# Supporting Information

# Appearance of β-lactam Resistance Genes in Agricultural Soils and Clinical Isolates over the 20th Century

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Content: Five tables and one figure.

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**Table S1.** qPCR quantification of mean 16S-rRNA, broad spectrum β-lactam resistance, and integron integrase genes (log abundances/g dry-weight soil) detected in archived soils from fields only provided inorganic fertilisers (IF) since 1894. Means of duplicates are reported. Measured duplicate samples were always < 0.5 log of each other.

| **Year** | 16S-rRNA | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 |
|---|---|---|---|---|---|---|
| 1923 | 9.44 | 5.00 | 3.99 | 4.68 | 4.77 | 3.78 |
| 1938 | 9.50 | 5.07 | 4.42 | 3.69 | 4.71 | 4.29 |
| 1945 | 9.49 | 4.91 | 4.10 | 3.98 | 3.37 | 3.80 |
| 1953 | 9.58 | 4.99 | 3.96 | 4.13 | 4.14 | 3.84 |
| 1964 | 9.74 | 4.66 | 3.41 | 3.97 | 3.43 | 4.11 |
| 1976 | 9.80 | 4.74 | 3.31 | 4.10 | 5.05 | 3.90 |
| 1985 | 9.40 | 4.34 | 4.16 | 3.67 | 4.66 | 3.74 |
| 1988 | 9.80 | 4.21 | 3.67 | 3.61 | 4.48 | 3.91 |
| 1992 | 9.89 | 5.21 | 3.59 | 3.69 | 5.67 | 3.80 |
| 1996 | 9.72 | 4.86 | 3.44 | 4.19 | 5.05 | 4.34 |
| 2000 | 10.0 | 5.00 | 3.10 | 4.36 | 5.27 | 4.60 |
| 2010 | 9.81 | 5.07 | 4.47 | 4.51 | 4.66 | 4.56 |
| **Mean** | **9.68** | **4.84** | **3.80** | **4.05** | **4.60** | **4.05** |
| **SD** | **0.20** | **0.30** | **0.45** | **0.35** | **0.68** | **0.32** |
| **95% CI** | **0.11** | **0.16** | **0.24** | **0.19** | **0.37** | **0.17** |

**Table S2.** qPCR quantification of mean 16S-rRNA, broad spectrum β-lactam resistance, and integron intergase genes (log abundances/g dry weight soil) detected in archived soils from fields provided only manure (M) since 1894. Means of duplicates are reported. Measured duplicate samples were always < 0.5 log of each other.

| **Year** | 16S-rRNA | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 |
|---|---|---|---|---|---|---|
| 1923 | 9.44 | 5.24 | 4.20 | 4.55 | 4.57 | 3.80 |
| 1938 | 9.62 | 5.04 | 4.46 | 4.4 | 4.38 | 4.01 |
| 1945 | 9.54 | 4.91 | 4.23 | 4.26 | 4.25 | 4.03 |
| 1953 | 9.63 | 4.88 | 4.11 | 4.34 | 4.67 | 4.05 |
| 1964 | 9.62 | 5.05 | 3.98 | 3.90 | 4.52 | 4.21 |
| 1976 | 9.14 | 5.10 | 4.63 | 4.83 | 4.32 | 4.25 |
| 1985 | 9.21 | 5.06 | 4.34 | 4.53 | 4.28 | 4.21 |
| 1988 | 10.4 | 5.55 | 4.24 | 5.20 | 6.65 | N/A[a] |
| 1992 | 9.92 | 5.21 | 4.13 | 4.20 | 5.91 | 4.84 |
| 1996 | 9.40 | 4.94 | 3.74 | 4.00 | 5.25 | 4.73 |
| 2000 | 9.56 | 5.14 | 4.22 | 4.27 | 5.17 | 5.00 |
| 2010 | 9.76 | 5.10 | 3.97 | 4.90 | 4.60 | 5.26 |
| **Mean** | **9.60** | **5.10** | **4.19** | **4.45** | **4.88** | **4.41** |
| **SD** | **0.32** | **0.18** | **0.23** | **0.38** | **0.74** | **0.46** |
| **95% CI** | **0.18** | **0.10** | **0.13** | **0.21** | **0.40** | **0.25** |

Notes: [a] N/A = Not available.

**Table S3.** Metal and nutrient concentrations in archived soils from fields provided inorganic fertilisers (IF) since 1894. Nutrient levels were determined at the time of historic sampling, whereas heavy metals were analysed on archived soils during this study.

| **Year** | As (ppm)[a] | Cd (ppm) | Co (ppm) | Cr (ppm) | Cu (ppm) | Hg (ppb) | Ni (ppm) | Pb (ppm) | Zn (ppm) | TC (g C/100 g) | TN (g N/100 g) | Ext P (mg P/100 g) | Ext K (mg K/100 g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1923 | 3.3 | 0.15 | 2.6 | 6.2 | 5.76 | 43 | 3.6 | 12.1 | 17.3 | 0.118 | 1.44 | 6.3 | 2.4 |
| 1938 | 4.0 | 0.16 | 2.4 | 5.9 | 4.20 | 63 | 3.2 | 12.3 | 15.8 | 0.111 | 1.49 | 3.9 | 4.3 |
| 1945 | 3.9 | 0.17 | 2.3 | 6.2 | 4.09 | 66 | 3.2 | 12.8 | 16.9 | 0.117 | 1.36 | 7.8 | 5.2 |
| 1953 | 3.9 | 0.19 | 2.6 | 5.8 | 3.67 | 60 | 2.9 | 12.5 | 15.7 | 0.115 | 1.36 | 12.3 | 3.6 |
| 1964 | 4.3 | 0.19 | 2.5 | 6.4 | 4.04 | 107 | 3.4 | 12.2 | 21.6 | 0.125 | 1.38 | 13.2 | 5.3 |
| 1976 | 4.0 | 0.20 | 2.9 | 6.7 | 4.18 | 74 | 3.6 | 13.1 | 21.0 | 0.108 | 1.46 | 15.6 | 8.3 |
| 1985 | 4.1 | 0.20 | 3.2 | 12.7 | 3.85 | 61 | 6.1 | 12.7 | 20.0 | 0.106 | 1.25 | 17.4 | 5.9 |
| 1996 | 3.9 | 0.23 | 2.5 | 9.5 | 4.31 | 73 | 4.9 | 14.2 | 23.7 | 0.12 | 1.31 | 16.2 | 6.9 |
| 2000 | 3.6 | 0.20 | 2.9 | 14.8 | 4.03 | 49 | 7.4 | 12.3 | 20.0 | 0.102 | 1.24 | N/A[b] | 5.4 |
| 2010 | 4.2 | 0.19 | 3.1 | 8.0 | 4.63 | 56 | 3.7 | 13.6 | 24.2 | 0.106 | 1.19 | 20.4 | 7.1 |
| **Mean** | **3.92** | **0.188** | **2.70** | **8.22** | **4.28** | **65.2** | **4.20** | **12.8** | **19.6** | **0.113** | **1.35** | **12.6** | **5.44** |
| **SD** | **0.290** | **0.023** | **0.31** | **3.16** | **0.58** | **17.6** | **1.48** | **0.7** | **3.10** | **0.007** | **0.10** | **5.53** | **1.74** |
| **95% CI** | **0.180** | **0.014** | **0.19** | **1.96** | **0.36** | **10.9** | **0.92** | **0.4** | **1.92** | **0.005** | **0.06** | **3.43** | **1.08** |

Notes: [a] Mass per mass of dry soil

[b] N/A = Not available.

**Table S4.** Metal and nutrient concentrations in archived soils from manure (M) fields since 1894. Nutrient levels were determined at the time of historic sampling, whereas heavy metals were analysed on archived soils during this study.

| **Year** | As (ppm)[a] | Cd (ppm) | Co (ppm) | Cr (ppm) | Cu (ppm) | Hg (ppb) | Ni (ppm) | Pb (ppm) | Zn (ppm) | TC (g C/100 g) | TN (g N/100 g) | Ext P (mg P/100 g) | Ext K (mg K/100 g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1923 | 1.7 | 0.11 | 2.1 | 5.0 | 4.38 | 27 | 2.7 | 9.9 | 13.5 | 0.133 | 1.53 | 4.2 | 1.4 |
| 1938 | 2.4 | 0.14 | 2.5 | 5.6 | 4.54 | 78 | 3.1 | 12.0 | 15.9 | 0.126 | 1.68 | 2.7 | 3 |
| 1945 | 2.9 | 0.14 | 2.4 | 5.6 | 4.18 | 59 | 3.1 | 12.0 | 17.6 | 0.131 | 1.5 | 6 | 5.7 |
| 1953 | 2.7 | 0.16 | 2.4 | 5.9 | 4.41 | 80 | 3.2 | 12.7 | 18.4 | 0.133 | 1.54 | 10.8 | 4.4 |
| 1964 | 2.9 | 0.16 | 2.5 | 6.2 | 4.21 | 130 | 3.2 | 13.3 | 23.6 | 0.128 | 1.6 | 13.8 | 4.2 |
| 1976 | 3.1 | 0.18 | 2.7 | 7.6 | 4.30 | 54 | 4.2 | 12.6 | 21.3 | 0.115 | 1.6 | 14.4 | 6.5 |
| 1985 | 3.4 | 0.19 | 4.2 | 22.5 | 4.63 | 65 | 10.6 | 14.4 | 23.4 | 0.122 | 1.37 | 18.3 | 7.5 |
| 1996 | 3.1 | 0.21 | 3.1 | 15.9 | 6.08 | 57 | 8.3 | 14.0 | 25.1 | 0.135 | 1.42 | 16.2 | 8.1 |
| 2000 | 3.0 | 0.18 | 2.6 | 11.8 | 6.23 | 46 | 5.8 | 12.8 | 22.1 | 0.122 | 1.38 | N/A[b] | 5.5 |
| 2010 | 3.4 | 0.18 | 3.5 | 7.8 | 9.16 | 53 | 4.4 | 13.5 | 28.3 | 0.127 | 1.31 | 19.2 | 7.8 |
| **Mean** | **2.86** | **0.165** | **2.80** | **9.39** | **5.21** | **64.9** | **4.86** | **12.7** | **20.9** | **0.127** | **1.49** | **11.7** | **5.41** |
| **SD** | **0.51** | **0.029** | **0.63** | **5.74** | **1.58** | **27.5** | **2.64** | **1.27** | **4.53** | **0.006** | **0.12** | **6.15** | **2.19** |
| **95% CI** | **0.31** | **0.018** | **0.39** | **3.56** | **0.98** | **17.0** | **1.64** | **0.79** | **2.81** | **0.004** | **0.07** | **3.81** | **1.35** |

Notes: [a] Mass per mass of dry soil

[b] N/A = Not available.

**Figure S1.** Relative mass of selected heavy metal and nutrients in archived soils compared with soils from before 1940 from manure (M) and inorganic fertiliser (IF) fields. Specific metals or nutrients are noted on y-axes and reported as ratios (i.e., mass of metal per date/mean mass of metal in samples from 1923 and 1938 samples).

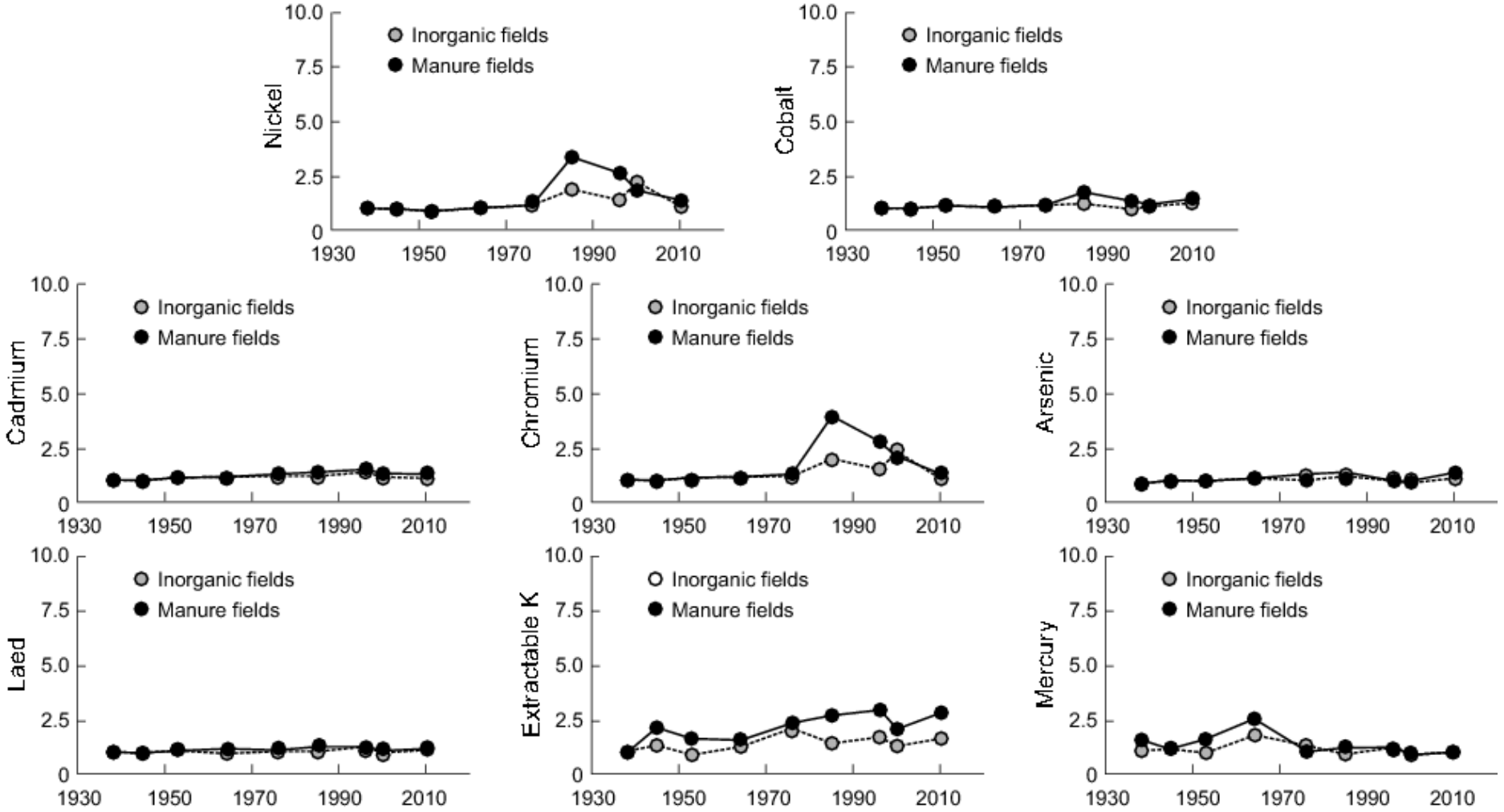

**Table S5.** Spearman's rank correlation coefficents between time, metals, nutrients and genes versus time or specific ARGs or *int*1 genes in archived soils from manure (M) and inorganic (IF) fertilised fields after 1940. Significant correlations are in bold and p-values in brackets.

| | **Inorganic** | | | | | | **Manure** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time[a] | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 | Time | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 |
| Time | - | -0.146 (0.688) | -0.127 (0.726) | 0.018 (0.960) | **0.673**[b] (0.033) | 0.467 (0.174) | - | 0.055 (0.881) | -0.491 (0.150) | -0.012 (0.973) | 0.418 (0.229) | **0.733**[b] (0.016) |
| Mo | 0.665[b] (0.036) | -0.07 (0.847) | 0.238 (0.508) | 0.043 (0.907) | 0.616 (0.058) | 0.427 (0.219) | **0.875**[c] (0.001) | -0.219 (0.544) | -0.669[b] (0.035) | -0.291 (0.415) | 0.474 (0.166) | 0.505 (0.137) |
| Cu | -0.103 (0.777) | 0.438 (0.206) | 0.624 (0.054) | **0.661**[b] (0.038) | -0.455 (0.187) | 0.515 (0.128) | **0.709**[b] (0.022) | -0.055 (0.881) | -0.273 (0.446) | -0.073 (0.841) | 0.103 (0.777) | **0.673**[b] (0.033) |
| Pb | 0.539 (0.108) | -0.292 (0.413) | -0.115 (0.751) | -0.055 (0.881) | 0.164 0.651) | 0.285 (0.425) | **0.806**[c] (0.005) | 0.176 (0.627) | -0.321 (0.365) | -0.110 (0.763) | 0.491 (0.150) | **0.648**[b] (0.043) |
| Zn | **0.736**[b] (0.015) | -0.055 (0.880) | 0.474 (0.166) | 0.109 (0.763) | 0.419 (0.228) | 0.584 (0.077) | **0.879**[c] (0.001) | 0.164 (0.651) | -0.382 (0.276) | 0.104 (0.776) | 0.321 (0.365) | **0.709**[b] (0.022) |
| Ni | **0.726**[b] (0.018) | 0.269 (0.452) | -0.116 (0.750) | -0.018 (0.960) | 0.628 (0.052) | 0.317 (0.372) | **0.872**[c] (0.001) | -0.012 (0.973) | -0.482 (0.159) | -0.245 (0.494) | **0.701**[b] (0.024) | 0.537 (0.110) |
| Co | 0.612 (0.060) | 0.160 (0.660) | -0.055 (0.880) | -0.141 (0.698) | 0.483 (0.157) | -0.171 (0.636) | **0.823**[c] (0.003) | -0.378 (0.281) | **-0.738**[b] (0.015) | -0.245 (0.494) | 0.555 (0.096) | 0.354 (0.316) |
| Mn | 0.236 (0.511) | 0.146 (0.688) | 0.515 (0.128) | 0.564 (0.090) | -0.103 (0.777) | 0.261 (0.467) | **0.891**[c] (0.001) | -0.200 (0.580) | **-0.648**[b] (0.043) | 0.024 (0.947) | 0.527 (0.117) | 0.479 (0.162) |
| Fe | 0.426 (0.220) | 0.409 (0.241) | 0.462 (0.179) | 0.176 (0.626) | 0.274 (0.444) | 0.340 (0.336) | **0.791**[c] (0.006) | -0.423 (0.223) | **-0.779**[c] (0.008) | -0.191 (0.596) | 0.423 (0.223) | 0.337 (0.340) |
| As | 0.302 (0.397) | -0.117 (0.747) | 0.554 (0.097) | -0.160 (0.659) | 0.246 (0.493) | -0.074 (0.839) | **0.869**[c] (0.001) | -0.263 (0.463) | **-0.765**[b] (0.010) | -0.222 (0.538) | 0.514 (0.129) | 0.422 (0.224) |

| | Time | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 | Time | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd | **0.777**[c] (0.008) | -0.330 (0.351) | -0.354 (0.315) | -0.379 (0.280) | 0.801[c] (0.005) | 0.093 (0.798) | **0.858**[c] (0.001) | -0.049 (0.892) | -0.549 (0.100) | -0.199 (0.582) | **0.735**[b] (0.016) | 0.482 (0.159) |
| Ca | **-0.752**[b] (0.012) | 0.294 (0.409) | -0.141 (0.698) | -0.128 (0.724) | -0.471 (0.169) | -0.343 (0.333) | -0.566 (0.088) | -0.043 (0.906) | 0.265 (0.460) | -0.650[b] (0.042) | -0.271 (0.449) | -0.357 (0.311) |
| P | 0.278 (0.437) | -0.257 (0.474) | 0.210 (0.561) | 0.265 (0.458) | -0.123 (0.734) | 0.395 (0.258) | **0.801**[c] (0.005) | 0.217 (0.546) | -0.304 (0.392) | -0.281 (0.431) | 0.255 (0.478) | **0.789**[c] (0.007) |
| Cr | **0.821**[c] (0.004) | 0.043 (0.907) | -0.158 (0.663) | -0.170 (0.638) | **0.681**[b] (0.030) | 0.347 (0.327) | **0.875**[c] (0.001) | -0.018 (0.960) | -0.486 (0.154) | -0.251 (0.485) | **0.693**[b] (0.026) | 0.529 (0.116) |
| Mg | -0.097 (0.790) | 0.463 (0.178) | -0.055 (0.879) | 0.354 (0.316) | 0.014 (0.970) | 0.139 (0.702) | **0.782**[c] (0.008) | -0.216 (0.549) | **-0.667**[b] (0.035) | 0.019 (0.958) | 0.578 (0.080) | 0.292 (0.412) |
| Ba | -0.311 (0.382) | -0.049 (0.893) | -0.207 (0.565) | 0.128 (0.724) | -0.384 (0.273) | 0.091 (0.802) | 0.573 (0.083) | 0.218 (0.545) | -0.187 (0.605) | -0.232 (0.519) | 0.237 (0.510) | **0.679**[b] (0.031) |
| Al | 0.049 (0.893) | -0.086 (0.812) | 0.351 (0.320) | 0.326 (0.358) | -0.351 (0.320) | 0.535 (0.111) | **0.765**[c] (0.010) | 0.144 (0.691) | -0.420 (0.227) | -0.129 (0.722) | 0.401 (0.250) | 0.609 (0.062) |
| Na | **-0.790**[c] (0.007) | -0.053 (0.885) | -0.117 (0.747) | 0.136 (0.708) | -0.599 (0.067) | -0.420 (0.227) | -0.354 (0.316) | 0.028 (0.939) | 0.402 (0.249) | 0.101 (0.781) | -0.451 (0.191) | -0.132 (0.717) |
| K | 0.497 (0.143) | -0.321 (0.366) | 0.569 (0.086) | 0.071 (0.845) | 0.213 (0.554) | 0.426 (0.219) | **0.646**[b] (0.044) | -0.190 (0.599) | -0.570 (0.086) | -0.306 (0.390) | 0.190 (0.599) | 0.494 (0.147) |
| Hg | -0.018 (0.960) | -0.578 (0.080) | 0.236 (0.511) | -0.467 (0.174) | 0.091 (0.803) | -0.079 (0.829) | -0.248 (0.489) | 0.273 (0.446) | 0.345 (0.328) | 0.183 (0.613) | 0.188 (0.603) | -0.115 (0.751) |
| TC | -0.511 (0.132) | -0.085 (0.815) | 0.389 (0.266) | 0.024 (0.947) | -0.195 (0.590) | 0.085 (0.815) | -0.305 (0.392) | 0.579 (0.079) | 0.482 (0.159) | 0.098 (0.787) | -0.439 (0.204) | 0.250 (0.486) |
| TN | **-0.802**[c] (0.005) | -0.046 (0.900) | 0.316 (0.374) | -0.067 (0.854) | -0.559 (0.093) | -0.474 (0.166) | **0.693**[b] (0.026) | -0.067 (0.854) | 0.371 (0.291) | 0.349 (0.324) | 0.012 (0.973) | **-0.693**[b] (0.026) |

| | Time | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 | Time | *bla*$_{TEM}$ | *bla*$_{SHV}$ | *bla*$_{OXA}$ | *bla*$_{CTX-M}$ | *int*1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ExtP | **0.967**[c] (0.001) | -0.176 (0.651) | 0.067 (0.865) | -0.183 (0.637 | **0.683**[b] (0.042) | 0.183 (0.637) | **0.967**[c] (0.001) | -0.067 (0.865) | -0.633 (0.067) | 0.025 (0.949) | 0.400 (0.286) | 0.600 (0.088) |
| ExtK | **0.806**[c] (0.005) | -0.316 (0.374) | 0.091 (0.803) | -0.200 (0.580) | 0.418 (0.229) | 0.285 (0.425) | **0.782**[c] (0.008) | -0.164 0.651) | **-0.745**[b] (0.013) | -0.317 (0.372) | 0.467 (0.174) | 0.442 (0.200) |
| *bla*$_{TEM}$ | -0.146 (0.688) | - | 0.298 (0.403) | 0.584 (0.077) | 0.061 (0.868) | 0.006 (0.987) | 0.055 (0.881) | - | **0.758**[b] (0.011) | 0.329 (0.353) | -0.115 (0.751) | 0.588 (0.074) |
| *bla*$_{SHV}$ | -0.127 (0.726) | 0.298 (0.403) | - | 0.370 (0.293) | -0.224 (0.533) | 0.188 (0.603) | -0.491 (0.150) | **0.758**[b] (0.011) | - | 0.390 (0.265) | -0.345 (0.328) | 0.103 (0.777) |
| *bla*$_{OXA}$ | 0.018 (0.960) | 0.584 (0.077) | 0.370 (0.293) | - | -0.236 (0.511) | 0.564 (0.090) | -0.012 (0.973) | 0.329 (0.353) | 0.390 (0.265) | - | -0.171 (0.637) | 0.165 (0.649) |
| *bla*$_{CTX-M}$ | **0.673**[b] (0.033) | 0.061 (0.868) | -0.224 (0.533) | -0.236 (0.511) | - | -0.030 (0.934) | 0.418 (0.229) | -0.115 (0.751) | -0.345 (0.328) | -0.171 (0.637) | - | -0.067 (0.855) |
| *int*1 | 0.467 (0.174) | 0.006 (0.987) | 0.188 (0.603) | 0.564 (0.090) | -0.030 (0.934) | - | **0.733**[b] (0.016) | 0.588 (0.074) | 0.103 (0.777) | 0.165 (0.649) | -0.067 (0.855) | - |

Notes: [a] Nutrients and metals = mass per mass of dry soil; genes = copies per mass of dry soil; time = years

[b] 95% Confidence correlation

[c] 99% Confidence correlation