Supplemental material

**Variations in soil bacterial communities and putative functions in a sugarcane soil following five years of chemical fertilization**

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Table S1. List of soil chemical properties after different fertilization treatments.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Control | PK | NK | NP | NPK |
| pH | 6.71±0.02a | 5.84±0.01b | 5.41±0.02e | 5.59±0.01d | 5.69±0.02c |
| NH4+-N(mg kg-1) | 10.48±0.27d | 5.47±0.34e | 29.24±0.02c | 55.82±1.58a | 43.79±0.80b |
| NO3--N(mg kg-1) | 62.73±5.72d | 32.36±2.75e | 281.45±9.46a | 185.89±2.37b | 153.90±2.99c |
| DOC(mg kg-1) | 32.79±2.53c | 35.34±1.42c | 53.43±2.53b | 78.39±2.22a | 76.52±2.11a |
| TN (g kg-1) | 0.62±0.005d | 0.69±0.006c | 0.84±0.01a | 0.79±0.01b | 0.78±0.01b |
| TP(g kg-1) | 0.52±0.009c | 1.88±0.058a | 0.52±0.01c | 1.09±0.005b | 1.02±0.012b |
| AP(mg kg-1) | 0.74±0.03c | 202.38±1.15a | 0.66±0.01d | 51.23±3.21b | 52.30±1.73b |
| AK(mg kg-1) | 122.67±2.08c | 365.33±5.77a | 343.33±4.16b | 87.33±2.52d | 329.33±10.07b |

Values are mean ± standard deviation (n=3). Different letters in each row indicate a significant difference at *p* < 0.05.

NH4+-N: ammonium-N; NO3--N: nitrate-N; DOC: dissolved organic carbon; TN: total nitrogen; TP: total phosphorus; AP: available phosphorus; AK: available potassium.

Table S2 Relative average abundances of the nineteen most abundant classes under different inorganic fertilizer treatments. Data are means ± standard deviation (n=3). Different letters indicate significant differences among the fertilizer treatments at *P* < 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Control | PK | NK | NP | NPK |
| Acidobacteria | 1.13±0.35ab | 2.05±1.08a | 0.12±0.02b | 0.33±0.11b | 0.55±0.32b |
| Blastocatellia | 1.87±0.59a | 1.86±0.30a | 0.10±0.02b | 0.35±0.37b | 0.13±0.04b |
| Holophagae | 1.40±0.22a | 1.05±0.42a | 0.02±0.01b | 0.02±0.01b | 0.07±0.02b |
| Solibacteres | 1.66±0.47a | 1.65±0.30a | 0.80±0.10b | 0.74±0.16b | 0.88±0.06b |
| Acidobacteria-Subgroup\_6 | 2.90±1.16a | 1.73±0.28a | 0.09±0.06c | 0.50±0.44bc | 0.44±0.08b |
| Acidimicrobiia | 1.41±0.50a | 0.99±0.05a | 0.44±0.10b | 0.28±0.16b | 0.28±0.13b |
| Actinobacteria | 6.45±2.95b | 6.17±1.73b | 10.85±1.99a | 9.38±2.02a | 9.42±0.38a |
| Thermoleophilia | 2.50±0.05ab | 2.55±0.84a | 0.38±0.14c | 1.37±0.45b | 1.20±0.11bc |
| Sphingobacteriia | 1.69±0.65b | 1.70±0.67b | 1.30±0.40b | 4.71±1.23a | 5.30±0.54a |
| Anaerolineae | 2.04±0.99a | 1.44±0.40a | 0.11±0.09b | 0.14±0.16b | 0.13±0.04b |
| Chloroflexia | 2.48±1.17a | 1.28±0.72ab | 0.22±0.08b | 0.87±1.01b | 0.49±0.04b |
| Chloroflexi-JG37-AG-4 | 4.72±2.36a | 7.50±4.21a | 0.26±0.12b | 0.24±0.25b | 0.28±0.19b |
| Thermomicrobia | 0.22±0.16b | 0.25±0.12b | 2.27±0.83a | 2.53±0.60a | 2.31±0.96a |
| Bacilli | 0.87±0.36b | 1.02±0.6b | 3.45±1.18a | 3.28±1.28a | 3.20±1.30a |
| Gemmatimonadetes | 1.36±0.58a | 1.39±0.45a | 0.19±0.02c | 0.51±0.16b | 0.35±0.06b |
| Alphaproteobacteria | 23.03±3.56a | 22.61±7.09a | 11.42±5.59b | 15.42±7.72ab | 10.07±0.46b |
| Betaproteobacteria | 7.80±3.25a | 7.34±1.95a | 9.12±4.63a | 11.67±0.48a | 12.30±2.96a |
| Deltaproteobacteria | 2.34±0.85a | 2.95±2.90a | 0.51±0.22a | 1.93±0.65a | 2.15±2.60a |
| Gammaproteobacteria | 3.82±1.46b | 4.74±2.10b | 10.30±1.63a | 31.73±17.08a | 29.83±11.56a |

Table S3 Correlations (R) analysis of soil properties and bacterial community structure using the partial mantel test.

|  |  |  |
| --- | --- | --- |
|  | R | *p* |
| pH | 0.27 | 0.022 |
| NH4+-N | 0.63 | 0.001 |
| NO3--N | 0.46 | 0.003 |
| DOC | 0.26 | 0.02 |
| TN | 0.70 | 0.001 |
| TP | 0.12 | 0.124 |
| AP | 0.24 | 0.091 |
| AK | -0.02 | 0.506 |

The values in the table are the Spearman’s correlation coefficients.

NH4+-N: ammonium-N; NO3--N: nitrate-N; DOC: dissolved organic carbon; TN: total nitrogen; TP: total phosphorus; AP: available phosphorus; AK: available potassium.

Table S4 Pearson’s correlation coefficients between soil properties and abundant classes (relative abundance >1%).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Class | pH | NH4+-N | NO3--N | DOC | TN | TP | AP | AK |
| Acidobacteria | 0.39 | -0.66\*\* | -0.77\*\*\* | -0.34 | -0.56\* | 0.60\*\* | 0.67\*\* | -0.18 |
| Blastocatellia | 0.89\*\*\* | -0.63 | -0.69\*\* | -0.44 | -0.91\*\* | -0.09 | -0.01 | -0.50 |
| Holophagae | 0.48\*\* | -0.71\*\* | -0.48 | 0.24 | 0.51 | -0.61\* | -0.19 | 0.56 |
| Solibacteres | 0.31 | -0.23 | -0.41 | -0.10 | -0.36 | 0.18 | 0.20 | -0.13 |
| Acidobacteria-Subgroup\_6 | 0.87\*\*\* | -0.64\* | -0.72\*\* | -0.42 | -0.89\*\*\* | -0.03 | 0.04 | -0.48 |
| Acidimicrobiia | 0.41 | -0.68\*\* | -0.59\* | -0.52\* | -0.52\* | 0.34 | 0.43 | -0.09 |
| Actinobacteria | -0.23 | 0.08 | 0.44 | -0.13 | 0.29 | -0.32 | -0.29 | 0.36 |
| Thermoleophilia | 0.57\* | -0.54\* | -0.86\*\*\* | -0.19 | -0.72\*\* | 0.52\* | 0.56\* | -0.50 |
| Sphingobacteriia | -0.27 | 0.69\*\* | 0.16 | 0.82\*\*\* | 0.27 | 0.09 | -0.05 | -0.43 |
| Anaerolineae | 0.79\*\*\* | -0.76\*\* | -0.76\*\* | -0.54\* | -0.85\*\*\* | 0.10 | 0.21 | -0.36 |
| Chloroflexia | 0.75\*\* | -0.48 | -0.61\* | -0.27 | -0.77\*\*\* | -0.04 | 0.004 | -0.51 |
| Chloroflexi-JG37-AG-4 | 0.82\*\*\* | -0.69\*\* | -0.82\*\*\* | -0.43 | -0.88\*\*\* | 0.16 | 0.24 | -0.46 |
| Thermomicrobia | -0.61\* | 0.54\* | 0.77\*\*\* | 0.23 | 0.74\*\* | -0.33 | -0.38 | 0.45 |
| Bacilli | -0.57\* | 0.72\*\* | 0.58\* | 0.58\* | 0.65\*\* | -0.16 | -0.26 | 0.18 |
| Gemmatimonadetes | 0.61\* | -0.57\* | -0.77\*\*\* | -0.27 | -0.74\*\* | 0.36 | 0.41 | -0.52 |
| Alphaproteobacteria | 0.65\*\* | -0.80\*\*\* | -0.85\*\*\* | -0.51\* | -0.78\*\*\* | 0.44 | 0.54\* | -0.41 |
| Betaproteobacteria | -0.32 | 0.61\* | 0.33 | 0.55\* | 0.38 | -0.09 | -0.19 | -0.05 |
| Deltaproteobacteria | 0.24 | -0.18 | -0.45 | 0.02 | 0.23 | 0.32 | 0.32 | -0.28 |
| Gammaproteobacteria | -0.44 | 0.83\*\*\* | 0.35 | 0.81\*\*\* | -0.56\* | 0.02 | -0.15 | -0.17 |

\**p* <0.05, \*\**p* <0.01, \*\*\**p* <0.001.

Table S5 Pearson’s correlation analysis between relative abundances of functional groups and soil chemical properties.

|  | pH | NH4+-N | NO3--N | DOC | TN | TP | AP | AK |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| methanol\_oxidation | 0.23 | -0.74\*\* | -0.12 | -0.83\*\*\* | -0.27 | -0.06 | 0.09 | 0.37 |
| methylotrophy | 0.24 | -0.70\*\* | -0.08 | -0.81\*\*\* | -0.26 | -0.11 | 0.036 | 0.37 |
| aerobic\_ammonia\_oxidation | -0.21 | 0.28 | 0.32 | 0.07 | 0.33 | -0.27 | -0.29 | 0.32 |
| aerobic\_nitrite\_oxidation | 0.84\*\*\* | -0.55\* | -0.81\*\*\* | -0.24 | -0.91\*\*\* | 0.14 | 0.19 | -0.29 |
| nitrification | 0.003 | 0.11 | 0.07 | -0.08 | 0.08 | -0.17 | -0.18 | 0.13 |
| sulfur\_respiration | 0.84\*\*\* | -0.84\*\*\* | -0.84\*\*\* | -0.60\* | -0.91\*\*\* | 0.17 | 0.29 | -0.35 |
| respiration\_of\_sulfur\_compounds | 0.84\*\*\* | -0.83\*\*\* | -0.84\*\*\* | -0.60\* | -0.91\*\*\* | 0.17 | 0.29 | -0.35 |
| nitrate\_denitrification | 0.75\*\* | -0.75\*\* | -0.92\*\*\* | -0.41 | -0.87\*\*\* | 0.41 | 0.49 | -0.43 |
| nitrite\_denitrification | 0.75\*\* | -0.75\*\* | -0.92\*\*\* | -0.41 | -0.87\*\*\* | 0.41 | 0.49 | -0.43 |
| nitrous\_oxide\_denitrification | 0.75\*\* | -0.75\*\* | -0.92\*\*\* | -0.41 | -0.87\*\*\* | 0.41 | 0.49 | -0.43 |
| denitrification | 0.75\*\* | -0.75\*\* | -0.92\*\*\* | -0.41 | -0.87\*\*\* | 0.41 | 0.49 | -0.43 |
| nitrogen\_fixation | 0.51\*\* | -0.48\*\* | -0.45\*\* | 0.40 | -0.15 | 0.41 | 0.33 | -0.30 |
| nitrite\_respiration | 0.43 | -0.49 | -0.77\*\*\* | -0.17 | -0.60\* | 0.57\* | 0.60\* | -0.42 |
| cellulolysis | -0.21 | -0.17 | -0.32 | 0.01 | 0.03 | 0.70\*\* | 0.70\*\* | 0.01 |
| dark\_oxidation\_of\_sulfur\_compounds | -0.47 | 0.65\*\* | 0.35 | 0.68\*\* | 0.42 | 0.05 | -0.07 | -0.21 |
| manganese\_oxidation | 0.12 | -0.24 | -0.06 | -0.31 | -0.10 | -0.06 | -0.01 | 0.12 |
| fermentation | -0.12 | 0.19 | -0.08 | 0.20 | 0.15 | 0.17 | 0.13 | 0.14 |
| aerobic\_chemoheterotrophy | -0.42 | 0.79\*\*\* | 0.35 | 0.78\*\*\* | 0.47 | -0.04 | -0.19 | -0.16 |
| human\_pathogens\_all | -0.09 | 0.26 | 0.22 | 0.25 | 0.14 | -0.19 | -0.23 | -0.14 |
| animal\_parasites\_or\_symbionts | -0.10 | 0.26 | 0.20 | 0.26 | 0.14 | -0.16 | -0.20 | -0.15 |
| aromatic\_hydrocarbon\_degradation | 0.51 | -0.29 | -0.20 | -0.28 | -0.45 | -0.27 | -0.23 | -0.20 |
| aromatic\_compound\_degradation | 0.64 | -0.56\* | -0.51\* | -0.46 | -0.62\* | -0.04 | 0.041 | -0.07 |
| aliphatic\_non\_methane\_hydrocarbon\_degradation | 0.49 | -0.27 | -0.18 | -0.26 | -0.42 | -0.28 | -0.23 | -0.17 |
| hydrocarbon\_degradation | 0.48 | -0.27 | -0.12 | -0.29 | -0.41 | -0.33 | -0.28 | -0.16 |
| iron\_respiration | 0.08 | 0.14 | -0.11 | 0.18 | -0.06 | -0.01 | -0.04 | 0.01 |
| nitrate\_respiration | 0.18 | 0.005 | -0.32 | 0.23 | -0.24 | 0.25 | 0.21 | 0.49 |
| nitrate\_reduction | 0.16 | 0.282 | -0.27 | 0.52\* | -0.18 | 0.17 | 0.08 | -0.65\*\* |
| nitrogen\_respiration | 0.18 | 0.005 | -0.32 | 0.23 | -0.24 | 0.25 | 0.21 | -0.49 |
| intracellular\_parasites | 0.80\*\*\* | -0.70\*\* | -0.75\*\*\* | -0.47 | -0.86\*\*\* | 0.10 | 0.19 | -0.44 |
| predatory\_or\_exoparasitic | 0.29 | 0.01 | -0.28 | 0.21 | -0.31 | 0.04 | 0.01 | -0.57\* |
| chloroplasts | 0.41 | -0.66\*\* | -0.65\*\* | -0.42 | -0.54\* | 0.40 | 0.49 | -0.14 |
| cyanobacteria | -0.50 | 0.80\*\*\* | 0.45 | 0.72\*\* | 0.56\* | -0.10 | -0.23 | 0.07 |
| anoxygenic\_photoautotrophy\_S\_oxidizing | 0.43 | -0.47 | -0.74\*\* | -0.15 | -0.59\* | 0.54\* | 0.57\* | -0.43 |
| anoxygenic\_photoautotrophy | 0.43 | -0.47 | -0.74\*\* | -0.15 | -0.59\* | 0.54\* | 0.57\* | -0.43 |
| oxygenic\_photoautotrophy | -0.50 | 0.80\*\*\* | 0.45 | 0.72\*\* | 0.56\* | -0.10 | -0.23 | 0.07 |
| photoautotrophy | -0.08 | 0.32 | -0.21 | 0.52\* | 0.01 | 0.36 | 0.26 | -0.29 |
| photoheterotrophy | 0.38 | -0.37 | -0.54\* | -0.14 | -0.50 | 0.35 | 0.37 | -0.43 |
| phototrophy | -0.16 | 0.45 | -0.009 | 0.56\* | 0.12 | 0.18 | 0.07 | -0.27 |
| ureolysis | 0.15 | -0.52\* | 0.09 | -0.68\*\* | -0.13 | -0.25 | -0.12 | 0.36 |
| chemoheterotrophy | -0.50 | 0.79\*\*\* | 0.59\* | 0.66\*\* | 0.60\* | -0.26 | -0.38 | 0.04 |

Figure S1 Principal coordinate analysis (PCoA) of bacterial community composition across all soil samples in different inorganic fertilization regimens.

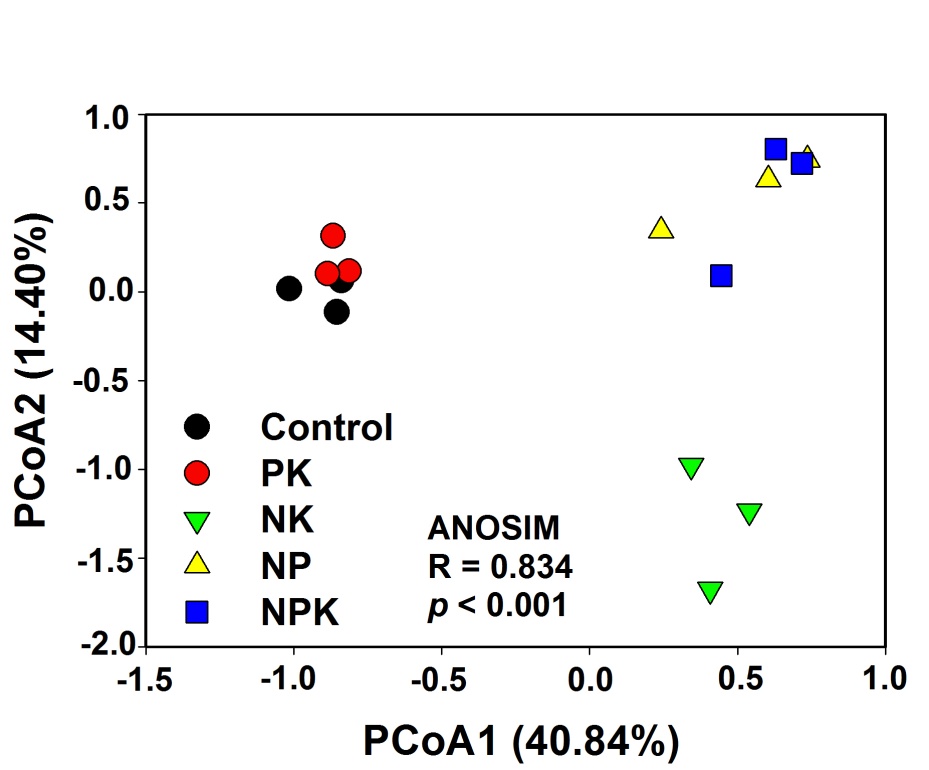


Figure S2 Variation partitioning analysis (VPA) of bacterial community composition explained by soil chemical parameters. NH4+-N: ammonium-N; NO3--N: nitrate-N; DOC: dissolved organic carbon; TN: total nitrogen.

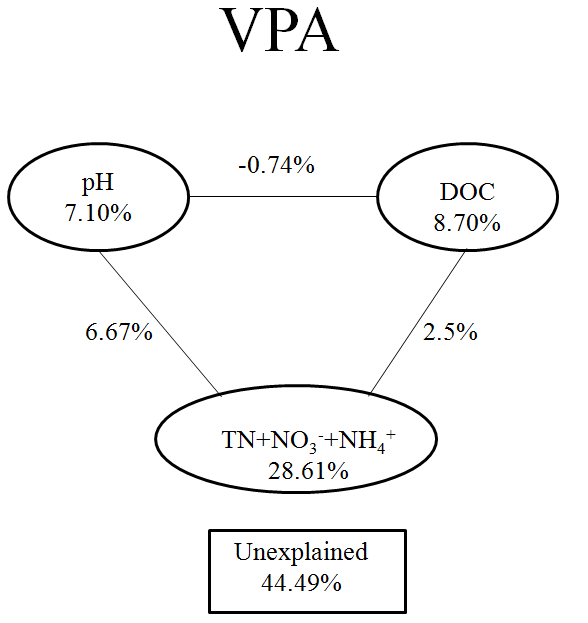


Figure S3 Heatmap of functional community profiles under different fertilizer treatments

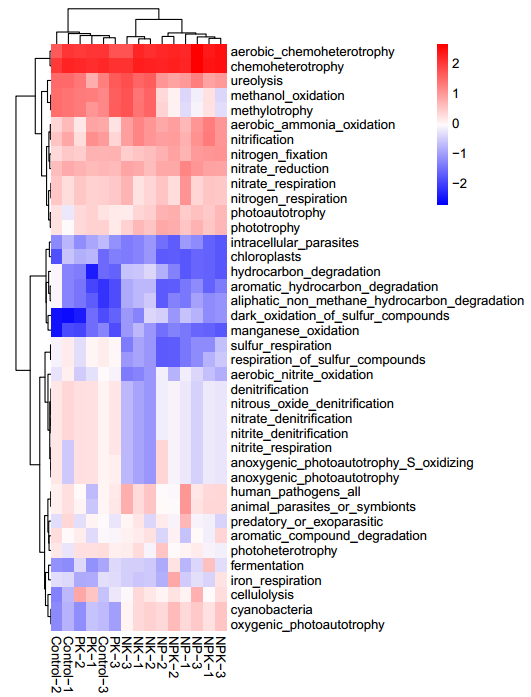


Figure S4 Relative abundances of the dominant genera under different fertilizer treatments.

