

# Effect of long-term fertilization in soil and soilless culture of tomatoes on the microbial and fungal community structure

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## Microbial diversity of soil and soilless culture systems

18 *Supplementary table 1: Overview of the chemical composition of soilplant and soilanimal before the start of the experiment (8/1/2015) and*  
 19 *throughout the whole experimental period. n=1. As bulk density 1.25 t ha<sup>-1</sup> (Vlaamse zandstreek; [Arthur et al. [58]]) was chosen for the 0.3*  
 20 *m top soil layer.*

| Treatment  | Date analysis | NO <sub>3</sub> -N<br>kg ha <sup>-1</sup> | NH <sub>4</sub> -N<br>kg ha <sup>-1</sup> | (N-mineral) |
|------------|---------------|---|---|-------------|
| SOILANIMAL | 8/01/2015     | 243                                       | < 4                                       | 247         |
| SOILPLANT  | 8/01/2015     | 196                                       | < 4                                       | 200         |
| SOILANIMAL | 3/2015        | 68  | <4  | 72          |
| SOILPLANT  | 3/2015        | 97  | 9   | 106         |
| SOILANIMAL | 5/2015        | 25  | <4  | 29          |
| SOILPLANT  | 5/2015        | 6   | 7   | 13          |
| SOILANIMAL | 6/2015        | 18  | <4  | 22          |
| SOILPLANT  | 6/2015        | 42  | 8   | 50          |
| SOILANIMAL | 27/7/2015     | 16  | 7   | 23          |
| SOILPLANT  | 27/7/2015     | 17  | 18  | 35          |
| SOILANIMAL | 4/11/2015     | 10  | <4  | 14          |
| SOILPLANT  | 4/11/2015     | 9   | <4  | 13          |

22 *Supplementary table 2: Overview of the chemical composition of the different fertilizers used. “-“ means that the elements was not analyzed*  
 23 *or specified. “\*” means according to the specifications of the supplier. “\*\*\*” means that the chemical composition was actually analyzed.*

| Fertilizer               | Composition (%)    |                                 |                                 |                               |                  |      |      |      |                 |
|--------------------------|--------------------|---------------------------------|---------------------------------|-------------------------------|------------------|------|------|------|-----------------|
|                          | N <sub>total</sub> | NO <sub>3</sub> <sup>-</sup> -N | NH <sub>4</sub> <sup>+</sup> -N | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | MgO  | CaO  | Cl   | SO <sub>3</sub> |
| Blood meal*              | 14                 | -                               | -                               | 0                             | 0                | -    | -    | -    | -               |
| Patentkali*              | -                  | -                               | -                               | -                             | 30               | 10   | -    | -    | 42              |
| Magnesium sulphate*      | -                  | -                               | -                               | -                             | -                | 16   | -    | -    | 32              |
| Malt sprouts*            | 3                  | -                               | -                               | 0                             | 0                | -    | -    | -    | -               |
| Antys MgS**              | 0                  | -                               | 0                               | 0                             | 0                | 9    | 0    | 0    | 18              |
| Biosyr**                 | 9.35               | -                               | 0.53                            | 3.96                          | 9.08             | 0.11 | 3.98 | 0.68 | 10.9            |
| Nutrikali**              | 2.4                | -                               | 0.1                             | 0.3                           | 4.9              | 0.1  | 0.1  | 1.1  | 1.1             |
| SP**                     | 0                  | -                               | 0                               | 0.001                         | 58.6             | 0    | 0    | 0    | 0               |
| Calcium chloride 33 %*   | 0                  | -                               | 0                               | 0                             | 0                | 0    | 16.5 | 21   | 0               |
| Calsal vlb 51%*          | 8.7                | -                               | -                               | -                             | -                | -    | 8.7  | -    | -               |
| Amnitra vlb 51%*         | 18                 | 9                               | 9                               | -                             | -                | -    | -    | -    | -               |
| Potassium nitrate*       | 13                 | 13                              | -                               | -                             | 45               | -    | -    | -    | -               |
| Magnesium sulphate*      | -                  | -                               | -                               | -                             | -                | 16   | -    | -    | 32              |
| Monopotassium phosphate* | -                  | -                               | -                               | 52                            | 34               | -    | -    | -    | -               |
| Potassium sulphate *     | -                  | -                               | -                               | -                             | 50               | -    | -    | -    | 45              |

25 *Supplementary table 3: Overview of the fertilizers used and the total amount used for the different*  
 26 *treatments. GBOF = soilless culture system with organic growing medium and organic fertilizer.*  
 27 *GBFISH = soilless culture system with organic growing medium and fish. SOILANIMAL= organic*  
 28 *soil with animal-derived material as fertilizer and SOILPLANT= organic soil with plant-derived*  
 29 *material as fertilizer*

| Treatment  | Type of fertilizer used                                    | Total amount of fertilizer used (g m <sup>-2</sup> ) |
|------------|--|--|
| GBFISH     | Calsal vlb 51% (Ca(NO <sub>3</sub> ) <sub>2</sub> )        | 1855   |
|            | Amnitra vlb 51% (NH <sub>4</sub> NO <sub>3</sub> )         | 557  |
|            | Potassium nitrate (KNO <sub>3</sub> )                      | 959  |
|            | Magnesium sulphate (MgSO <sub>4</sub> )                    | 279  |
|            | Monopotassium phosphate (KH <sub>2</sub> PO <sub>4</sub> ) | 368  |
|            | Potassium sulphate (K <sub>2</sub> SO <sub>4</sub> )       | 288  |
| GBOF       | Antys MgS  | 827  |
|            | Biosyr   | 1292   |
|            | Nutrikali  | 1870   |
|            | SP   | 404  |
|            | CaCl <sub>2</sub>  | 921  |
|            | Libremix   | 49   |
| SOILANIMAL | Blood meal   | 180  |
|            | Patentkali   | 163  |
| SOILPLANT  | Malt sprouts   | 1001   |
|            | Patentkali   | 163  |

## systems

37 *Supplementary table 4: Overview of the chemical composition of the four different organic fertilizers*  
 38 *(Nutrikali. ANTYS MgS. Biosyr and SP). “-“: means that this element was not determined in the*  
 39 *fertilizer*

|                              | Nutrikali          | ANTYS MgS         | Biosyr            | SP                  |
|------------------------------|--------------------|-------------------|-------------------|---------------------|
| Total Nitrogen (%)           | $2.35 \pm 0.12$    | -                 | $9.35 \pm 0.23$   | -                   |
| Organic nitrogen (%)         | $2.25 \pm 0.11$    | -                 | $8.81 \pm 0.22$   | -                   |
| $\text{NH}_4^+\text{-N}$ (%) | $0.056 \pm 0.003$  | -                 | $0.532 \pm 0.027$ | -                   |
| $\text{NO}_3^-\text{-N}$ (%) | $0.042 \pm 0.002$  | -                 | $0.012 \pm 0.001$ | -                   |
| $\text{P}_2\text{O}_5$ (%)   | $0.257 \pm 0.013$  | -                 | $3.96 \pm 0.20$   | $0.0007 \pm 0.0001$ |
| $\text{K}_2\text{O}$ (%)     | $4.93 \pm 0.25$    | -                 | $9.08 \pm 0.23$   | $58.6 \pm 1.5$      |
| $\text{CaO}$ (%)             | $60.113 \pm 0.006$ | -                 | $3.98 \pm 0.20$   | -                   |
| $\text{MgO}$ (%)             | $0.121 \pm 0.006$  | $0.725 \pm 0.036$ | $0.110 \pm 0.005$ | -                   |
| $\text{SO}_3^{2-}$ (%)       | $1.11 \pm 0.06$    | $1.28 \pm 0.06$   | $10.9 \pm 0.3$    | -                   |
| $\text{Na}_2\text{O}$ (%)    | $1.97 \pm 0.10$    | -                 | $0.836 \pm 0.042$ | -                   |
| $\text{Cl}$ (%)              | $0.839 \pm 0.042$  | -                 | $0.676 \pm 0.034$ | -                   |
| Organic matter (%)           | $0.839 \pm 0.042$  | -                 | $57.4 \pm 1.4$    | -                   |

46 *Supplementary table 5: Correlations between microbial community composition and chemical characteristics in four different tomato*  
 47 *cultivating systems across time points. indicated by the Multiple Factor Analysis. Dimensions of the MFA can be described by the categorical*  
 48 *variables included in the analysis. For each categorical variable (growing medium and time point). a one-way analysis of variance was*  
 49 *performed with the coordinates of the samples on the axis. explained by the time point or growing medium type. Then. for each level of the*  
 50 *category (i.e. time point 1. time point 2 or time point 3 or growing medium GB). a Hotelling  $T^2$ -test was used to compare the average of the*  
 51 *category with the general average (using the constraint  $P_i a_i = 0$ .  $a_i = 0$ ). For instance. the coordinates of the relative abundance of family*  
 52 *“x” at GB at time point 1 were compared with the average coordinates of the relative abundance of family “x” in GB. The P value associated*  
 53 *to this test is transformed to a normal quantile to assess whether the mean of the category is significantly less or greater than 0. Negative*  
 54 *values indicate negative correlations.*

| Dimension | Variance | Descriptor | Estimate ( $R^2$ ) | P value  | Taxon and or chemical characteristics | Correlation | P value  |
|-----------|----------|------------|--------------------|----------|---------------------------------------|-------------|----------|
| DIM 1     | 1.5492   | GBOF       | 1.5492             | 0.00261  | K                                     | 0.9505387   | <0.0001  |
|           |          |            |                    |          | Protozoa                              | 0.882805    | <0.0001  |
|           |          |            |                    |          | EC                                    | 0.8713766   | <0.0001  |
|           |          |            |                    |          | Fungi.18.3                            | 0.7156968   | 1E-07    |
|           |          | GBFISH     | 1.451668           | 0.015256 | Na                                    | 0.7015117   | 3E-07    |
|           |          |            |                    |          | Total                                 | 0.6796157   | 0.000001 |
|           |          |            |                    |          | Ca                                    | 0.6779943   | 1.1E-06  |
|           |          |            |                    |          | P                                     | 0.6499159   | 4.3E-06  |
|           |          |            |                    |          | NO <sub>3</sub> <sup>-</sup> N        | 0.6348602   | 8.3E-06  |
|           |          |            |                    |          | Fungi.18.2                            | 0.5960548   | 3.92E-05 |
|           |          |            |                    |          | Cl                                    | 0.548466    | 0.000205 |
|           |          |            |                    |          | Mg                                    | 0.4452108   | 0.003536 |
|           |          |            |                    |          | SO <sub>4</sub> <sup>2-</sup>         | 0.4374311   | 0.004237 |
|           |          |            |                    |          | Actinomycetes.                        | -0.3618954  | 0.020071 |
|           |          |            |                    |          | pH(H <sub>2</sub> O)                  | -0.6273887  | 1.13E-05 |
|           |          |            |                    |          | Bacteria Fungi18.2 ratio.             | -0.6355404  | 8.00E-06 |
|           |          |            |                    |          | Gram-positive bacteria                | -0.7987867  | <0.0001  |
| DIM 2     |          |            |                    |          | AMF                                   | 0.729531    | 1E-07    |
|           |          |            |                    |          | Gram-negative bacteria                | 0.7288208   | 1E-07    |

# Microbial diversity of soil and soilless culture systems

|       |            |            |          |                                 |            |          |
|-------|------------|------------|----------|---------------------------------|------------|----------|
|       |            |            |          | Bacteria Fungi ratio.18.2.      | 0.670518   | 1.6E-06  |
|       | tpt4       | 1.178248   | 0.014011 | Ca                              | 0.5255753  | 0.000418 |
|       | SOILPLANT  | 0.8708145  | 0.016697 | SO <sub>4</sub> <sup>2-</sup>   | 0.4707461  | 0.001895 |
|       | SOILANIMAL | 0.7376762  | 0.103984 | P                               | 0.4189329  | 0.006407 |
|       |            |            |          | Gram-positive bacteria          | 0.4164783  | 0.006757 |
|       |            |            |          | pH(H <sub>2</sub> O)            | 0.4021037  | 0.009157 |
|       |            |            |          | Na                              | 0.3751969  | 0.015642 |
|       |            |            |          | Cl                              | 0.3520146  | 0.024004 |
|       |            |            |          | Fungi.18.3                      | -0.3528578 | 0.023645 |
|       |            |            |          | NH <sub>4</sub> <sup>+</sup> -N | -0.517281  | 5.34E-04 |
|       |            |            |          | Actinomycetes                   | -0.5900243 | 4.91E-05 |
|       |            |            |          | Fungi.18.2                      | -0.6236232 | 1.33E-05 |
|       |            |            |          | Fungi.18.1                      | -0.8010289 | <0.0001  |
| DIM 3 |            |            |          | NH <sub>4</sub> <sup>+</sup> -N | 0.675832   | 1.2E-06  |
|       |            |            |          | Total                           | 0.6403078  | 6.5E-06  |
|       | GBOF       | 1.27019    | 5.96E-05 | Cl                              | 0.5425307  | 0.000248 |
|       |            |            |          | pH(H <sub>2</sub> O)            | 0.4863632  | 0.001263 |
|       |            |            |          | Mg                              | 0.3706436  | 0.017054 |
|       |            |            |          | P                               | 0.3569491  | 0.021966 |
|       |            |            |          | Fungi.18.2                      | -0.446345  | 0.003443 |
|       |            |            |          | Fungi.18.3                      | -0.49035   | 1.14E-03 |
|       |            |            |          | NO <sub>3</sub> <sup>-</sup> -N | -0.722229  | 1.00E-07 |
| DIM 4 |            |            |          | Actinomycetes                   | 0.5783302  | 7.49E-05 |
|       | SOILANIMAL | -0.6524775 | 0.005355 | Gram-negative bacteria          | 0.5103223  | 0.000653 |
|       |            |            |          | SO <sub>4</sub> <sup>2-</sup>   | 0.4863936  | 0.001262 |
| DIM 5 |            |            |          | NH <sub>4</sub> <sup>+</sup> -N | 0.4423815  | 0.003778 |
|       | tpt3       | 0.4160831  | 0.051796 | Mg                              | 0.3565815  | 0.022113 |
|       |            |            |          | Gram-negative bacteria          | 0.3443766  | 0.027467 |
|       |            |            |          | Cl                              | -0.313677  | 0.045812 |

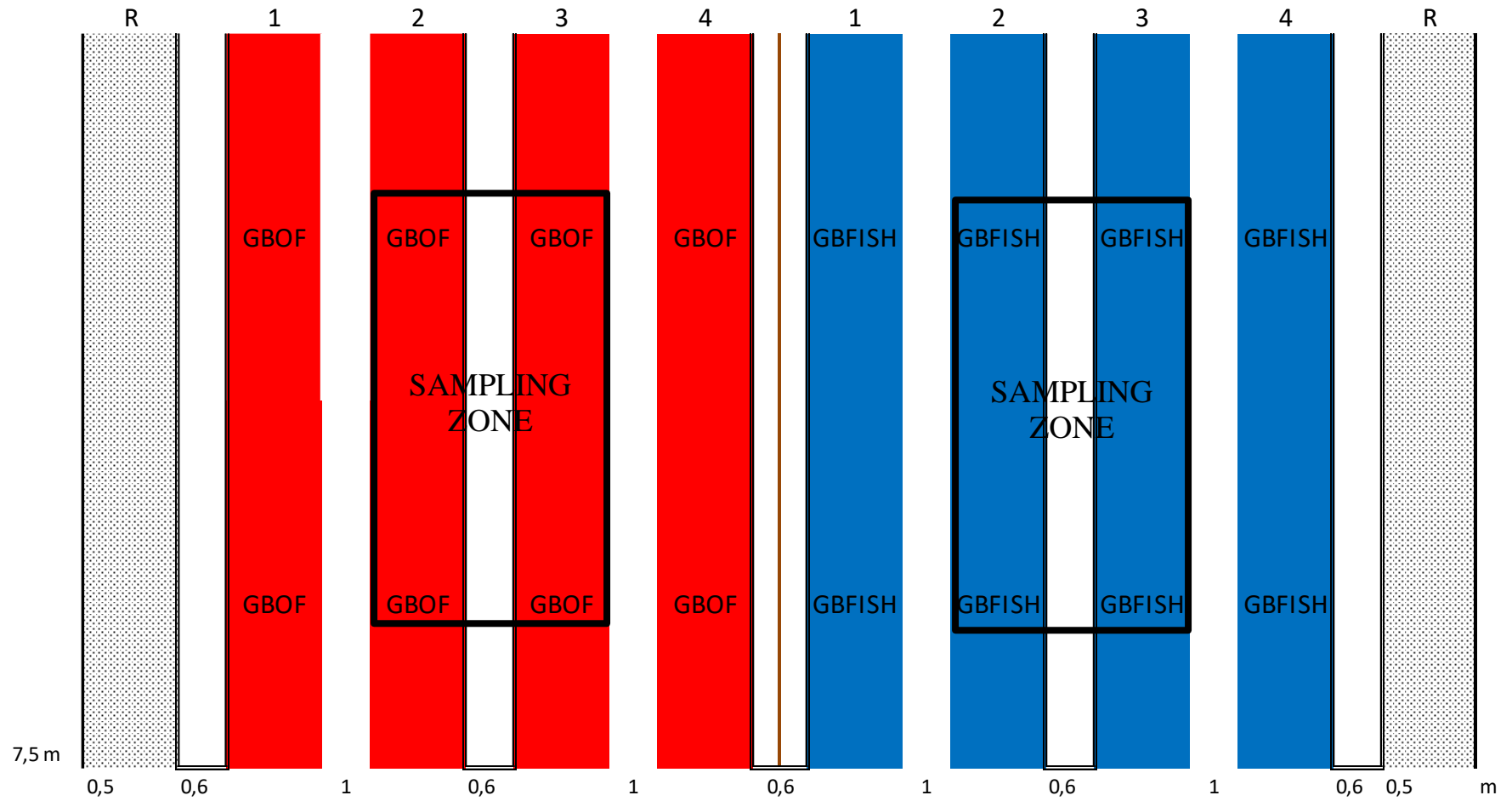
**Microbial diversity of soil and soilless culture systems**

|            |           |          |
|------------|-----------|----------|
| Na         | -0.320351 | 0.041154 |
| Fungi.18.1 | -0.347512 | 0.025998 |
| P          | -0.39069  | 0.011552 |

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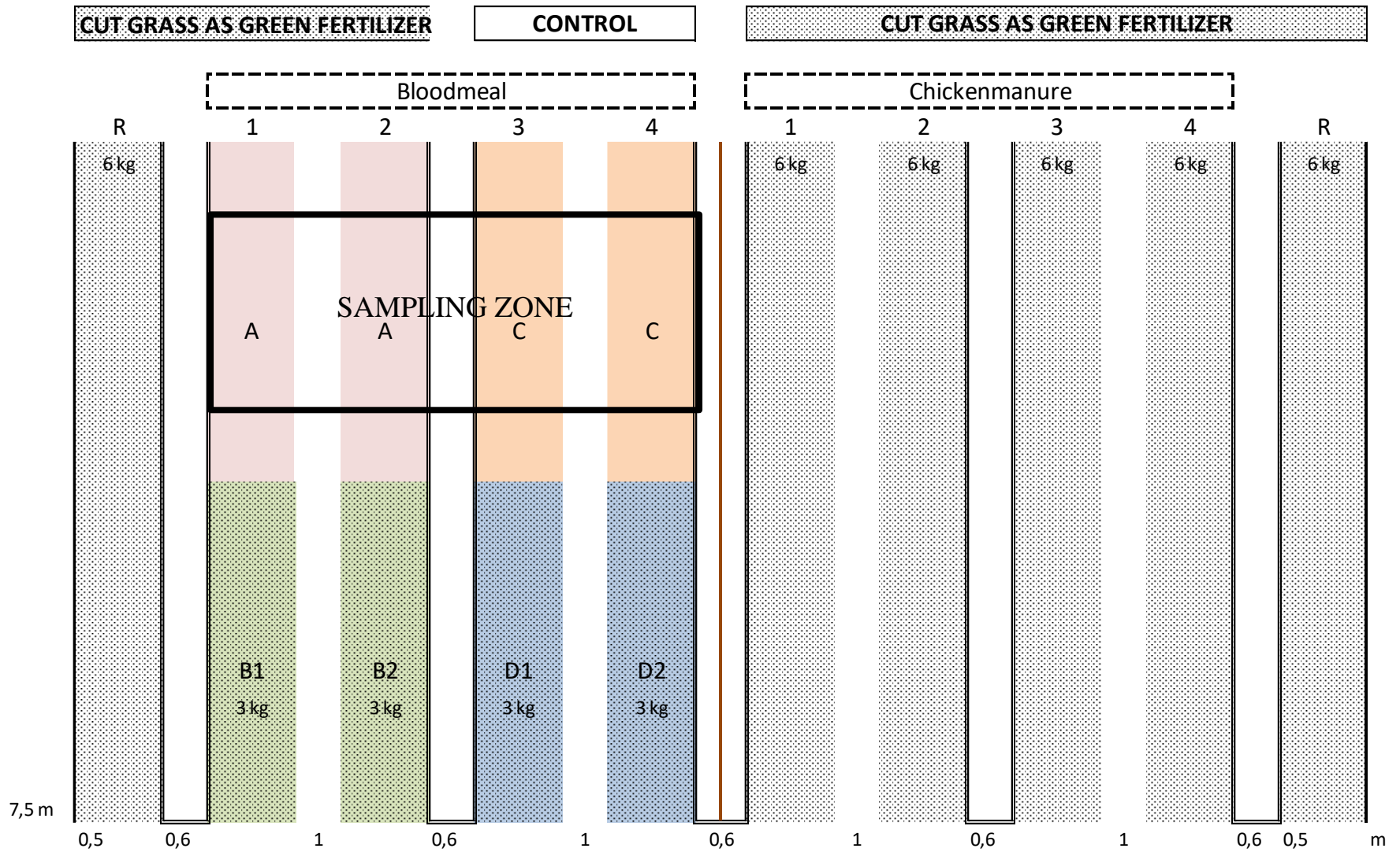


## 9a SOILLESS CULTURE

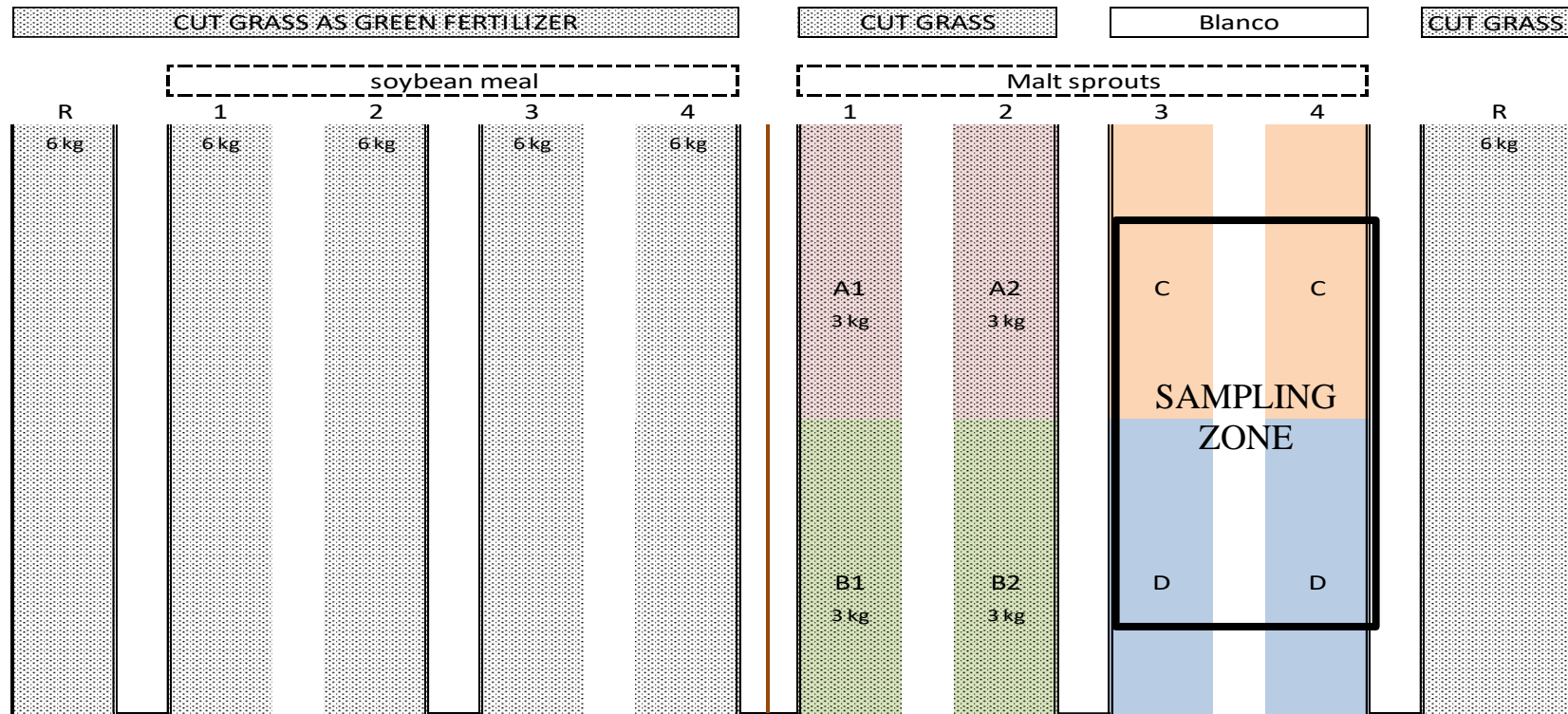


# Microbial diversity of soil and soilless culture systems

## 9b SOILANIMAL



## 9c SOILPLANT



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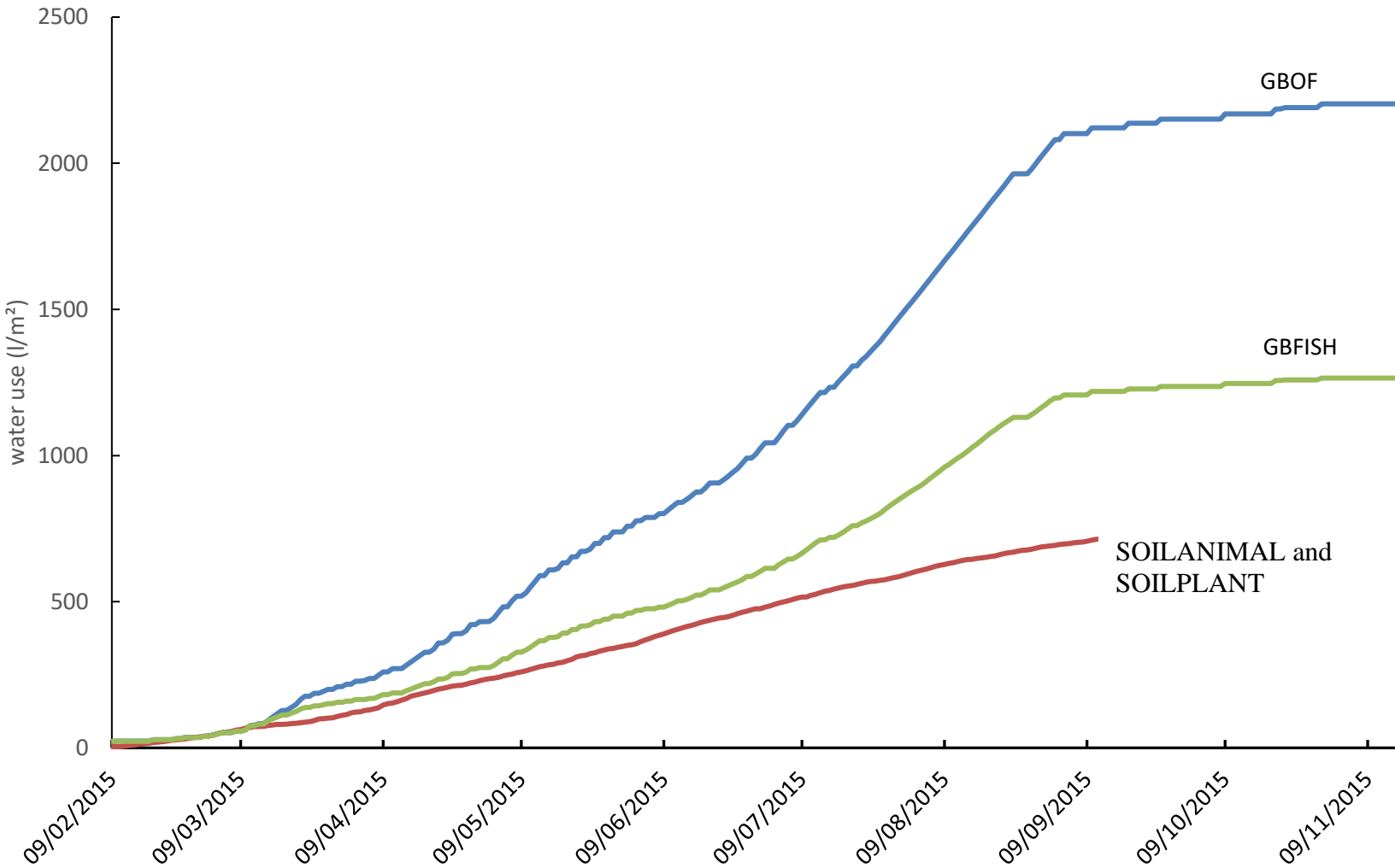
60 *Supplementary figure 1: Overview of the experimental set-up. The glass house was divided into three compartments (S91 = 9a GBOF and*  
 61 *GBFISH. S92=9b soilanimal and S93 = 9c soilplant) with a surface of 80 m<sup>2</sup>. S91 was subdivided into two part. i.e. red color = GBOF and*  
 62 *blue color = GBFISH; S92 was organic soil with animal (blood meal) derived material and S92 was the organic soil with plant-derived*  
 63 *material (malt sprouts). In S92 and S93 the previous cultures were tomato in 2014. pepper in 2013 and cucumber in 2012. R= outer rows.*  
 64 *Plant density was the same for all the treatment 2.65 plants m<sup>-2</sup>. Samples were taken in the sampling zone with 40 plants per sampling zone.*

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Microbial diversity of soil and soilless culture systems



Supplementary figure 2: Overview of the cumulative water dosage (L per m²) for the different tomato cultivating systems.