

Supplementary Materials

Table S1. Depth distribution of soil enzyme activities expressed per TOC unit; mean (\pm SE), n = 5.

| Genetic horizon | Depth | *UR/TOC | *NR/TOC | *PRO/TOC | **NAG/TOC |
|-------------------------------------|---------|-------------------|------------------|-------------------|------------------|
| Profile 1. Haplic Luvisol (Cutanic) | | | | | |
| Ap | 0-30 | 608 \pm 33 D | 30.5 \pm 0.8 A | 1320 \pm 40 D | 25.3 \pm 0.5 C |
| E1 | 30-55 | 793 \pm 36 C | 17.2 \pm 0.7 C | 4262 \pm 105 B | 62.9 \pm 1.4 A |
| E2 | 55-82 | 2179 \pm 76 A | 22.9 \pm 1.1 B | 7335 \pm 340 A | 42.8 \pm 1.9 B |
| Bt | 82-144 | 2021 \pm 100 A | 15.0 \pm 1.0 C | 4772 \pm 297 B | 12.1 \pm 0.7 D |
| BC | 144-150 | 1589 \pm 55 B | 12.9 \pm 1.1 C | 2145 \pm 123 C | 5.1 \pm 0.4 E |
| Profile 2. Mollic Stagnic Gleysol | | | | | |
| Ap | 0-30 | 2479 \pm 51 A | 81.4 \pm 3.5 A | 1765 \pm 107 C | 12.1 \pm 0.4 A |
| A2 | 30-53 | 1329 \pm 72 B | 5.6 \pm 0.5 C | 2377 \pm 118 B | 8.3 \pm 1.0 B |
| 2ACgg | 53-70 | 982 \pm 86 C | 48.9 \pm 2.6 B | 6669 \pm 230 A | 6.9 \pm 0.1 BC |
| 3G1 | 70-110 | 759 \pm 30 D | 7.4 \pm 0.4 C | 1463 \pm 52 C | 7.1 \pm 0.1 B |
| 3G2 | 110-150 | 376 \pm 35 E | 6.4 \pm 0.1 C | 1577 \pm 82 C | 5.1 \pm 0.6 CD |
| Profile 3. Haplic Luvisol (Cutanic) | | | | | |
| Ap | 0-32 | 436 \pm 30 B | 58.4 \pm 24 A | 2767 \pm 108 D | 53.3 \pm 2.1 A |
| E | 32-46 | 363 \pm 29 B | 10.6 \pm 0.2 B | 3153 \pm 127 C | 44.7 \pm 1.5 B |
| EB | 46-58 | 1401 \pm 17 A | 5.5 \pm 0.5 C | 8426 \pm 86 A | 46.0 \pm 0.8 B |
| Bt | 58-135 | 263 \pm 11 C | 2.5 \pm 0.4 D | 6956 \pm 122 B | 59.5 \pm 1.1 A |
| Ck | 135-150 | 192 \pm 41 D | 2.4 \pm 0.4 D | 2376 \pm 50 D | 23.8 \pm 0.7 C |
| Profile 4. Cambic Stagnic Phaeozem | | | | | |
| Ap | 0-35 | 3456 \pm 87 A | 200 \pm 4.3 A | 3204 \pm 109 CB | 24.5 \pm 0.5 B |
| BCKg | 35-67 | 1082 \pm 31 D | 38.7 \pm 1.5 C | 8818 \pm 74 B | 12.7 \pm 0.7 C |
| Gk | 67-90 | 1406 \pm 145 CD | 34.2 \pm 0.9 C | 3101 \pm 428 C | 12.1 \pm 0.2 C |
| 2Gk1 | 90-123 | 1805 \pm 68 C | 59.3 \pm 4.6 B | 10393 \pm 328 A | 14.9 \pm 0.5 C |
| 2Gk2 | 123-150 | 2833 \pm 254 B | 32.9 \pm 2.6 C | 32848 \pm 392 C | 55.4 \pm 1.8 A |

* mg substrate kg⁻¹ TOC h⁻¹, ** mM substrate kg⁻¹ TOC h⁻¹, UR – urease, NR – nitrate reductase, PRO – proteases, NAG - N-acetyl-glucosaminidase. The uppercase letters indicate significant differences (P< 0.05) between genetic horizons within the same soil profile.

Table S2. Depth distribution of soil enzyme activities expressed per MBC unit; mean (\pm SE), n = 5.

| Genetic horizon | Depth | *UR/MBC | *NR/MBC | *PRO/MBC | **NAG/MBC |
|-------------------------------------|---------|--------------------|--------------------|--------------------|-------------------|
| Profile 1. Haplic Luvisol (Cutanic) | | | | | |
| Ap | 0-30 | 50.8 \pm 2.15 B | 2.55 \pm 0.08 A | 110.6 \pm 6.0 E | 2.11 \pm 0.07 B |
| Eet1 | 30-55 | 73.9 \pm 1.11 B | 0.97 \pm 0.06 C | 236.8 \pm 10.9 C | 3.49 \pm 0.05 A |
| Eet2 | 55-82 | 124.3 \pm 5.32 A | 1.31 \pm 0.07 B | 418.4 \pm 20.9 A | 2.44 \pm 0.13 B |
| Bt | 82-144 | 140.6 \pm 6.03 A | 1.04 \pm 0.06 C | 333.1 \pm 27.1 B | 0.84 \pm 0.07 C |
| BC | 144-150 | 122.8 \pm 6.70 A | 1.00 \pm 0.07 C | 165.1 \pm 6.1 D | 0.39 \pm 0.02 D |
| Profile 2. Mollic Stagnic Gleysol | | | | | |
| Ap | 0-30 | 288.9 \pm 11.4 A | 9.52 \pm 0.57 A | 206.7 \pm 11.4 B | 1.41 \pm 0.07 A |
| A2 | 30-53 | 110.5 \pm 0.46 B | 0.46 \pm 0.04 C | 197.4 \pm 8.82 B | 0.69 \pm 0.09 B |
| 2ACgg | 53-70 | 71.7 \pm 5.08 C | 3.52 \pm 1.61 B | 488.4 \pm 12.6 A | 0.50 \pm 0.01 B |
| 3G1 | 70-110 | 67.5 \pm 1.57 C | 0.67 \pm 0.05 C | 130.3 \pm 3.0 C | 0.63 \pm 0.02 B |
| 3G2 | 110-150 | 46.2 \pm 4.96 C | 0.79 \pm 0.03 C | 194.1 \pm 2.7 B | 0.64 \pm 0.11 B |
| Profile 3. Haplic Luvisol (Cutanic) | | | | | |
| Ap | 0-32 | 38.4 \pm 18.9 B | 51.5 \pm 0.52 A | 244.3 \pm 8.0 C | 4.70 \pm 0.05 A |
| E | 32-46 | 19.5 \pm 1.04 C | 0.57 \pm 0.03 B | 177.9 \pm 82.4 D | 2.40 \pm 0.08 D |
| EB | 46-58 | 78.3 \pm 4.17 A | 0.31 \pm 0.04 C | 470.5 \pm 18.7A | 2.57 \pm 0.09 D |
| Bt | 58-135 | 15.4 \pm 0.38 C | 0.15 \pm 0.02 B | 419.9 \pm 22.9 B | 3.58 \pm 0.12 C |
| Ck | 135-150 | 32.6 \pm 5.56 B | 0.40 \pm 0.05 C | 409.3 \pm 17.6 B | 4.10 \pm 0.16 B |
| Profile 4. Cambic Stagnic Phaeozem | | | | | |
| Ap | 0-35 | 220.5 \pm 57.1 A | 12.7 \pm 0.36 A | 203.1 \pm 7.2 A | 1.56 \pm 0.04 A |
| BCkg | 35-67 | 31.3 \pm 10.7 B | 1.09 \pm 0.07 B | 239.7 \pm 13.3 A | 0.36 \pm 0.20 B |
| Gk | 67-90 | 36.1 \pm 3.99 B | 0.88 \pm 0.03 BC | 79.7 \pm 11.7 B | 0.31 \pm 0.01 B |
| 2Gk1 | 90-123 | 16.9 \pm 1.41 C | 0.55 \pm 0.02 C | 96.8 \pm 2.7 B | 0.14 \pm 0.01 C |
| 2Gk2 | 123-150 | 19.5 \pm 2.04 C | 0.23 \pm 0.03 D | 223.9 \pm 21.1 A | 0.38 \pm 0.02 B |

* mg substrate kg⁻¹ MBC h⁻¹, ** mM substrate kg⁻¹ MBC h⁻¹, UR – urease, NR – nitrate reductase, PRO – proteases, NAG - N-acetyl-glucosaminidase. The uppercase letters indicate significant differences (P< 0.05) between genetic horizons within the same soil profile.

Table S3. Root length and surface in the studied profiles (the average values and selected intervals).

| Horizons | Root mass (g dm ⁻³) | Root length (mm dm ⁻³) | | | | | Root surface (mm ² dm ⁻³) | | | | |
|------------------------------------|------------------------------------|------------------------------------|-------------|-----------|-----------|---------|--|----------|-----------|-----------|-----------|
| | | all | ^Ø < 0.5 | Ø 0.5-2,5 | Ø 2.5-5.0 | Ø > 5 | all | Ø < 0.5 | Ø 0.5-2,5 | Ø 2.5-5.0 | Ø > 5 |
| Profile 1. Haplic Luvisol | | | | | | | | | | | |
| Ap | 5.14 | 5768 ± 630 | 4756 ± 523 | 941 ± 104 | 44 ± 4 | 27 ± 1 | 788 ± 82 | 299 ± 39 | 266 ± 20 | 77 ± 8 | 146 ± 56 |
| E1 | 1.59 | 424 ± 35 | 282 ± 26 | 87.0 ± 8 | 45 ± 4 | 11 ± 3 | 117 ± 10 | 23 ± 2 | 21 ± 1 | 25 ± 2 | 49 ± 7 |
| E2 | 1.11 | 474 ± 21 | 320 ± 14 | 133 ± 6 | 19 ± 2 | 3 ± 0.4 | 100 ± 11 | 28 ± 2 | 41 ± 6 | 27 ± 3 | 5 ± 1 |
| Bt | 1.14 | 396 ± 31 | 213 ± 23 | 138 ± 15 | 32 ± 3 | 14 ± 2 | 105 ± 8 | 19 ± 2 | 42 ± 2 | 41 ± 3 | 3 ± 1 |
| BC | 0.10 | 132 ± 13 | 91.8 ± 11.7 | 35 ± 4 | – | 6 ± 1 | 19 ± 1 | 8 ± 1 | 10 ± 1 | – | 1.5 ± 0.4 |
| Profile 2. Mollic Stagnic Gleysol | | | | | | | | | | | |
| Ap | 14.22 | 3138 ± 246 | 2264 ± 170 | 780 ± 59 | 39 ± 5 | 54 ± 14 | 688 ± 75 | 163 ± 12 | 228 ± 32 | 3 ± 3 | 266 ± 91 |
| A2 | 1.20 | 180 ± 14 | 91 ± 9 | 57 ± 5 | 21 ± 2 | 12 ± 1 | 78 ± 7 | 7 ± 1 | 21 ± 1 | 24 ± 2 | 26 ± 6 |
| 2ACgg | 0.74 | 215 ± 12 | 112 ± 6 | 80 ± 4 | 23 ± 2 | 1 ± 0.2 | 64 ± 7 | 10 ± 1 | 24 ± 2 | 19 ± 2 | 11 ± 6 |
| 3G1 | 0.20 | 249 ± 18 | 164 ± 17.0 | 73 ± 8 | 1.4 ± 0 | 12 ± 7 | 42 ± 2 | 12 ± 2 | 22 ± 2 | 7 ± 1 | 2 ± 0.4 |
| 3G2 | 0.04 | 83 ± 10 | 52 ± 6 | 29 ± 3 | – | 2 ± 1 | 12 ± 2 | 5. ± 1 | 6 ± 1 | – | 0.4 ± 0 |
| Profile 3. Haplic Luvisol | | | | | | | | | | | |
| Ap | 2.1 | 1259 ± 286 | 1047 ± 234 | 176 ± 46 | 17 ± 4 | 19 ± 8 | 129 ± 31 | 68 ± 15 | 42 ± 12 | 2.4 ± 1 | 16 ± 8 |
| E | 0.5 | 811 ± 40 | 665 ± 33 | 121 ± 6 | 19 ± 1 | 6 ± 2 | 91 ± 7 | 48 ± 5 | 30 ± 4 | 0.3 ± 0.1 | 13 ± 6 |
| EB | 0.8 | 843 ± 64 | 660 ± 55 | 131 ± 11 | 23 ± 2 | 29 ± 5 | 88 ± 8 | 42 ± 4 | 33 ± 3 | 0.6 ± 0.1 | 12 ± 7 |
| Bt | 1.0 | 932 ± 113 | 817 ± 120 | 103 ± 15 | 1.4 ± 0.2 | 11 ± 2 | 85 ± 15 | 52 ± 8 | 20 ± 2 | 0.5 ± 0.1 | 12 ± 7 |
| Ck | 0.4 | 422 ± 35 | 389 ± 41 | 21 ± 2 | – | 12 ± 3 | 33 ± 3 | 24 ± 2 | 4 ± 0.4 | – | 5 ± 1 |
| Profile 4. Cambic Stagnic Phaeozem | | | | | | | | | | | |
| Ap | 1.9 | 2679 ± 404 | 2220 ± 339 | 442 ± 68 | 10 ± 2 | 7 ± 2 | 291 ± 44 | 143 ± 23 | 103 ± 10 | 10 ± 1 | 35 ± 14 |
| BCg | 1.0 | 1749 ± 151 | 1539 ± 146 | 187 ± 18 | 0.2 ± 0.0 | 22 ± 10 | 174 ± 22 | 115 ± 12 | 40 ± 4 | 0.2 ± 0 | 24 ± 12 |
| Gk | 1.0 | 1278 ± 178 | 1092 ± 168 | 164 ± 25 | 0.6 ± 0.1 | 22 ± 12 | 141 ± 20 | 84 ± 12 | 35 ± 4 | 0.5 ± 0.1 | 21 ± 11 |
| 2Gk1 | 0.4 | 362 ± 23 | 306 ± 37 | 35 ± 4 | – | 22 ± 9 | 36 ± 3 | 23 ± 3 | 6.9 ± 0.5 | – | 6 ± 1 |
| 2Gk2 | 0.3 | 207 ± 22 | 171 ± 23 | 30 ± 4 | – | 7 ± 4 | 22 ± 1 | 12 ± 1 | 6.0 ± 0.5 | – | 3 ± 0.5 |

^ roots diameter. * no roots were found

Table S4. Correlation matrix between the studied properties (n = 100).

| Enzyme | Horizons | TOC | TN | MBC | MBN | Clay | pH in KCl | CEC | NO ₃ ⁻ | NH ₄ ⁺ |
|--------|---------------|-------|-------|-------|-------|--------|--------------|-------|------------------------------|------------------------------|
| UR | *Surface | 0.829 | 0.821 | 0.812 | 0.843 | – | 0.731 | 0.825 | – | – |
| | **Sub-surface | ^– | – | 0.364 | 0.407 | – | – | 0.393 | 0.326 | 0.324 |
| | All | 0.835 | 0.851 | 0.818 | 0.842 | – | – | – | – | 0.676 |
| NR | Surface | – | – | – | – | – | 0.559 | – | 0.938 | 0.617 |
| | Sub-surface | 0.476 | – | 0.873 | 0.880 | 0.372 | 0.374 | 0.802 | 0.596 | 0.636 |
| | All | 0.657 | 0.692 | 0.627 | 0.625 | – | – | – | – | 0.764 |
| PRO | Surface | 0.674 | 0.723 | 0.857 | 0.852 | – | 0.782 | 0.927 | – | – |
| | Sub-surface | 0.369 | – | 0.575 | 0.731 | – | – | 0.308 | – | 0.341 |
| | All | 0.820 | 0.819 | 0.885 | 0.907 | – | – | – | – | 0.711 |
| NAG | Surface | 0.536 | 0.643 | 0.595 | 0.572 | –0.658 | – | – | 0.847 | 0.842 |
| | Sub-surface | 0.534 | 0.352 | – | – | – | –0.498 | – | – | 0.621 |
| | All | 0.767 | 0.800 | 0.718 | 0.730 | –0.332 | – | – | – | 0.895 |

p < 0.05; * - Ap horizons; ** - all horizons below the Ap horizon; ^ – not significant; UR—urease; NR—nitrate reductase; PRO—proteases; NAG— N-acetyl-glucosaminidase; TOC— total organic carbon; NT—total nitrogen; MBC—microbial biomass carbon, ; MBN—microbial biomass nitrogen; CEC—cation exchange capacity; N-NO₃⁻ nitrate nitrogen; N-NH₄⁺ - ammonium nitrogen; Units of properties are those given under previous tables.

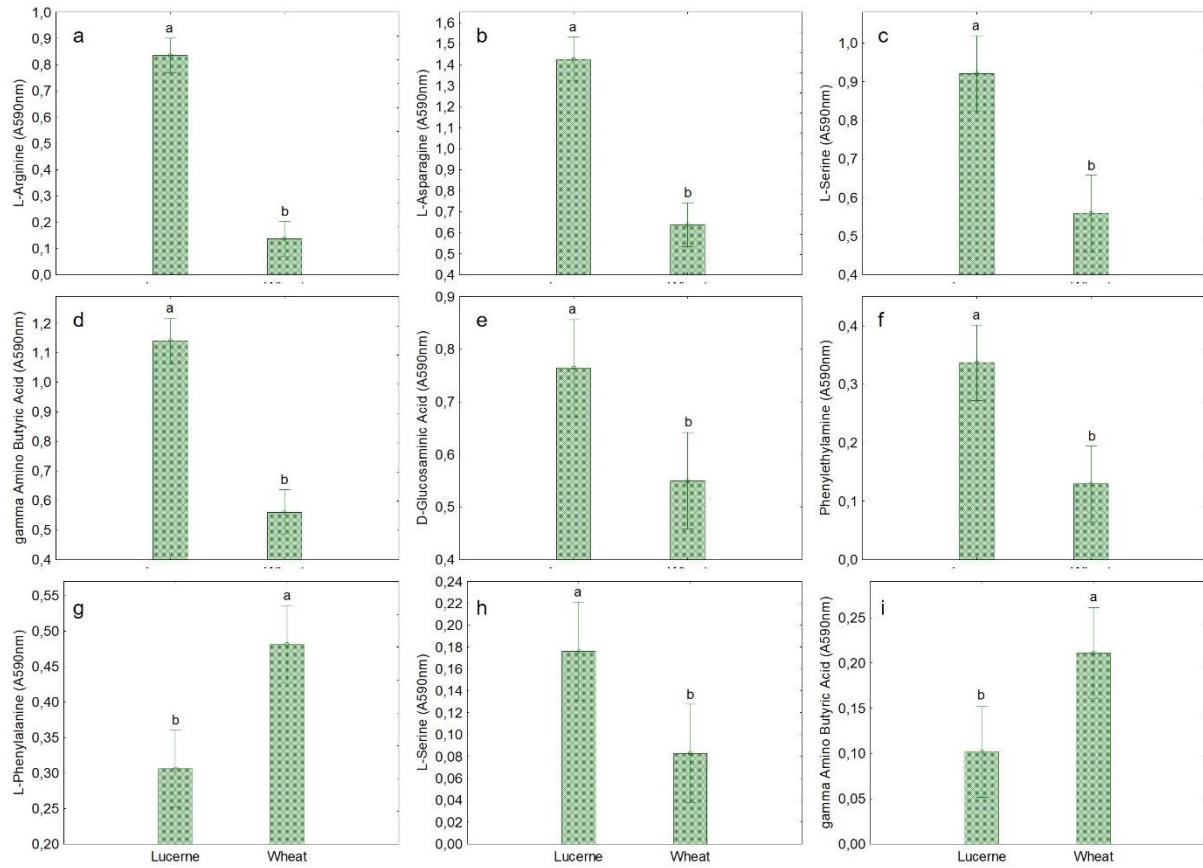


Figure S1. Capacity of the soil microbial community to use different N-substrates in aerobic (a – L-Arginine, b – L-Asparagine, c – L-Serine, d – gamma Amino Butyric Acid, e – D-Glucosaminic Acid, f – Phenylethylamine, g – L-Phenylalanine) and anaerobic (h – L-Serine, i – gamma Amino Butyric Acid) conditions under lucerne and wheat cultivation. Displayed are the means of the three replicates from all tested depths of soil; error bars show the 95% confidence intervals. Different letters above bars indicate significant differences between the tested plants ($p < 0.05$).

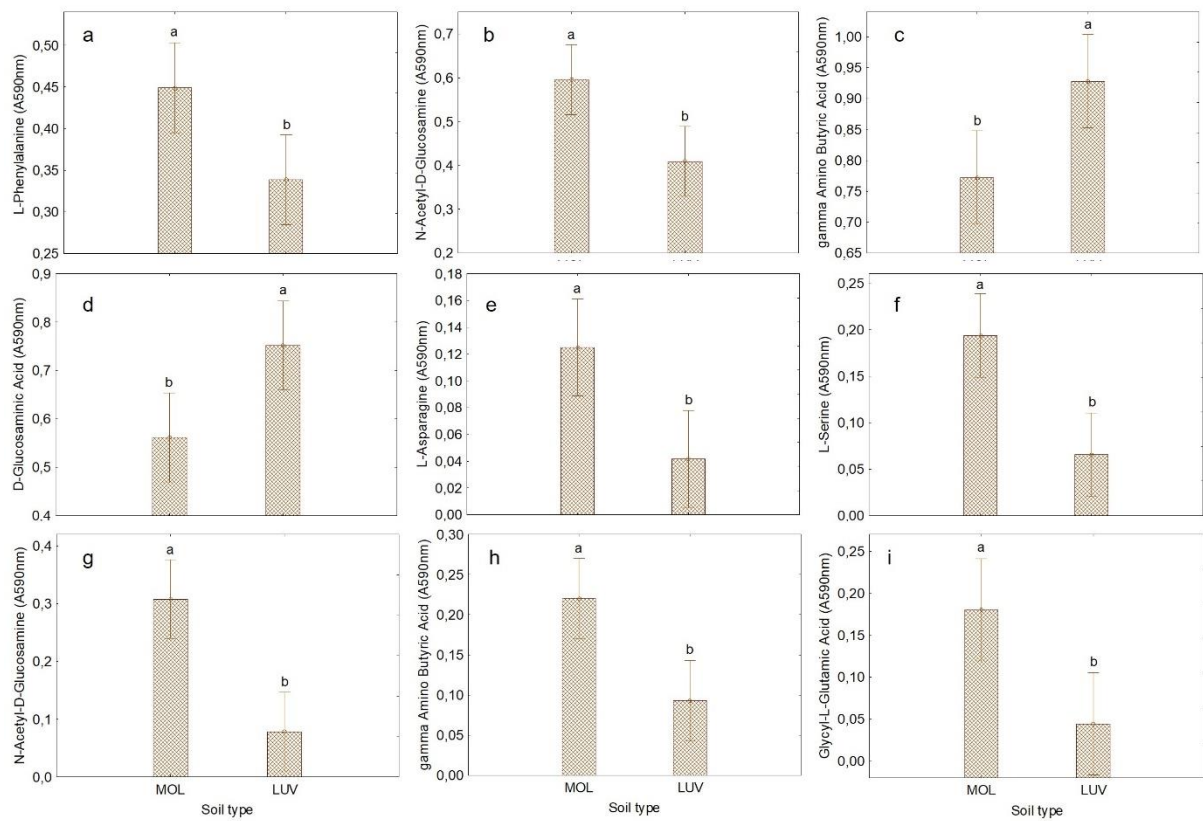


Figure S2. Capacity of the soil microbial community to use different N-substrates in aerobic (a – L-Phenylalanine, b – N-Acetyl-D-Glucosamine, c – gamma Amino Butyric Acid, d – D-Glucosaminic Acid) and anaerobic (e – L-Asparagine, f – L-Serine, g – N-Acetyl-D-Glucosamine, h – gamma Amino Butyric Acid, i – Glycyl-L-Glutamic Acid) conditions in soils with mollic layers – Mollic Staginc Gleysol and Cambic Stagnic Phaeozem (MOL) and Haplic Luvisol (LUV). Displayed are the means of the three replicates from all tested depths of soil; error bars show the 95% confidence intervals. Different letters above bars indicate significant differences between the tested soil types ($p < 0.05$).