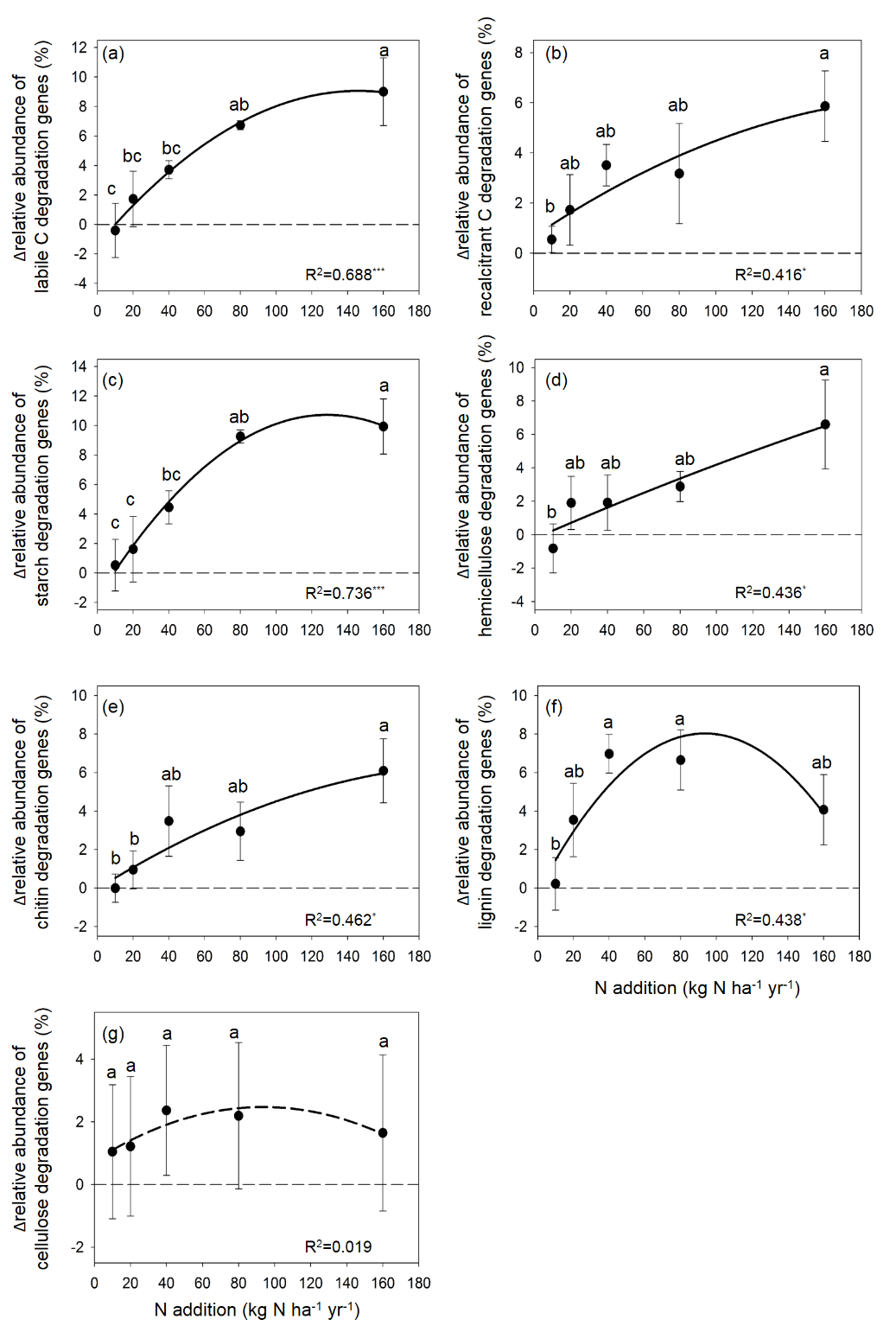
**Fig. S1** The experimental design in the Nam Co Station. In May 2009, three homogenous plots were randomly assigned as replicates in an alpine steppe. In each plot, six subplots (~13 m2) were arranged in a circle, with a buffer zone of at least 2 m between adjacent subplots.



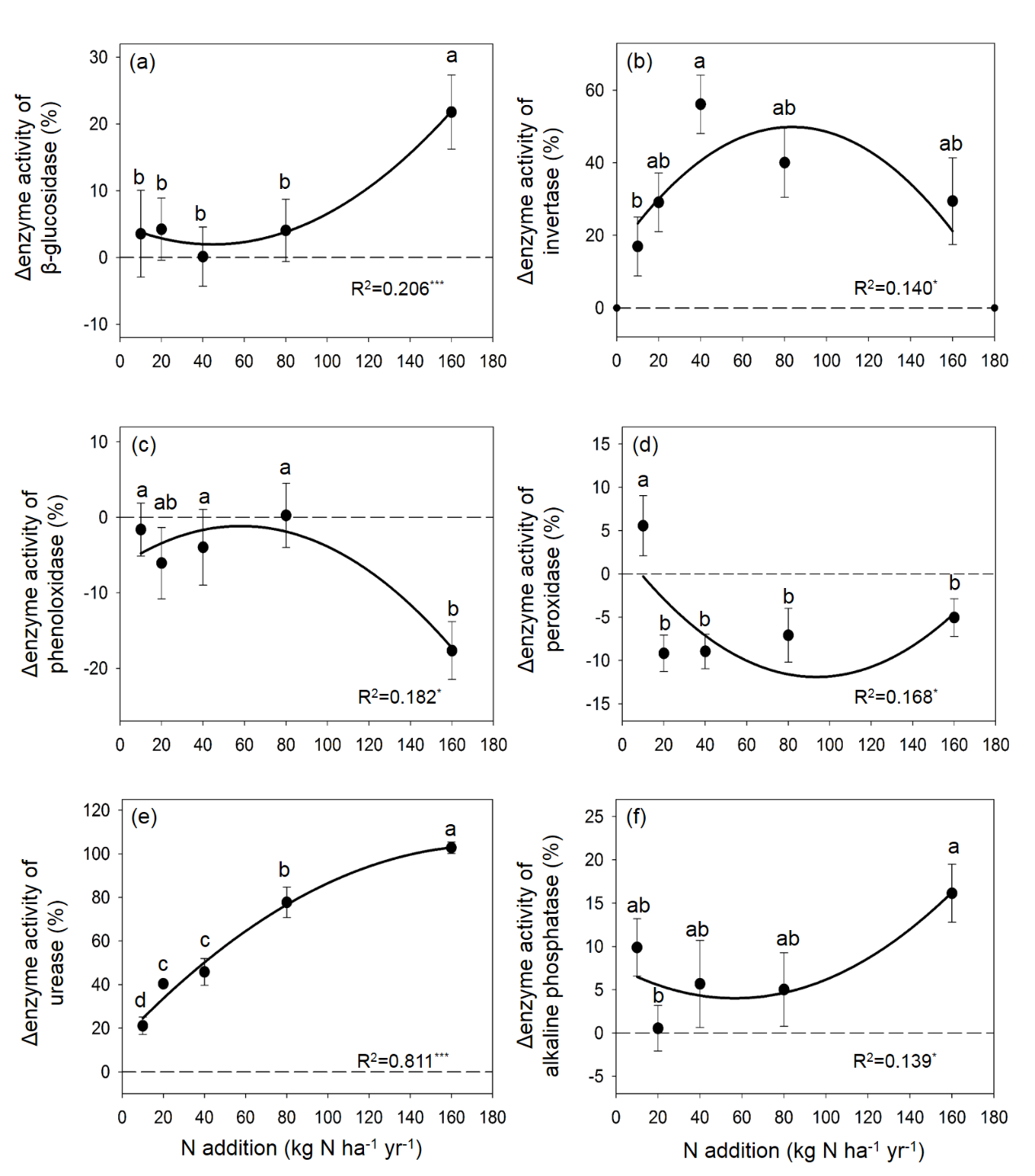
**Fig. S2** Compositional differences of Geochip data between low and high nitrogen addition rates. For each site, the low and high nitrogen addition treatments were defined as the treatments below and above(include include current rate) current nitrogen addition rate, respectively. The significant differences between the Geochip data of low and high N addition treatments were tested by a permutational multivariate analysis of variance (PERMANOVA) with pseudo-F statistic and were shown as solid and open circles for significance (*p*<0.05) or non-significance (*p*>0.05), respectively.

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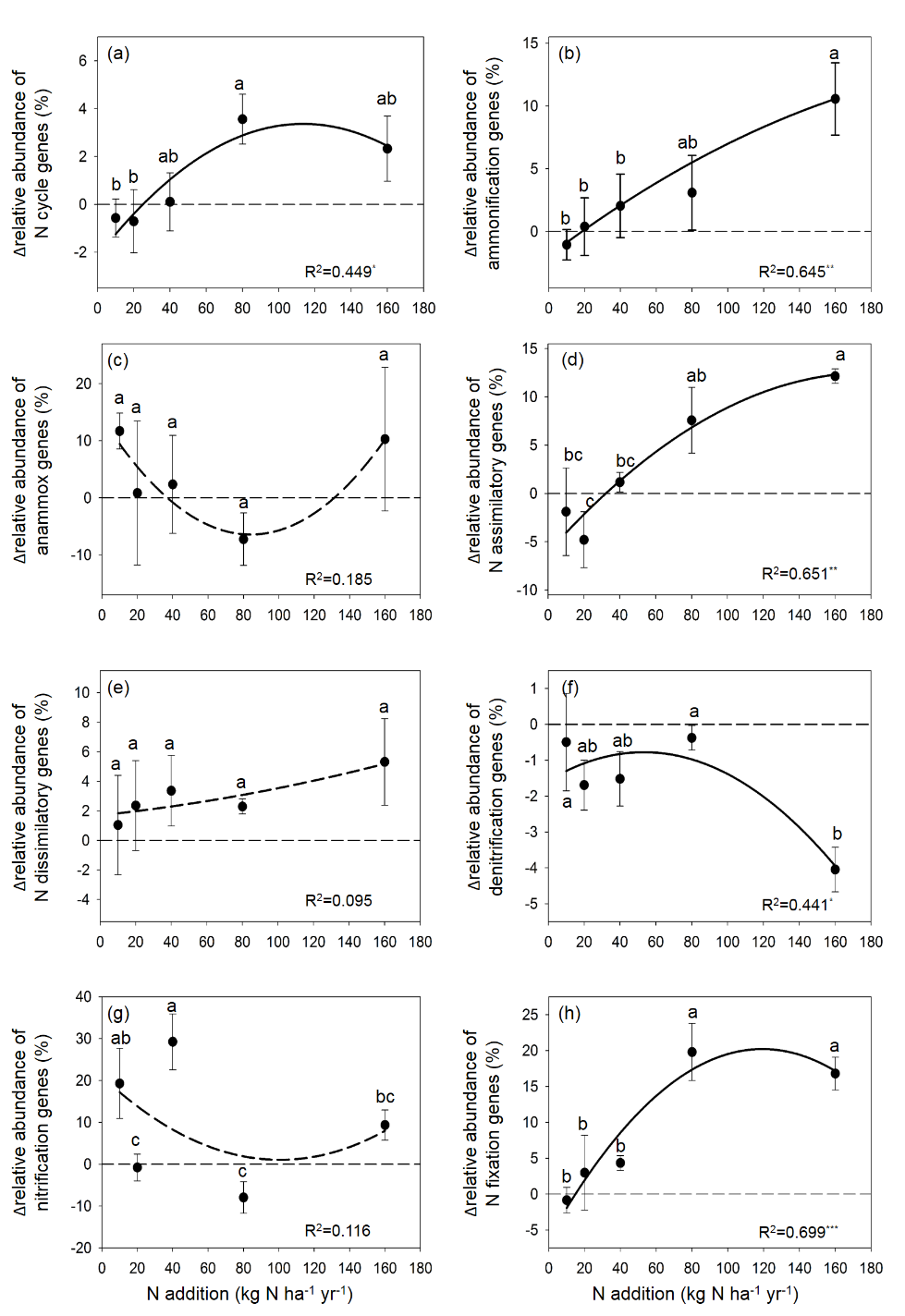
**Fig. S3** The difference in the abundance of C-cycle genes (%) between each treatment and control. (a) Total C cycle genes; (b) C sequestration genes; (c) Methane cycling genes; (d) C degradation genes. Different lowercase letters indicate a significant difference (*p*<0.05) according to ANOVA analysis. Asterisks represent significant correlations (\* *p*<0.05, \*\* *p*<0.01, \*\*\**p*<0.001).



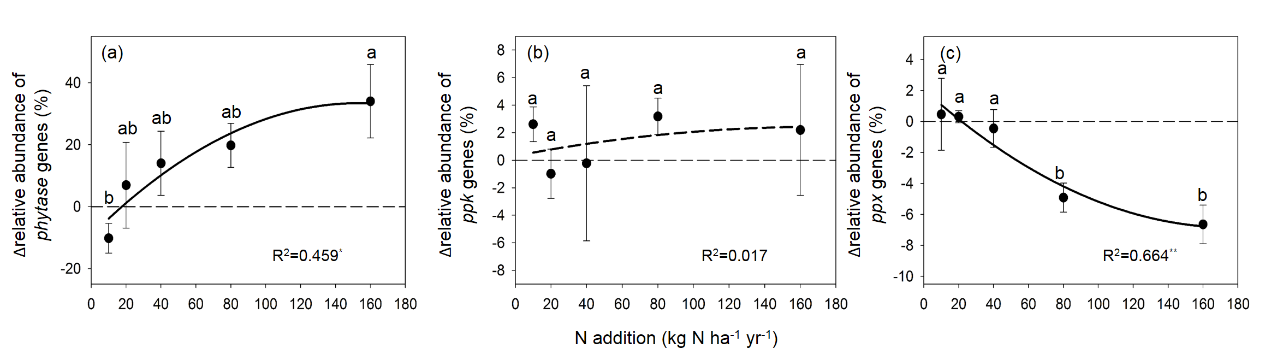
**Fig. S4** The difference in the abundance of C degradation genes (%) between each treatment and control. (a) Labile C degradation; (b) Recalcitrant C degradation; (c) Starch degradation; (d) Hemicellulose degradation; (e) Chitin degradation; (f) Lignin degradation; (g) Cellulose degradation. Different lowercase letters indicate a significant difference (*p*<0.05) according to ANOVA analysis. Asterisks represent significant correlations (\* *p*<0.05, \*\* *p*<0.01, \*\*\**p*<0.001).



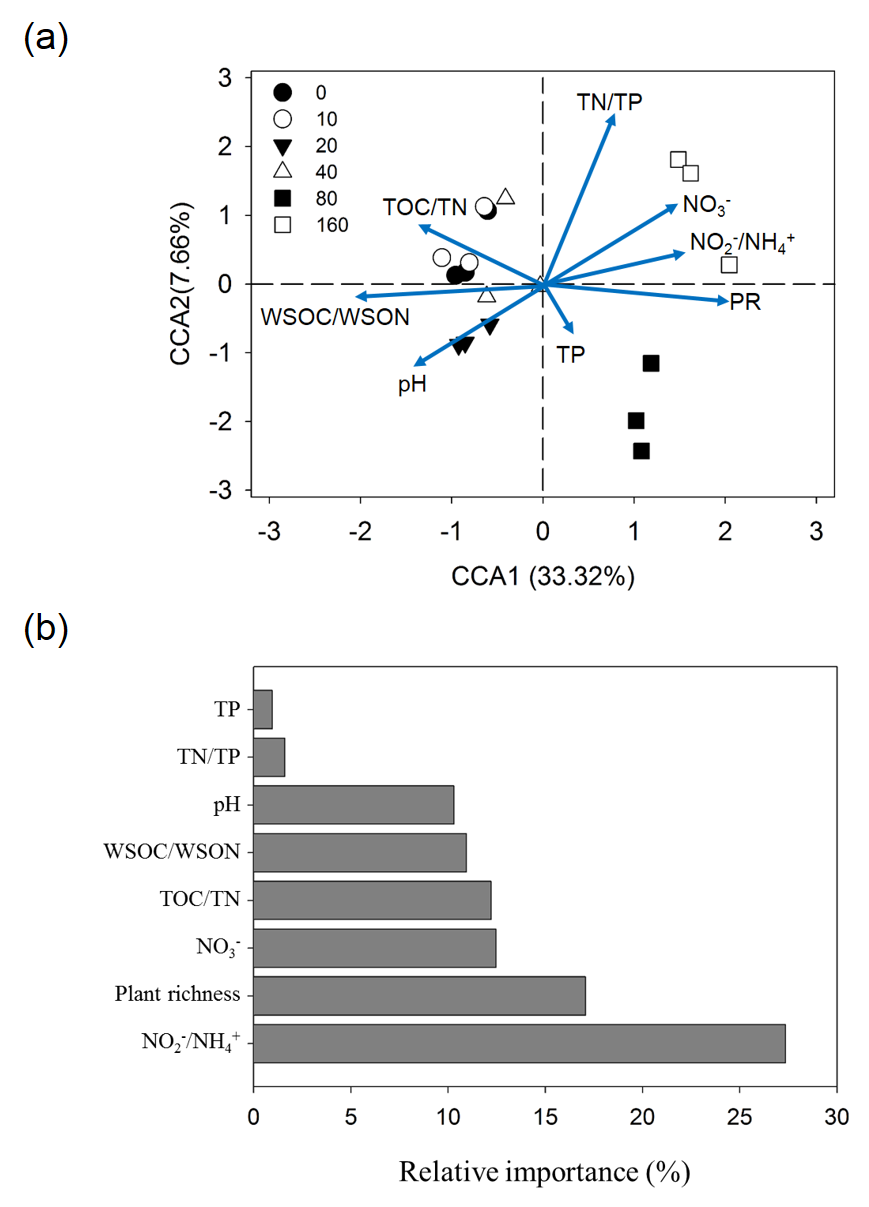
**Fig. S5** The difference in the enzyme activity (%) between each treatment and control. (a) Invertase; (b) β-glucosidase; (c) Phenoloxidase; (d) Peroxidase; (e) Urease; (f) Alkaline phosphatase. Different lowercase letters indicate a significant difference (*p*<0.05) according to ANOVA analysis. Asterisks represent significant correlations (\* *p*<0.05, \*\* *p*<0.01, \*\*\**p*<0.001).



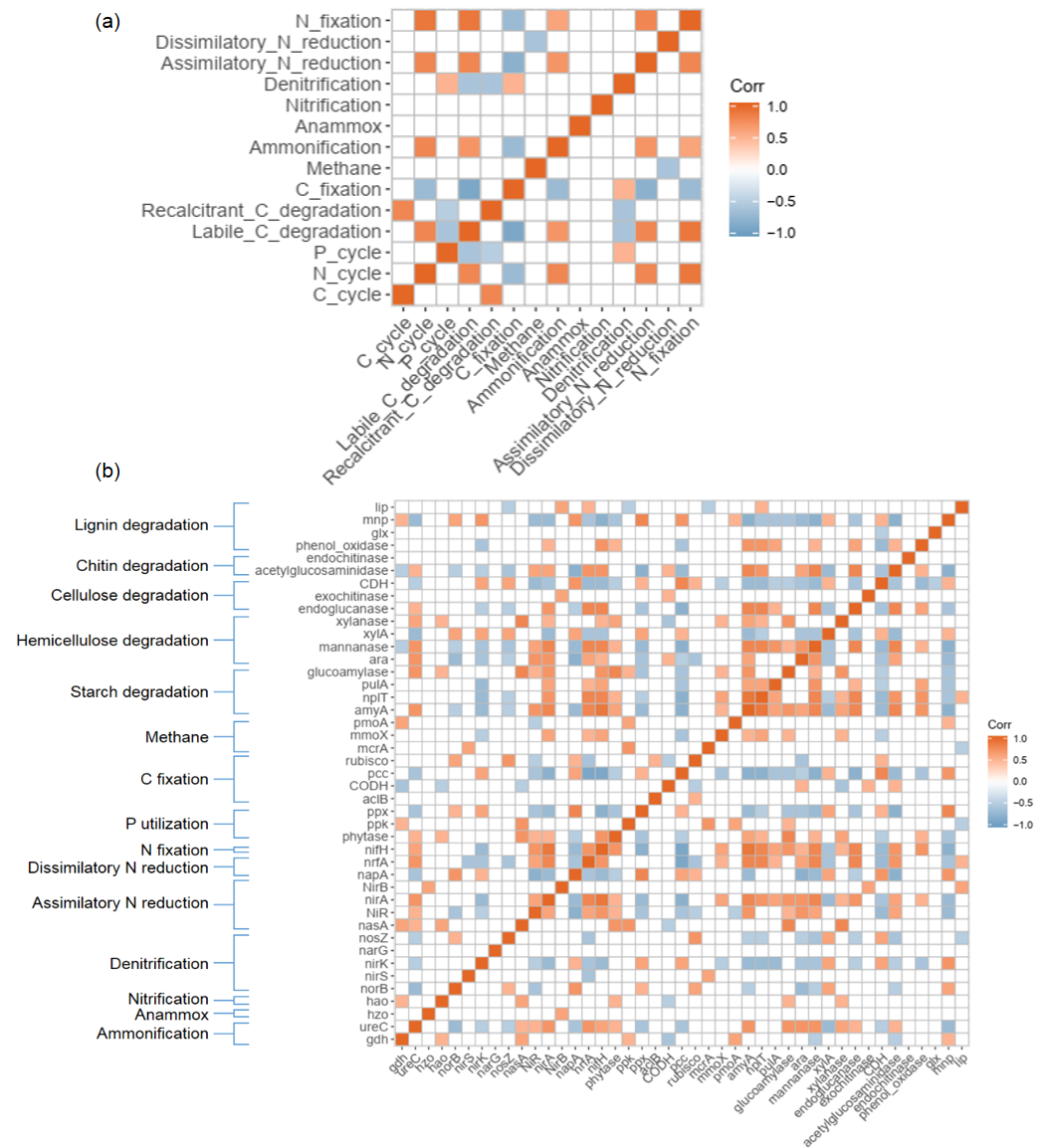
**Fig. S6** The difference in the abundance of N-cycle genes (%) between each treatment and control. (a) Total N cycle genes; (b) Ammonification genes; (c) Assimilatory N reduction genes; (d) Nitrogen fixation genes; (e) Anammox genes; (f) Nitrification genes; (g) Dissimilatory N reduction genes; (h) Denitrification genes. Different lowercase letters indicate a significant difference (*p*<0.05) according to ANOVA analysis. Asterisks represent significant correlations (\* *p*<0.05, \*\* *p*<0.01, \*\*\**p*<0.001).



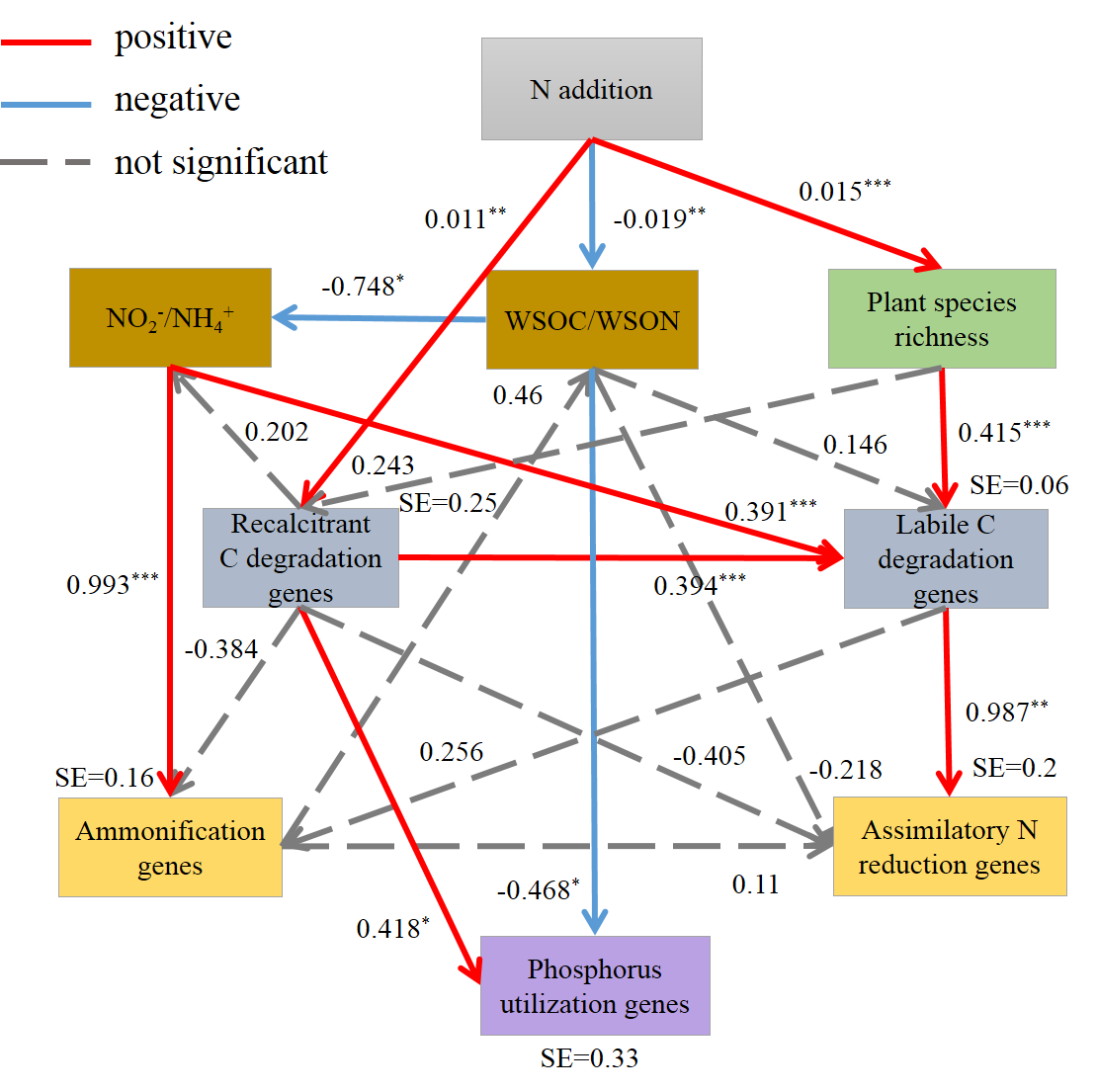
**Fig. S7** The difference in the abundance of P utilization genes (%) between each treatment and control. (a) *phytase* genes; (b) *ppk* genes; (c) *ppx* genes. Different lowercase letters indicate a significant difference (*p*<0.05) according to ANOVA analysis. Asterisks represent significant correlations (\* *p*<0.05, \*\* *p*<0.01, \*\*\**p*<0.001).

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**Fig. S8** Canonical correlation analysis (CCA) and multiple regression analysis of the microbial function genes and environmental variables. (a) pH, TP, NO2-, NO3-, NH4+,TOC/TN,TN/TP, WSOC/WSON and Plant richness. The percentage of variation explained by each axis is shown. (b) The relative importance (%) of each variable for functional genes is shown as bar plots. The functional genes composition were indicated by first axis of detrended correspondence analyses (DCA).



**Fig. S9** Correlations between C, N, and P cycle genes. (a) Correlations between the sums of functional gene abundance; (b) Correlations between individual C, N, and P-cycle related gene abundance.

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**Fig. S10** Structural equation modelling (SEM) of function genes and environmental factors. The red solid lines represent significant positive correlations, blue solid lines represent significant negative correlations, and the dashed black lines represent non-significant correlations. Chi-square (χ2) =14.165; *p*=0.586; CMIN/DF=0.885, GFI=0.846, RMSEA<0.001, AIC=72.165.