Supplementary Material

Supplementary Table and Figure captions, Table S3, S4, Figure S1 and S2 are uploaded separately on submission.

Table S1. Significantly increased taxa by relative abundance group in 100% chemical N fertilizer, biogas slurry replacing chemical N fertilizer at 50% (CBS) and 100% (BS) soils.

Table S2. Effects of the replacement of biogas slurry for chemical N fertilizer on soil bacterial functional groups related to N, C and S cycle.

Table S3. Summary of functional group assignments by FAPROTAX method.

Table S4. Changes of relations among soil bacterial functional groups with soil physico-chemical parameters, leaf nutrients and fruit parameters of apple following the increment of replacing rate by biogas slurry for chemical N fertilizer. Table S4A, correlation coefficient, r; Table S4B, significant value, p. CK, control, no chemical fertilizer and biogas slurry; CF, 100% chemical nitrogen (N) fertilizer; CBS and BS, biogas slurry replacing 50% and 100% of chemical N fertilizer, respectively.

Figure S1. Changes of functional groups with significantly different relative abundance fol-lowing the increment of replacing rate by biogas slurry for chemical N fertilizer.

Figure S2. Contribution (r2) of plant (A and B) and soil (C and D) variables to bacterial community composition and functional structure.

Table S1. Significantly increased taxa by relative abundance group in 100% chemical N fertilizer, biogas slurry replacing chemical N fertilizer at 50% (CBS) and 100% (BS) soils.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Taxa** | Abundance group, % | Number of taxa (relative abundance, %) | CK to CF  | CF to CBS | CF to BS |
| CK | CF | CBS | BS | Increase | CK | CF | Increase | CF | CBS | Increase | CF | BS |
| **Phylum** | >1 | 12(97.9) | 12(97.8) | 10(95.7) | 11(96.8) | 2(8.40) | 23.4b | 31.8a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.1-1 | 4(1.8) | 8(2.1) | 8(4.1) | 5(2.9) | 2(0.15) | 0.17b | 0.32a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.01-0.1 | 7(0.3) | 3(0.1) | 5(0.2) | 7(0.3) | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a |
|  | <0.01 | 11(0.0) | 11(0.0) | 11(0.0) | 11(0.0) | 1(0.003) | 0.001b | 0.004a | 0(0.00) | 0.00b | 0.00b | 0(0.00) | 0.00b | 0.00b |
|  | **Sum** | **34(100)** | **34(100)** | **34(100)** | **34(100)** | **5(8.55)** | **23.6b** | **32.1a** | **0(0.00)** | **0.00c** | **0.00c** | **0(0.00)** | **0.00c** | **0.00c** |
| **Class** | >1 | 16(92.6) | 16(91.9) | 15(91.3) | 16(92.5) | 3(6.82) | 17.5b | 24.3a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.1-1 | 16(6.6) | 17(7.4) | 18(8.1) | 17(6.8) | 3(0.94) | 1.19b | 2.13a | 1(0.17) | 0.56c | 0.73c | 1(0.05) | 0.08d | 0.13d |
|  | 0.01-0.1 | 16(0.7) | 16(0.6) | 14(0.6) | 14(0.6) | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c | 2(0.04) | 0.09b | 0.13a |
|  | <0.01 | 21(0.1) | 21(0.1) | 21(0.1) | 23(0.1) | 2(0.006) | 0.001b | 0.01a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | **Sum** | **69(100)** | **70(100)** | **68(100)** | **70(100)** | **8(7.76)** | **18.7b** | **26.4a** | **1(0.17)** | **0.56a** | **0.73a** | **3(0.09)** | **0.17a** | **0.26a** |
| **Order** | >1 | 17(92.1) | 17(91.1) | 15(90.0) | 17(92.4) | 3(3.38) | 4.69b | 8.07a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.1-1 | 21(7.3) | 20(8.1) | 24(9.4) | 20(6.8) | 5(1.10) | 1.41b | 2.51a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.01-0.1 | 18(0.6) | 19(0.7) | 16(0.5) | 19(0.7) | 1(0.02) | 0.05c | 0.06b | 1(0.02) | 0.06b | 0.08a | 0(0.00) | 0.00d | 0.00d |
|  | <0.01 | 29(0.1) | 31(0.1) | 29(0.1) | 31(0.1) | 1(0.003) | 0.001b | 0.004a | 0(0.00) | 0.00b | 0.00b | 0(0.00) | 0.00b | 0.00b |
|  | **Sum** | **85(100)** | **87(100)** | **84(100)** | **87(100)** | **11(4.50)** | **6.2b** | **10.6a** | **1(0.02)** | **0.06c** | **0.08c** | **0(0.00)** | **0.00c** | **0.00c** |
| **Family** | >1 | 12(83.9) | 12(82.8) | 9(82.0) | 11(84.4) | 2(2.15) | 2.4b | 4.5a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.1-1 | 48(13.5) | 49(14.7) | 51(15.5) | 43(12.7) | 11(1.82) | 3.2b | 5.0a | 0(0.00) | 0.00c | 0.00c | 0(0.00) | 0.00c | 0.00c |
|  | 0.01-0.1 | 62(2.3) | 59(2.2) | 57(2.2) | 67(2.7) | 2(0.03) | 0.05c | 0.08c | 1(0.02) | 0.04c | 0.06c | 2(0.14) | 0.62b | 0.76a |
|  | <0.01 | 86(0.3) | 91(0.3) | 93(0.3) | 90(0.3) | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a |
|  | **Sum** | **208(100)** | **211(100)** | **210(100)** | **211(100)** | **15(4.00)** | **5.6b** | **9.6a** | **1(0.02)** | **0.04d** | **0.06d** | **2(0.06)** | **0.62c** | **0.76c** |
| **Genus** | >1 | 13(79.9) | 11(76.4) | 8(75.1) | 10(78.1) | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a | 0(0.00) | 0.00a | 0.00a |
|  | 0.1-1 | 52(13.2) | 64(17.4) | 61(18.5) | 53(15.3) | 16(1.77) | 2.7b | 4.5a | 1(0.17) | 0.54e | 0.71de | 5(0.49) | 0.91c | 1.40c |
|  | 0.01-0.1 | 171(5.7) | 169(5.1) | 150(5.2) | 157(5.4) | 15(0.31) | 0.32b | 0.63a | 2(0.07) | 0.09e | 0.16d | 4(0.10) | 0.13de | 0.23c |
|  | <0.01 | 406(1.2) | 396(1.1) | 432(1.3) | 432(1.2) | 5(0.011) | 0.004c | 0.015b | 0(0.00) | 0.00c | 0.00c | 6(0.02) | 0.01b | 0.03a |
|  | **Sum** | **642(100)** | **640(100)** | **651(100)** | **652(100)** | **36(2.09)** | **3.08b** | **5.17a** | **3(0.15)** | **0.63e** | **0.86de** | **15(0.62)** | **1.05d** | **1.67c** |

Different letters following relative abundance indicate significant difference in the same row (*p* < 0.05); The “Increase” column were the relative abundance difference between CF VS CK, CBS VS CF, and BS VS CF, respectively; CK, control, no chemical fertilizer and biogas slurry; CF, 100% chemical nitrogen (N) fertilizer; CBS and BS, biogas slurry replacing 50% and 100% of chemical N fertilizer, respectively.

Table S2. Effects of the replacement of biogas slurry for chemical N fertilizer on soil bacterial functional groups related to N, C and S cycle.

|  |  |
| --- | --- |
| Functional group | Linear mixed effect model |
| F | P | R2 |
| Aerobic ammonia oxidation | 2.30 | 0.177 | 0.464 |
| **Aerobic nitrite oxidation** | **42.7** | **0.000** | **0.932** |
| Anammox | 0.38 | 0.770 | 0.460 |
| Denitrification | 4.00 | 0.052 | 0.600 |
| Nitrate ammonification | 0.41 | 0.748 | 0.134 |
| Nitrate denitrification | 2.91 | 0.101 | 0.522 |
| **Nitrate reduction** | **6.54** | **0.015** | **0.710** |
| **Nitrate respiration** | **4.89** | **0.047** | **0.644** |
| Nitrification | 2.41 | 0.166 | 0.480 |
| Nitrite ammonification | 0.37 | 0.780 | 0.120 |
| Nitrite denitrification | 4.00 | 0.052 | 0.600 |
| **Nitrite respiration** | **4.52** | **0.039** | **0.629** |
| **Nitrogen fixation** | **16.2** | **0.003** | **0.851** |
| **Nitrogen respiration** | **6.20** | **0.029** | **0.695** |
| Nitrous oxide denitrification | 4.00 | 0.052 | 0.600 |
| Ureolysis | 2.60 | 0.147 | 0.494 |
| Aerobic anoxygenic phototrophy | 3.28 | 0.080 | 0.551 |
| Aerobic chemoheterotrophy | 3.33 | 0.077 | 0.556 |
| Aliphatic non methane hydrocarbon degradation | 0.48 | 0.708 | 0.151 |
| Anoxygenic photoautotrophy | 2.55 | 0.129 | 0.489 |
| Anoxygenic photoautotrophy H2 oxidizing | 1.09 | 0.409 | 0.290 |
| Anoxygenic photoautotrophy S oxidizing | 2.26 | 0.159 | 0.458 |
| Aromatic compound degradation | 0.47 | 0.713 | 0.182 |
| Aromatic hydrocarbon degradation | 0.17 | 0.914 | 0.072 |
| **Cellulolysis** | **4.54** | **0.039** | **0.630** |
| **Chemoheterotrophy** | **5.31** | **0.026** | **0.666** |
| Fermentation | 0.41 | 0.753 | 0.132 |
| **Fumarate respiration** | **6.45** | **0.016** | **0.707** |
| Hydrocarbon degradation | 0.72 | 0.574 | 0.251 |
| **Hydrogenotrophic methanogenesis** | **4.51** | **0.039** | **0.629** |
| **Ligninolysis** | **4.44** | **0.041** | **0.625** |
| **Methanogenesis** | **4.46** | **0.040** | **0.626** |
| Methanogenesis by CO2 reduction with H2 | 0.99 | 0.458 | 0.352 |
| **Methanogenesis by reduction of methyl compounds with H2** | **4.50** | **0.040** | **0.628** |
| Methanol oxidation | 0.94 | 0.466 | 0.260 |
| Methanotrophy | 0.19 | 0.899 | 0.071 |
| Methylotrophy | 0.94 | 0.479 | 0.264 |
| Photoautotrophy | 2.55 | 0.129 | 0.489 |
| Photoheterotrophy | 2.95 | 0.098 | 0.525 |
| Phototrophy | 2.42 | 0.141 | 0.476 |
| Reductive acetogenesis | 0.70 | 0.576 | 0.209 |
| Xylanolysis | 3.98 | 0.053 | 0.599 |
| Chitinolysis | 3.57 | 0.066 | 0.573 |
| Dark oxidation of sulfur compounds | 2.37 | 0.146 | 0.471 |
| Dark sulfide oxidation | 1.83 | 0.243 | 0.423 |
| Dark sulfur oxidation | 2.76 | 0.134 | 0.510 |
| Dark thiosulfate oxidation | 2.62 | 0.123 | 0.496 |
| **Respiration of sulfur compounds** | **13.7** | **0.002** | **0.837** |
| **Sulfate respiration** | **12.6** | **0.002** | **0.825** |
| Sulfite respiration | 3.04 | 0.093 | 0.533 |
| Sulfur respiration | 0.58 | 0.648 | 0.244 |
| Thiosulfate respiration | 0.90 | 0.481 | 0.253 |