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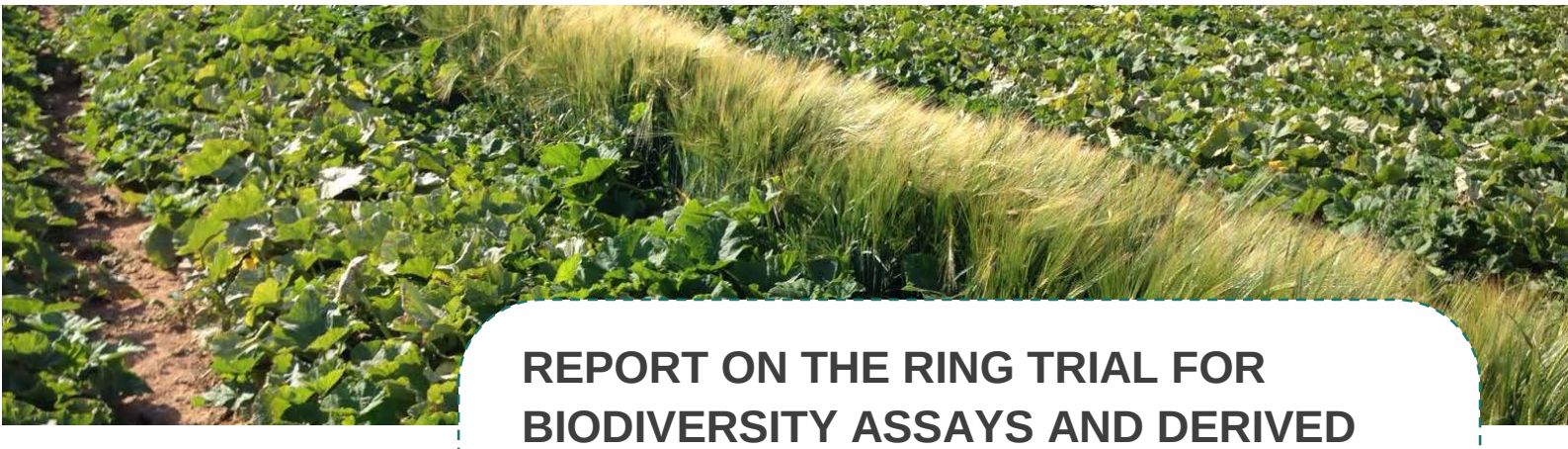
Crop diversification and low-input farming across Europe: from practitioners' engagement and ecosystems services to increased revenues and value chain organisation



REPORT ON THE RING TRIAL FOR BIODIVERSITY ASSAYS AND DERIVED RECOMMENDATIONS

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Paavolan kotijuustola	PK	Finland
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Executive summary

The different case studies setup in Diverfarming will be used to identify positive effects and drawbacks on aboveground and belowground biodiversity by adoption of diversified cropping systems in comparison to conventional systems. To effectively measure and compare the functioning of the agro-ecosystem and its microbiological diversity across different regions, soil types and agro-ecosystems, standardisation is indispensable. An important output of WP4 was the dissemination between the partners of methods and protocols and the consolidation of standard (or standardisable) protocols to efficiently compare and correlate the results. Thus, a suite of soil microbiological parameters was listed in the proposal of the Diverfarming project. These parameters and respective methods for their analyses were further defined at the Workshop on Plant and Soil Procedures and Protocols, held in Trier, Germany from 18-20 January 2018.

Since different methods exist even for the analysis of single parameters, it was necessary to set methods' protocols that are binding for all participating groups. All these methods and protocols were compiled in the "Handbook on WP4 protocols for sampling, sampling procedure and analysis" (Deliverable D4.1), also published as an open-access book (https://zenodo.org/record/2553445#.XQI_a4gzZPY). Since several methods and even more the specific protocols were new for some groups, it was decided to select some of the analytical methods to undergo a preliminary inter-laboratory ring test with the aim of assuring high quality, comparable data. A ring test (also called as proficiency test) is an inter-laboratory test that permits to assess the performance of different testing laboratories which are measuing the same property, based on analysis of the same sample with the same method. So, results should be very similar. The aim is to enable laboratories to assess and improve their feed analysis performance.

The ring trial was performed using one soil sample from an arable field at Cartagena, Spain (corresponding with case study LT1) and three methods that represent the various methods on soil microbial properties used within WP4. Four laboratories and partners within Diverfarming, respectively, contributed to the ring trial.

The ring trial revealed that for each individual laboratory and parameter, the reproducibility was good, standard deviations were small and no outliers were produced. Standard deviation between replicate samples was within the same range or even lower compared to previously reported results. Analyses showed that the microbial enzyme activity was very low and gene abundance was small of the selected soil sample. This could be attributed to the semiarid Mediterranean climate of the region. Anyhow, laboratories were able to master this challenging task. Consistency of data was good, when comparing the results and deviations with typical data reported in the literature. Furthermore, it must be emphasized again that analyses were done with a soil sample with very low microbial activity and microbial biomass, and analytical data were in part near the detection limit of the methods. Hence, small deviations in absolute values resulted in apparently large relative deviations. In total, the ring trial showed that initial, methodical problems could be solved and that all groups were able to master the methods. Consistent data were obtained by the different groups so that partners and methods on soil microbial structure and function are ready for use within the DiverFarming project.

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1. Methodology

The ring trial was started in March 2018. It was decided to test two enzyme activities: dehydrogenase (determined by colorimetric method) and ß-glucosidase (determined by fluorimetric method), and the abundance of one functional gene: *amo*A. In previous discussions, it was agreed that these three selected tests represent best all other analytical techniques on soil microbial parameters that are performed in WP4. Microbial activity methods are represented by the tests on dehydrogenase and ß-glucosidase, one test using colorimetric determination, the other fluorimetric determination. Thus, the two distinct methodical ways of analysing microbial activity are covered by the two tests. The determination of the abundance of the functional gene *amo*A requires extraction, purification and quantification (quality assurance) of DNA extraction, which is also representative for all other DNA-based methods (e.g. with subsequent sequencing). The quantitative time-resolved PCR (qPCR) technique to further analyse the abundance of the *amo*A gene was identified as a specifically challenging task because different qPCR devices were available at the participating instiutions and working groups, respectively. Including methods such as DNA sequencing, earthworm analysis and determination of vegetation cover was considered to be not expedient. This is because sequencing is not done by all participants but only by two selected, and well experienced laboratories/partners, sequencing one beneficiary bacteria and the other fungi; determination of earthworms and of vegetation cover can only be done in the field and not in a small soil sample.

The four testing laboratories involved were Luke Institute, Finland (Tero Tuomivirta); CREA, Italy (Flavia Pinzari, Loredana Canfora, Melania Migliore, Silvia Dell'Orco); CSIC/UPCT, Spain (Margarita Ros, José Antonio Pascual / Onurcan Özbolat, Raúl Zornoza, Marcos Egea-Cortines); University of Trier, Germany (Felix Dittrich, Sören Thiele-Bruhn).

A soil sample was taken by UPCT team from an arable field at Cartagena, Spain, coinciding with the case study LT1. The field moist sample was prepared and homogenized, following the methods described in the "Handbook on WP4 protocols for sampling, sampling procedure and analysis" (D4.1), split in four equal portions and sent by mail to the other three laboratories using dry ice to keep the initial soil characteristics.

The three parameters (dehydrogenase activity, ß-glucosidase activity and the abundance of the *amo*A gene) were determined in the moist soil sample following the protocols explained in the handbook (D4.1 and open access book: https://zenodo.org/record/2553445#.XQI_a4gzZPY). All samples were investigated in triplicate. For some groups and methods, specific training of staff was necessary. During analyes, some technical problems were encountered by some groups. For example, new methods required ordering of necessary chemicals, which constituted an unexpected administrative burden and delay. We realized during this procedure that some details in the handbook of protocols were unclear or not fully unequivocal, making clarifications when necessary. Excel templates for the calculation of final concentrations from measurement data and the evaluation of the standard curves for calibration were created and made available to be used by all the participants. Once the four different testing laboratories

had their results on the three microbiological properties, uploaded the results on Excel files to the Diverfarming common cloud on Microsoft365 OneDrive, so WP4 coordinator could have access to them and assess the results obtained.

2. Results and discussion

The results from the four testing laboratories and the three parameters investigated are shown in Figures 1, 2 and 3. Names of the four laboratories were anonymised by randomly assigning the letters A to D; assignment is similar in all three figures, though. Data on *amo*A gene abundance were determined using the plasmid approach. It can be seen that all groups were able to do the required analyes and received respective results. For each testing laboratory and parameter, the reproducibility was good, standard deviations were small and no outliers were produced.

Standard deviation was ≤ 3.65 (coefficient of variation, CVr ≤ 11) for dehydrogenase activity and ≤19 (CVr ≤ 26) for ß-glucosidase activity. The CVr was ≤ 23 for *amo*A gene abundance. The CVr reported for enzyme actvities and *amo*A gene abundance in references for individual laboratories are in the same range (CVr mean at 15 and maximum at 30) as determined in the ring trial, while standard deviations are – due to higer activities and gene abundances – much larger (for enzyme actvities mean at 40 and maximum at 80) (see references in Figs. 1-3 and Marques et al., 2018).

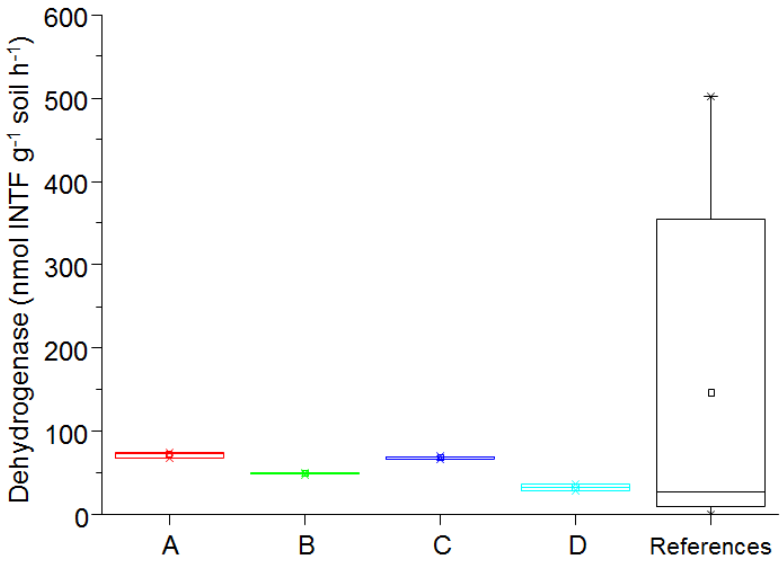

Figure 1. Dehydrogenase activity in an arable field topsoil from Cartagena, Spain determined by four different laboratories (A to D) and in comparison to data on different soils from other studies ('references: Thiele-Bruhn and Beck, 2005; Lahl et al., 2012; unpublished data from Thiele-Bruhn group).

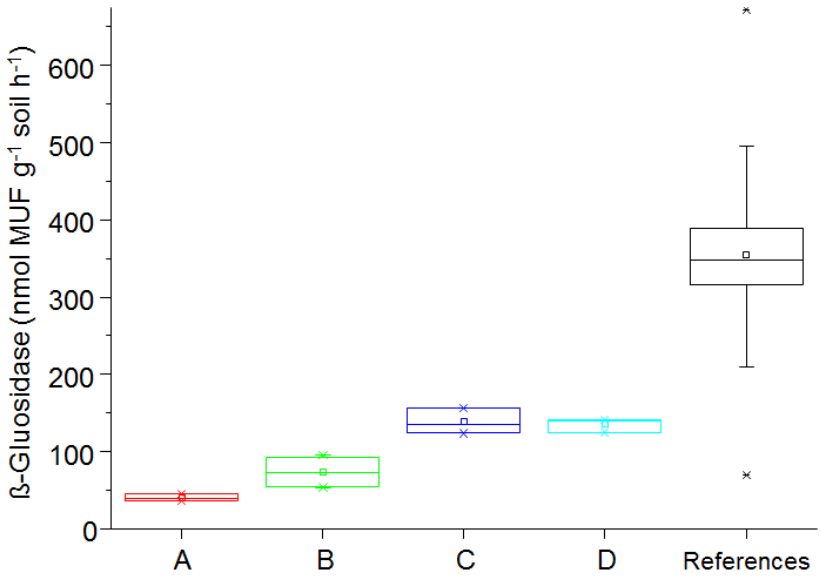

Figure 2. ß-Glucosidase activity in an arable field topsoil from Cartagena, Spain determined by four different laboratories (A to D) and in comparison to data reported in the literature on different soils ('references': Bandick and Dick, 1999; Lahl et al., 2012).

Deviations among groups were in part substantially larger. The overall CVr between groups ranged from 28 (dehydrogenase), 43 (ß-glucosidase) to 93 (*amo*A gene). However, this result is not significant and apperas accurate when comparing the results of this ring trial with the specific soil sample with typical data reported in the literature (see Figs. 1-3). It becomes obvious that the microbial enzyme activity was very low and gene abundance in the soil sample of this ring trial was rather small (note the logarithmic scale in Fig. 3), owing to the semiarid Mediterranean climate of the region where the soil was collected. Reported ranges of the three investigated parameters are most often much larger and the same applies to standard deviations for replicate samples (Marques et al., 2018). So, the observed variation among groups may be due to the low microbial activity and microbial biomass in the selected soil, with values near the detection limit of the method. However, the analysis performance in the different groups was accurate and showed the quality of the different laboratories to continue performing all soil microbial analyses for Diverfarming. Furthermore, this ensures data generated by the different groups can be comparable.

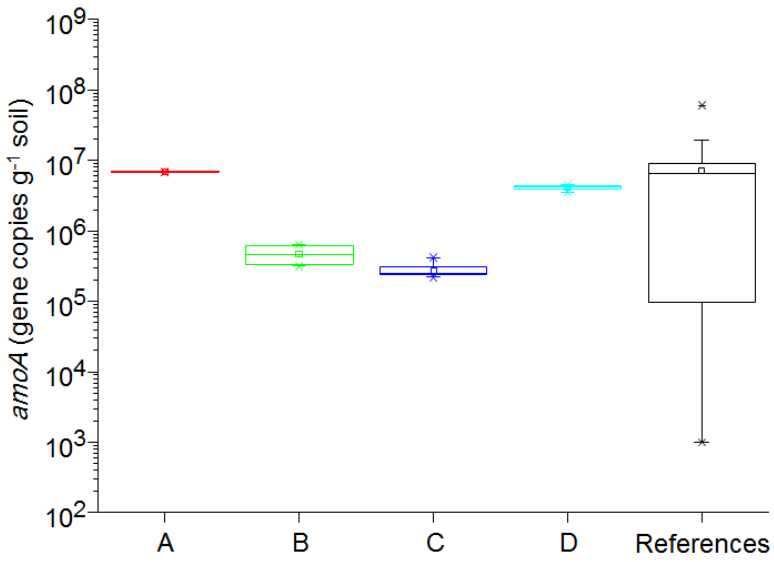

Figure 4. The *amo*A functional gene abundance in an arable field topsoil from Cartagena, Spain, determined by four different laboratories (A to D) and in comparison to data reported on different soils in the literature ('references': Jia and Conrad, 2009; Nicol et al., 2008; Schauss et al., 2009; Wessén et al., 2010).

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