# Response of rhizosphere soil microbial to *Deyeuxia angustifolia* encroaching in two different vegetation communities in alpine tundra

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TableS1. Sampling sites, geographical location and dominant species that use plant coverage (%) to indicate.

| Types | Altitude(m) | Latitude | Longitude | Dominant species & Coverage (%) |
|---|---|---|---|---|
| NH | 2182 | 41°59′25.57″ | 128°1′0.23″ | *Ligularia jamesii (Hemsl.) Kom.* 43%, *Sanguisorba sitchensis*46%, *Veratrum nigrum L.* 7% |
| EH | 2182 | 41°59′25.57″ | 128°1′0.23″ | *D. angustifolia* 40%, *Ligularia jamesii (Hemsl.) Kom*. 22%, *Sanguisorba sitchensis* 26% |
| NS | 2182 | 41°59′25.57″ | 128°1′0.23″ | *Rhododendron aureum* Georgi 60%,*Vaccinium uliginosum* Linn. 28% |
| ES | 2182 | 41°59′25.57″ | 128°1′0.23″ | *D. angustifolia 35%, Rhododendron aureum* Georgi 35%, *Vaccinium uliginosum Linn.*25% |
| DA | 2182 | 41°59′25.57″ | 128°1′0.23″ | *D. angustifolia* 92% |
| NS | 2178 | 41°59′20.99″ | 128°0′52.65″ | *Vaccinium uliginosum Linn.*68%, *Phyllodoce caerulea (L.) Bab*.24% |
| ES | 2178 | 41°59′20.99″ | 128°0′52.65″ | *D. angustifolia* 49%, *Vaccinium uliginosum Linn.*28%, *Phyllodoce caerulea (L.) Bab.* 15% |
| NS | 2178 | 41°59′20.99″ | 128°0′52.65″ | *Rhododendron aureum Georgi* 83%, *Vaccinium uliginosum Linn.*8% |
| ES | 2178 | 41°59′20.99″ | 128°0′52.65″ | *D. angustifolia* 49%, *Rhododendron aureum Georgi* 42% |
| DA | 2178 | 41°59′20.99″ | 128°0′52.65″ | *D. angustifolia* 90% |
| NS | 2143 | 41°59′25.02″ | 128°0′45.68″ | *Rhododendron aureum Georgi* 95%, *Vaccinium uliginosum Linn.*3% |
| ES | 2143 | 41°59′25.02″ | 128°0′45.68″ | *D. angustifolia* 45%, *Rhododendron aureum Georgi* 51% |
| NS | 2143 | 41°59′25.02″ | 128°0′45.68″ | *Vaccinium uliginosum Linn.*92% |
| ES | 2143 | 41°59′25.02″ | 128°0′45.68″ | *D. angustifolia* 55%, *Vaccinium uliginosum Linn.*36% |
| NH | 2142 | 41°59′25.02″ | 128°0′45.68″ | *Ligularia jamesii (Hemsl.) Kom*. 82%, *Trollius chinensis Bunge* 7%, *Veratrum nigrum L.* 5% |
| EH | 2143 | 41°59′25.02″ | 128°0′45.68″ | *D. angustifolia* 45%, *Ligularia jamesii (Hemsl.) Kom*42%, *Trollius chinensis Bunge* 6% |
| DA | 2143 | 41 °59′25.02″ | 128°0′45.68″ | *D. angustifolia* 93% |
| NS | 2034 | 41°59′20.92″ | 128°0′9″ | *Phyllodoce caerulea (L.) Bab.*34%, *Rhododendron aureum Georgi* 52%, *Vaccinium uliginosum Linn.*9% |
| ES | 2034 | 41°59′20.92″ | 128°0′9″ | *D. angustifolia* 47%, *Phyllodoce caerulea (L.) Bab*. 15%,*Rhododendron aureum Georgi* 23%, *Vaccinium uliginosum Linn.*7% |
| NH | 2034 | 41°59′20.92″ | 128°0′9″ | *Sanguisorba sitchensis* 60%, *Trollius chinensis Bunge* 22%, *Aconitum monanthum Nakai*8%, *Veratrum nigrum* L. 5% |
| EH | 2034 | 41°59′20.92″ | 128°0′9″ | *D. angustifolia* 55%,*Sanguisorba sitchensis* 30%,*Trollius chinensis Bunge* 8%,*Aconitum monanthum Nakai* 5% |
| NH | 2034 | 41°59′20.92″ | 128°0′9″ | *Sanguisorba sitchensis* 30%, *Aconitum monanthum Nakai* 21%, *Veratrum nigrum L*. 15%, *Trollius chinensis Bunge* 18%, *Ligularia jamesii (Hemsl.) Kom* 10% |
| EH | 2034 | 41°59′20.92″ | 128°0′9″ | *D. angustifolia* 42%,*Sanguisorba sitchensis* 18%, *Aconitum monanthum Nakai* 12%, *Trollius chinensis Bunge* 8%, *Veratrum nigrum L*. 5% |
| DA | 2034 | 41°59′20.92″ | 128°0′9″ | *D. angustifolia 90%,Veratrum nigrum* L. 5% |
| NH | 2026 | 41°59′15.72″ | 128°0′6.29″ | *Trollius chinensis Bunge* 35%, *Sanguisorba sitchensis* 30%, *Aconitum monanthum Nakai* 12% *Veratrum nigrum L*. 8%, *Ligularia jamesii (Hemsl.) Kom 12%* |
| EH | 2026 | 41°59′15.72″ | 128°0′6.29″ | *Trollius chinensis Bunge* 25%, *Sanguisorba sitchensis* 9%, *Aconitum monanthum Nakai* 7%, *Veratrum nigrum L*. 4% |
| DA | 2026 | 41°59′15.72″ | 128°0′6.29″ | *D. angustifolia* 83%, *Trollius chinensis Bunge* 6% |

TableS2. Primers and real-time PCR conditions used in this experiment.

| function genes | primers | | real-time PCR protocol |
|---|---|---|---|
| *nifH* | PolF | 5′-TG CGAY CCS AAR GCB GAC TC-3′ | 94℃ for 5 min; 40 cycles of 94℃ for 30s, 57℃ for 45s(54℃ for *nifH* gene, 60℃ for AOB), 72℃ for 1 min. |
| | PolR | 5′-ATS GCC ATC ATY TCR CCG GA-3′ | |
| AOA | Arch-amoAF | 5′-STA ATG GTC TGG CTT AGA CG-3′ | |
| | Arch-amoAR | 5′-GCG GCC ATC CAT CTG TAT GT-3′ | |
| AOB | amoA-1F | 5′-GGG GTT TCT ACT GGT GGT-3′ | |
| | amoA-2R | 5′-CCC CTC KGS AAA GCC TTC TTC-3′ | |
| *nosZ* | nosZ-F | 5′-CGY TGT TCM TCG ACA GCC AG-3′ | |
| | nosZ 1622R | 5′-CGS ACC TTS TTG CCS TYG CG-3′ | |

TableS3. Pearson correlation coefficient (*r*) between soil properties and enzymatic activities, Asterisk indicates significant differences *indicate significant ($P<0.05$) effects, ** indicate significant ($P<0.01$) effects.

| | TN | TOC | TP | TK | $NO_3^-$ | $NH_4^+$ | Moisture | pH | MBC | MBN | AN | AP | AK | C/N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Urease | 0.616** | 0.715** | 0.329* | -0.288 | -0.153 | 0.082 | 0.567** | -0.336 | 0.016 | -0.076 | 0.515** | 0.290 | 0.422* | 0.606** |
| Sucrase | 0.484** | 0.714** | 0.177 | -0.260 | -0.278 | 0.275 | 0.402* | -0.168 | 0.094 | -0.318 | 0.317 | 0.084 | 0.324* | 0.683** |
| ACP | 0.062 | 0.242 | 0.490** | -0.086 | -0.030 | 0.240 | 0.310 | -0.409 | 0.037 | -0.087 | 0.297 | 0.651** | 0.543** | 0.233 |
| Catalase | 0.748** | 0.731** | 0.387* | 0.034 | 0.013 | 0.305 | 0.592** | -0.244 | 0.262 | -0.100 | 0.440* | 0.336* | 0.413* | 0.561** |

TableS4. Pearson correlation coefficient (*r*) between N cycling function genes abundance and soil properties, just list significant (*P*<0.05) correlation. Bolded values indicate significant (*P*<0.05) effects.

| | AOB | | AOA | | *nosZ* | | *nifH* | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| **Total** | **n=27** | | | | | | | |
| $NO_3^-$ | **-0.404** | **0.036** | **0.451** | **0.018** | -0.073 | 0.719 | -0.050 | 0.806 |
| AP | **-0.410** | **0.040** | -0.061 | 0.764 | 0.291 | 0.141 | -0.324 | 0.099 |
| TP | -0.245 | 0.218 | 0.298 | 0.131 | 0.039 | 0.847 | **0.461** | **0.016** |
| $NH_4^+$ | -0.118 | 0.557 | -0.132 | 0.512 | **0.560** | **0.002** | -0.207 | 0.300 |
| Moisture | 0.020 | 0.922 | -0.096 | 0.634 | **0.490** | **0.009** | 0.013 | 0.950 |
| **Herbaceous** | **n=10** | | | | | | | |
| AP | **-0.636** | **0.048** | -0.240 | 0.505 | -0.341 | 0.334 | -0.463 | 0.178 |
| TOC | 0.249 | 0.489 | **0.833** | **0.003** | 0.207 | 0.506 | **0.815** | **0.004** |
| TP | -0.232 | 0.518 | **0.672** | **0.033** | -0.023 | 0.950 | **0.668** | **0.035** |
| C/N | 0.293 | 0.412 | **0.701** | **0.024** | 0.190 | 0.599 | **0.698** | **0.025** |
| TK | -0.514 | 0.128 | **-0.671** | **0.034** | 0.193 | 0.592 | **-0.632** | **0.048** |
| MBC | -0.425 | 0.221 | -0.213 | 0.554 | **-0.633** | **0.049** | -0.469 | 0.171 |
| MBN | -0.452 | 0.190 | -0.357 | 0.311 | **-0.665** | **0.036** | -0.542 | 0.106 |
| Moisture | 0.475 | 0.165 | **0.626** | **0.530** | 0.456 | 0.185 | **0.633** | **0.037** |
| **Shrub** | **n=12** | | | | | | | |
| AN | 0.420 | 0.174 | **-0.591** | **0.043** | -0.029 | 0.928 | 0.533 | 0.074 |
| AK | -0.173 | 0.582 | **-0.643** | **0.024** | **0.334** | **0.288** | 0.431 | 0.161 |
| $NH_4^+$ | -0.231 | 0.471 | -0.437 | 0.155 | **0.620** | **0.031** | -0.332 | 0.291 |
| MBC | -0.240 | 0.460 | -0.143 | 0.658 | **0.841** | **0.001** | 0.134 | 0.679 |

TableS5. Alpha Diversity Metrics for Native sites (NH, NS), Encroaching sites (EH, ES) in two vegetation types and *D. angustifolia* (DA) bacteria.

| | **chao1** | **observed species** | **PD whole tree** | **shannon** | **simpson** | **goods coverage** |
|---|---|---|---|---|---|---|
| NS | 1100.42 | 857.5 | 54.29 | 7.86 | 0.99 | 0.99 |
| ES | 925.80 | 826.83 | 48.02 | 7.40 | 0.98 | 0.99 |
| NH | 1037.12 | 865.40 | 52.10 | 7.45 | 0.98 | 0.99 |
| EH | 998.05 | 834.80 | 51.02 | 7.36 | 0.98 | 0.99 |
| DA | 863.95 | 747.75 | 48.38 | 7.62 | 0.98 | 0.99 |

TableS6. A non-parametric test showed differences between encroaching and native sites in two treatments (NS/ES and NH/EH), compare the microbial diversity *D. angustifolia* to native samples (DA/NS/NH) and to encroaching samples (DA/ES/EH). Bolded values indicate significant ($\alpha<0.05$) effects.

| Alpha name | NS/ES | NH/EH | DA/NS/NH | DA/ES/EH |
|---|---|---|---|---|
| chao1 | **0.004** | 0.55 | **0.05** | 0.09 |
| observed species | 0.618 | 0.31 | 0.26 | 0.17 |
| PD whole tree | **0.025** | 0.84 | 0.48 | 0.36 |
| shannon | **0.037** | 0.84 | 0.70 | 0.82 |
| simpson | 0.113 | 1.00 | 0.33 | 0.76 |
| goods coverage | 0.857 | 0.84 | **0.03** | 0.22 |

TableS7. Phylum with a greater relative abundance associated with native and encroaching sites under woody and herbaceous treatment to have a significant effect based on ANOSIM Analysis. Bolded values indicate significant ($P<0.05$) effects.

| Phylum | NS/ES | NH/EH | DA/NS/NH | DA/ES/EH |
|---|---|---|---|---|
| Acidobacteria | 0.07 | 0.43 | 0.17 | 0.17 |
| Proteobacteria | **0.05** | **0.05** | 0.12 | 0.66 |
| Actinobacteria | 0.11 | 0.43 | 0.43 | 0.15 |
| Bacteroidetes | 0.09 | 0.25 | **0.04** | 0.58 |
| Chloroflexi | 0.61 | 0.66 | 0.41 | 0.71 |
| Candidatus Saccharibacteria | 0.69 | 0.93 | 0.08 | 0.20 |
| Planctomycetes | **0.04** | 1.00 | 0.42 | 0.31 |
| Firmicutes | 0.61 | 0.93 | 0.35 | 0.45 |
| Gemmatimonadetes | **0.02** | 0.79 | **0.04** | 0.64 |
| Verrucomicrobia | 0.08 | 0.54 | 0.51 | 0.61 |
| Nitrospirae | 0.50 | 1.00 | **0.04** | 0.63 |
| Latescibacteria | 1.00 | 0.40 | **0.05** | 0.68 |
| Cyanobacteria/Chloroplast | 0.17 | 0.27 | 0.31 | 0.47 |
| Chlamydiae | 0.26 | 0.51 | 0.61 | 0.70 |

TableS8. The correlation between community composition and environmental variables for bacteria. Values in bold indicate significant correlation (*P*<0.05).

| **Variable** | ***r*** | ***Pr* (>*r*)** |
|---|---|---|
| **TN** | **0.353** | **0.009 **** |
| **Moisture** | **0.262** | **0.030 *** |
| **pH** | **0.261** | **0.024 *** |
| **MBN** | **0.274** | **0.029 *** |
| MBN | 0.204 | 0.068 |
| AK | 0.174 | 0.096 |
| Elevation | 0.153 | 0.128 |
| TOC | 0.157 | 0.129 |
| $NH_4^+$ | 0.100 | 0.266 |
| AP | 0.055 | 0.520 |
| AN | 0.034 | 0.663 |
| TK | 0.026 | 0.722 |
| $NO_3^-$ | 0.022 | 0.745 |
| TP | 0.020 | 0.794 |

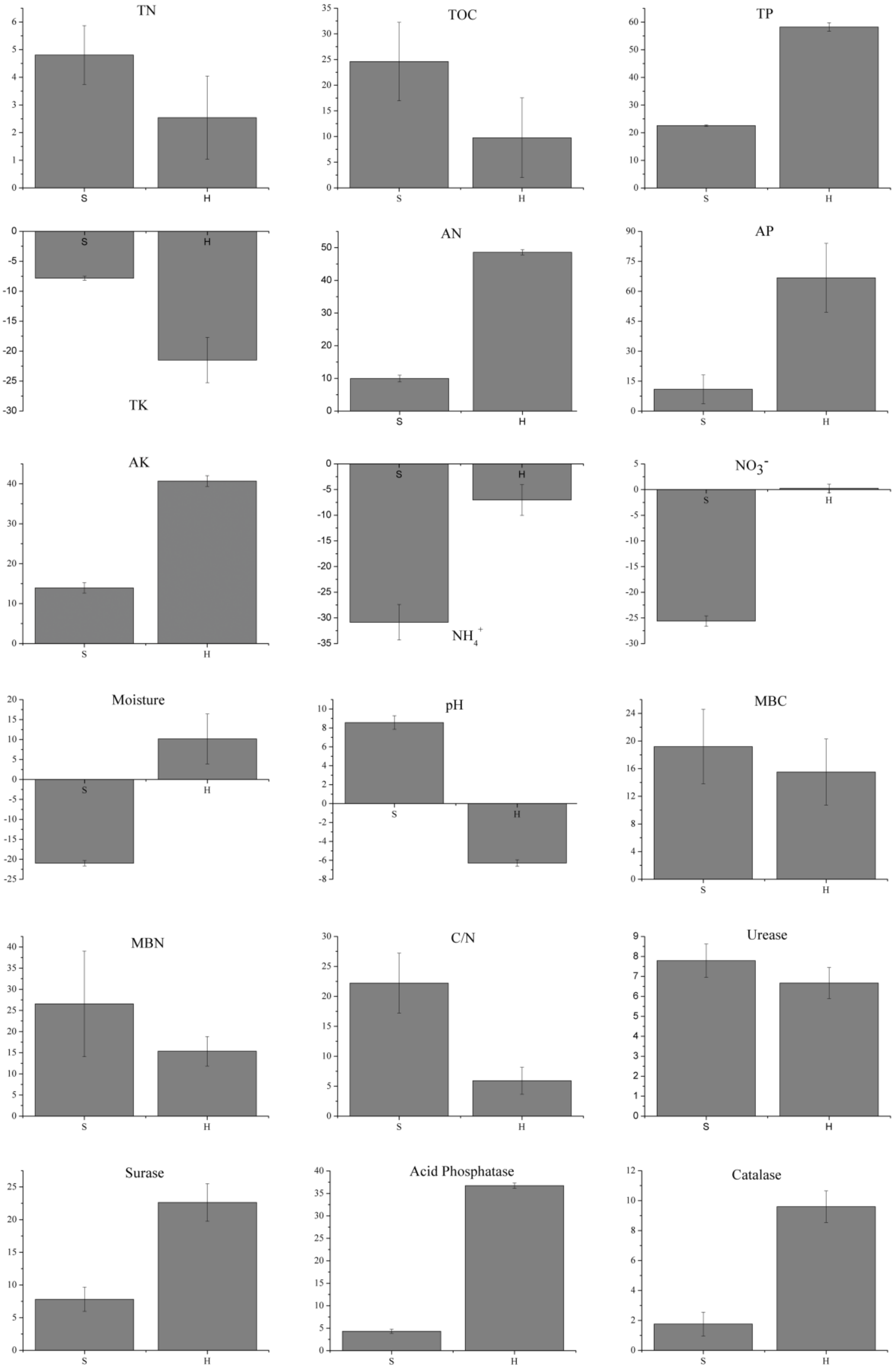

Fig.S1. The effect of *D. angustifolia* encroaching on soil nutrients are shown. Data are pooled over vegetation types and were generated by looking at the percentage loss or gain of a particular attribute *D. angustifolia* encroaching relative to the native control values. Bars are standard errors.

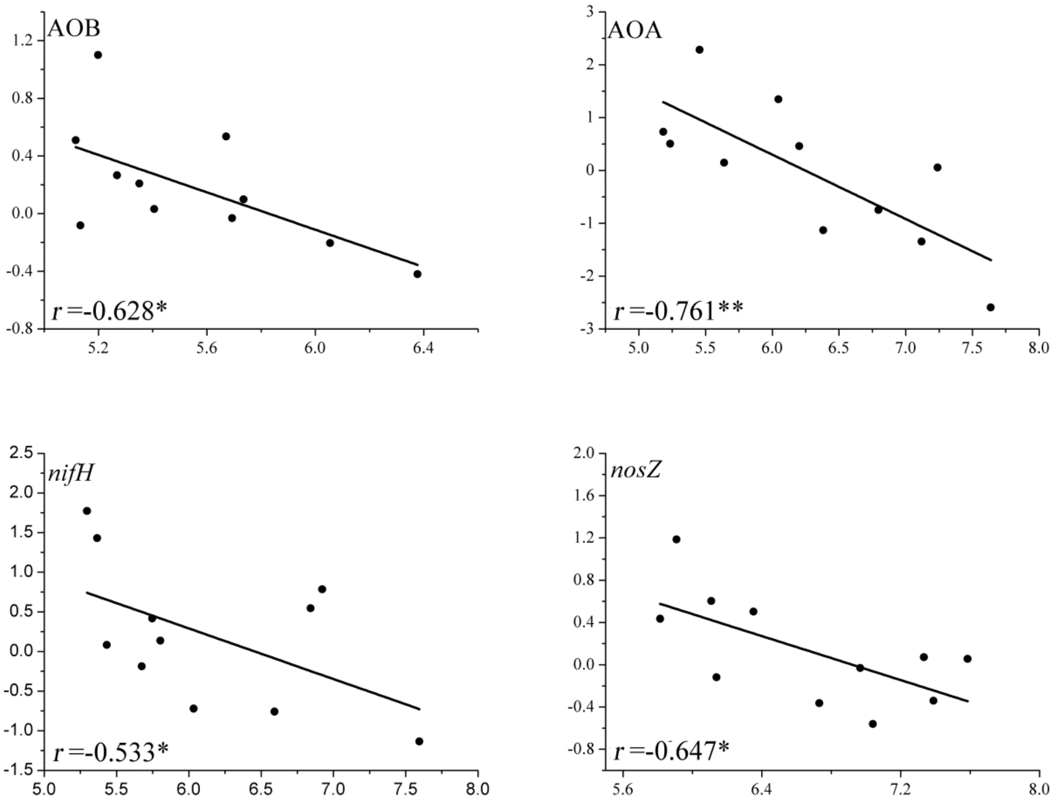

Fig.S2. Impact of invasive species on N-transforming function genes in native plots (*N*). Impact is calculated as the difference between the value of the parameter in encroaching plots (*E*) minus the value of the parameter in native plots (*N*). A positive value of *E-N* indicates an increased concentration of that element in the invaded soil Pearson correlation coefficient (*r*) between *N* and *E -N*. Significance levels(**P* < 0.05, ***P* < 0.01).

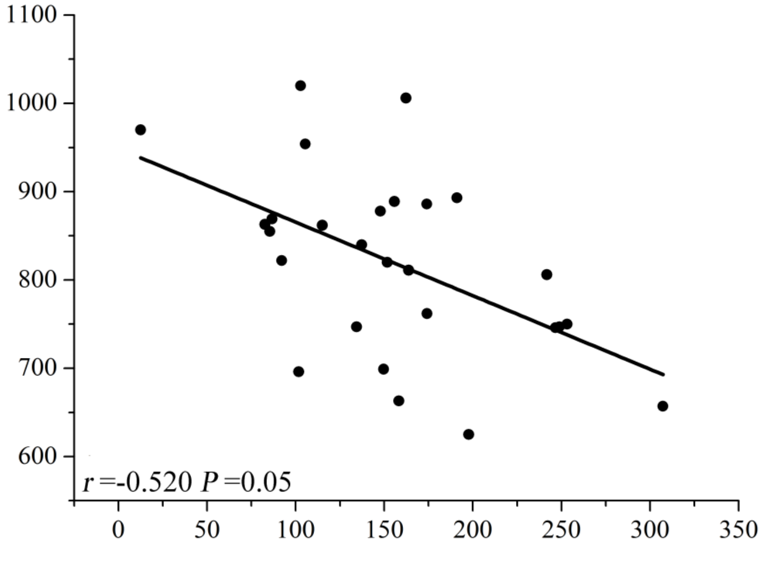

Fig.S3. Pearson correlation coefficient (*r*) between OTU richness and TOC.