Supplementary Files

A second NMDS approach was applied to analyze in depth both the functional guilds and groups based on the community position in the initial NMDS ordination. For this type of analysis were constructed four different datasets that contain each only the data that are present in the same quadrant of the initial NMDS in relation to Axis 1 and 2 (Q1 -/-; Q2-/+; Q3 +/+; Q4 +/-). This resulted in four different ordinations that show the groups and guilds associated with a reduced number of treatments and makes the assessment of general differences more focused (Figure S1–S4). To further explore how treatments affect soil functional microbiomes, the four-quadrant additional NMDS ordination method was used. Each of the four new NMDS graphs were projected based on the variant position in the general NMDS. The results enabled a deep analysis of visual differences between variants that showed a similarity in the activities, and the masked effects of the treatments. The entire set of data for each Quadrant NMDS was run again, respecting the procedure for the general NMDS.

The first NMDS (Figure S1.), focuses on the functional space defined by –/– quadrant of the primarily NMDS analysis. This ordination was done based on all types of treatments, but with a better position in space that reflects a distinct metabolic assemblage induced by long-term treatments. The N based treatments are positioned in the right area of the graph, separated by Axis 1.

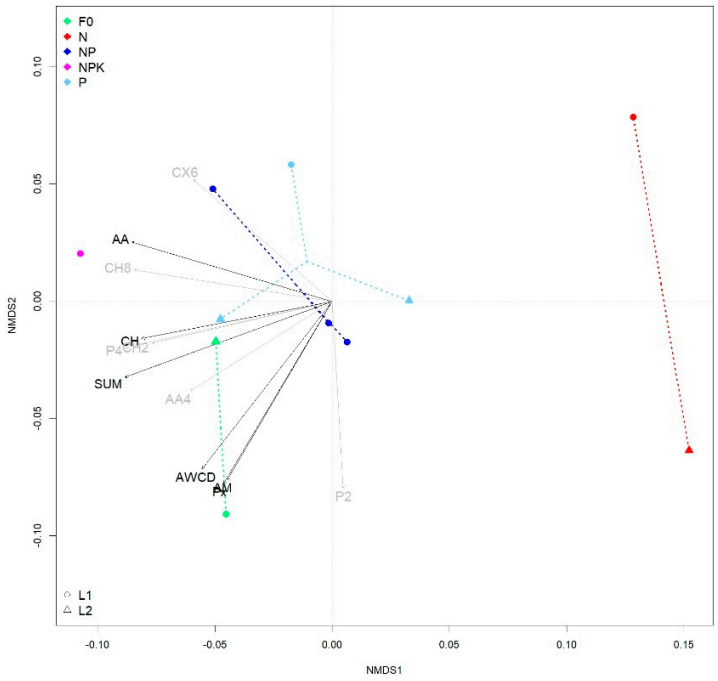


Figure S1. Partial NMDS ordination for Q1 dataset of soil decomposition microbiome. Legend: L1–liming with 2.5 t ha $^{-1}$ calcium carbonate, L2 – liming with 5 t ha $^{-1}$ calcium carbonate; F0–unfertilized, N– N_{100}, P– P_{70}, NP–$N_{100}P_{70}$, NPK–$N_{100}P_{70}K_{60}$ (according to the detailed description in Materials and Methods, Table A1); SUM – sum of activities, AWCD – Average well color development; Functional guilds: AA – Amino acids, AM – Amines/amides, CH – Carbohydrates, Px – Polymers; Functional groups: AA4 – l-Serine, CH2 – d-Cellobiose, CH8 – N-Acetyl-d-glucosamine, CX6 – γ-Hydroxy butyric acid, P2 – Tween 80, P4 – Glycogen.

The lack of direct correlation with specific guild indicate the broad community alteration impact and the requirement of synergy with liming to stimulate specialized guild activity. For the left part of the ordination multiple phenomena were visible. The exterior of the group-guild association was assured by the angle between CX6 and P2 vectors. Only two points are outside this area, both associated with P fertilizers. One interesting aspect is the NP treatment positioning along CX6 vector, indicating the sensitivity of the group to this type of input. The AM, Px and AWCD vectors were oriented toward the –/– quadrant and associated with medium liming and the absence of fertilizers. Both CH and sum of activities vectors were near to Axis 1 and associated with intensive liming and both the P fertilizers and the unfertilized variants. This indicates the shift between generalist to specialist and the maintenance of similar functional activities. The two vectors were sustained by P2 and CH4 vectors, while NPK application was associated with the AA and CH8 activities, which indicate that complexity of fertilizers promote both diversity and specialized metabolic preferences.

The second NMDS projection use the points from –/+ quadrant (general NMDS) and showed a complete point dispersion within the entire ordination space (Figure S2.). The untreated variant was near to the first axis of the graph, but it was not correlated with any group or guild vectors. This confirms the reduced functionality of native soil compared to the specialized and intense activity in managed ones. Most of the N and NPK points were positioned within the AA1 and CH4 vectors, which show a large angle of variation and suggest the presence of metabolic flexibility in these communities.

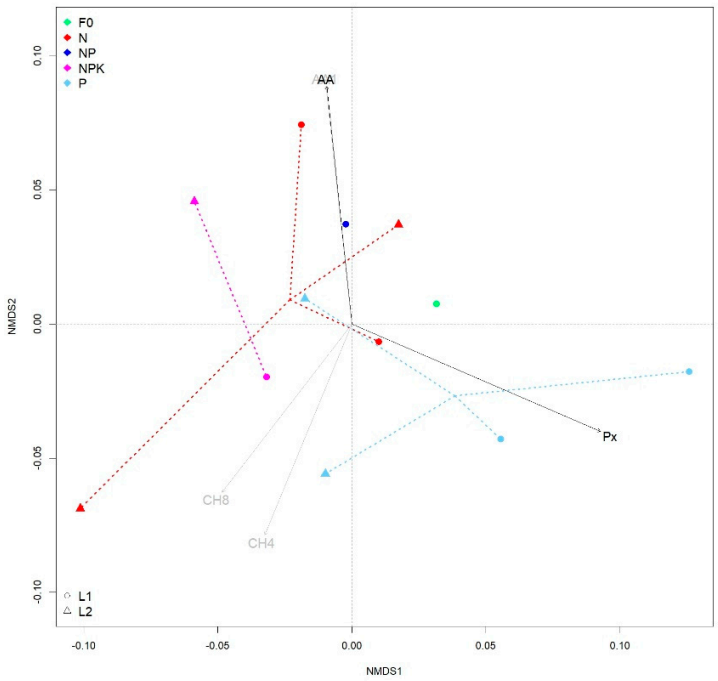


Figure S2. Partial NMDS ordination for Q2 dataset of soil decomposition microbiome. Legend: L1–liming with 2.5 t ha $^{-1}$ calcium carbonate, L2 – liming with 5 t ha $^{-1}$ calcium carbonate; F0–unfertilized, N– N_{100}, P– P_{70}, NP–$N_{100}P_{70}$, NPK–$N_{100}P_{70}K_{60}$ (according to the detailed description in Materials and Methods, Table A1); Functional guilds: AA – Amino acids, Px – Polymers; Functional groups: CH4 – β-Methyl-d-glucoside, CH8 – N-Acetyl-d-glucosamine.

The AA1 vector was associated with an NP variant, the only one present in this ordination, and indicates the high potential of this treatment to modify the activity of AA1 microbiome. Phosphorus application and the two levels of liming create a cross image of variation, with one of the gradients along P4 and CX9 vectors, and the other one at the opposite. Also, the polymers vector crosses the area occupied by these microbiomes.

The third projection (Figure S3.), showed both synergistic assemblage of group and guild activities due to the application of treatments and a large dispersion of data. Most of the points were positioned in the reverse direction of the vectors, sustaining the synergy of activities and more diverse microbiomes. One N treated variant was positioned near to SUM and CH vectors, indicating that this treatment will affect directly the activity of CH guild and the dominant position of this guild in the entire microbiome. Another point, untreated variant, was positioned between three polymers and one AA3 vectors, indicating that these groups and guild face the most important changes in the activities for native soils. Another important aspect is the star shaped positioning of NPK treated soils. For this treatment, the application of medium liming determines the direction of activities parallel to the P2 vector, while intensive liming conduced to a convergence with AM1 vector. The third point of this treatment was positioned at the opposite of AWCD and SUM vectors, indicating the high perturbance potential of the treatment for the entire microbiome and shifted between generalist and specialists in the microbial communities.

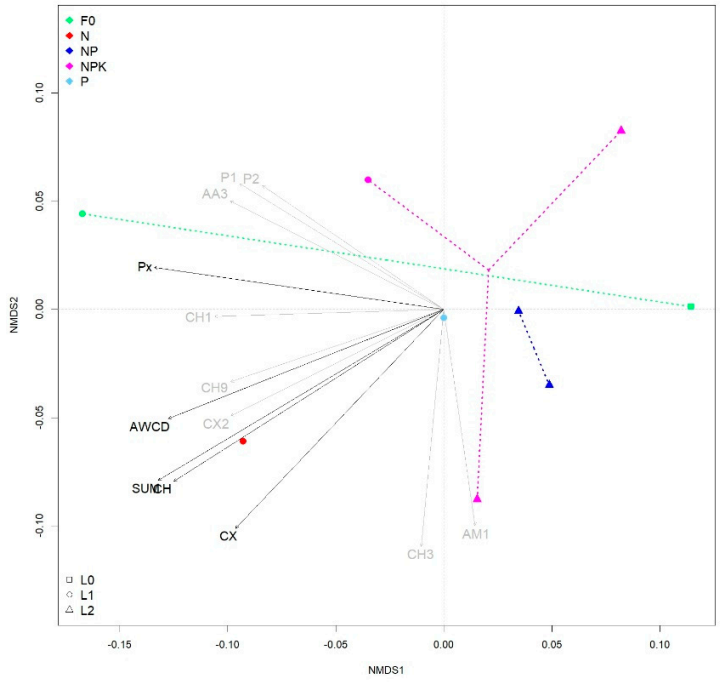


Figure S3. Partial NMDS ordination for Q3 dataset of soil decomposition microbiome. Legend: L0–no liming, L1–liming with 2.5 t ha $^{-1}$ calcium carbonate, L2 – liming with 5 t ha $^{-1}$ calcium carbonate; F0–unfertilized, N– N_{100}, P– P_{70}, NP–$N_{100}P_{70}$, NPK– $N_{100}P_{70}K_{60}$ (according to the detailed description in Materials and Methods, Table A1); SUM – sum of activities, AWCD – Average well color development; Functional guilds: CH – Carbohydrates, CX – Carboxylic and acetic acids Px – Polymers; Functional groups: AA3 – l-Phenylalanine, AM1 – Phenylethylamine, CH1 – Pyruvic acid methyl ester, CH3 – α-d-Lactose, CH9 – Glucose-1-phosphate, CX2 – d-Galactonic acid γ-lactone, P1 – Tween 40, P2 – Tween 80.

The last NMDS (Figure S4.), which score the points from the +/– quadrant of the general graph, showed the most dispersed image of vectors and points. This is consistent with the most visible punctual differences in the functional microbiomes, with liming effects associated with Axis 1 and fertilizers correlated with Axis 2. The absence of fertilizers (F0) enables the visualization of the liming application effect. This type of agronomic input showed multiple punctual changes in the functional activities by projecting the points all over the ordination and along Axis 1. The multiple corner star image of these microbiomes was crossed by NP and NPK treatments, each one positioned on the left and right of the Axis 2. The entire NMDS projection showed the Axis 1 associated with liming and Axis 2 with fertilizers. The vectors of AM and CX guilds were associated with NP highly liming variants, while both AWCD and sum of activities vectors were associated with this type of combined treatment. An interesting case was the analysis of group–variant association, where there were multiple potential scenarios of changes in the functional microbiomes. The CH7 was one of the most probable functional groups that suffer changes due to high liming, while a similar change can be found in CH5 group. The absence of liming leads to significant changes for the AM2 and AA1 groups, along with a global change in terms of the sum of activities. The application of N fertilizer can produce different changes of the soil microbiome, that overcome the ones that were associated with any other inputs, a phenomenon that showed the transition from generalists to specialists in microbiome.

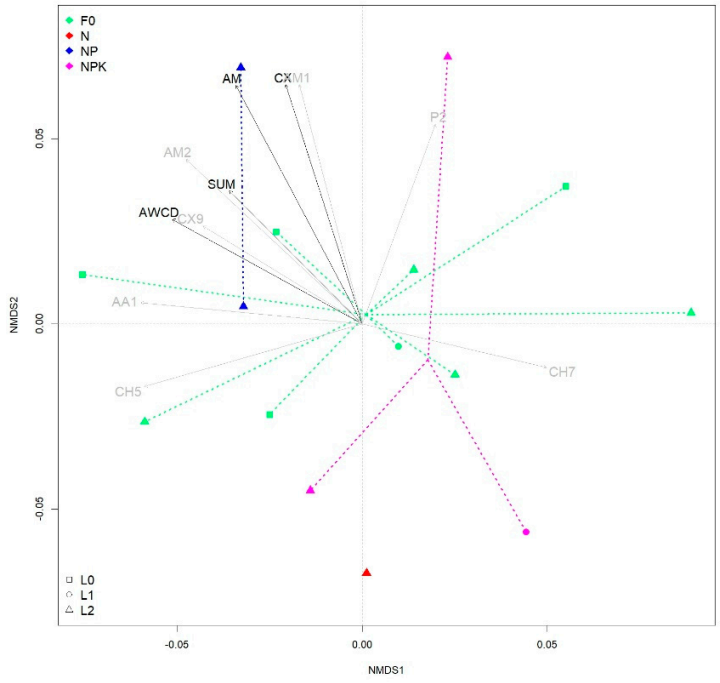


Figure S4. Partial NMDS ordination for Q4 dataset of soil decomposition microbiome. Legend: L0–no liming, L1–liming with 2.5 t ha $^{-1}$ calcium carbonate, L2 – liming with 5 t ha $^{-1}$ calcium carbonate; F0–unfertilized, N– N_{100}, P– P_{70}, NP–$N_{100}P_{70}$, NPK– $N_{100}P_{70}K_{60}$ (according to the detailed description in Materials and Methods, Table A1); SUM – sum of activities, AWCD – Average well color development; Functional guilds: AM – Amines/amides, CX – Carboxylic and acetic acids; Functional groups: AA1 – l-Arginine, AM1 – Phenylethylamine, AM2 – Putrescine, CH5 – d-Xylose, CH7 – d-Mannitol, CX9 – d-Malic acid, P2 – Tween 80.