Catchment scale spatial distribution of soil enzyme activities in a mountainous German coniferous forest

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Abstract

Topography features within catchments influence soil properties, nutrient status, microbial dynamics and ultimately enzyme activities. Extracellular soil enzymes are essential for the decomposition of organic substrates and play a central role in global biogeochemical cycles. How topography and soil properties drive the spatial expression of enzyme activities at the catchment scale is still underexplored, especially in coniferous forest ecosystems. This study investigated the activity of four extracellular soil enzymes: β-glucosidase (β-glu), β-cellobiosidase (β-cello), acid phosphatase (pho) and leucine-aminopeptidase (l-leu) in Oh and Ah horizons of a (27 ha) mountainous coniferous forest catchment (Wüstebach, Eifel National Park, Germany). Spatial patterns and "hot spots" of activities of these four enzymes (involved in C-,N-, and P- cycling) were examined in connection to catchment units differing in slope, exposure and soil type (Cambisol vs. Gleysol), and multiple soil parameters (i.e., moisture content, pH, C, N, P, K, Fe, Mn content, C:N, C:P and N:P ratio). Catchment enzyme activities were overall, except for β-cello, significantly higher in the Oh than Ah horizon. Lower β-glu, and l-leu activities were found where more anaerobic soil conditions did occur, e.g., the river valleys (RV). Neither enhanced Oh horizon erosion on steeper Eastern (ES) and Northeastern (NES) slopes nor larger spatial soil nutrients heterogeneity on Northern (NS) and Western (WS) slopes, did significantly affect enzyme activity. Landscape topography did lead to a spatial variation of the activity of the four enzymes examined. The site-specific variation in C-cycling enzymes (β-glu and β-cello) was most marked at drier East, Northeast and Northern slopes, for P-cycling (pho) within the central wetter river valley but was for N-cycling (l-leu) enzyme activity more homogeneously distributed over the whole catchment. Overall, enzyme activities were strongly correlated to soil properties (especially soil moisture and organic carbon), but locations NS (Wüstebach source area) and RV (Wüstebach river flow path) showed less site-specific correlations. Further refinement of site-specific soil and external factors driving spatial

distribution of enzyme activities at catchment scales and beyond will help to further tool up this research at larger spatial scales.

Keywords: enzyme activity, landscape topography, soil moisture, organic carbon, nutrients

1. Introduction

Topography features within catchments are well known to influence soil properties, nutrient status, microbial dynamics and ultimately also enzyme activities (Glendell et al., 2014; Chiwa et al., 2016; Fairbanks et al. 2020). Soil enzymes and their functional diversity play a key role in the relational flows between resource availability, microbial community structure and ecosystem nutrition processes (Caldwell 2005, Kandeler et al. 1996). The enzyme content of soils varies widely, as each soil type has diverse levels and qualities of organic matter, variable nutrient status, differential composition and activity of living organisms and overall intensity of biological processes (Jie et al. 2016, Heitkötter et al. 2017, Piotrowska-Dlugosz et al. 2022). In soils, enzyme activities are essential for degradation of organic matter, mineralization activity, energy conversion and the overall maintenance of nutrient cycles (Dick and Kandeler 2005, Hendriksen et al. 2015, Ottow 2011). Extracellular enzymes have various functions in soil by breaking down and degrading complex organic material into simpler forms and processing complex organic compounds into assimilable subunits (Burns et al. 2003, Caldwell 2005, Sinsabaugh 1994). They are “the proximate agents of organic matter decomposition and measures of their activities can be used as indicators of microbial demand” (Sinsabaugh et al. 2008). As such, they are also a direct expression of the soil community for its metabolic and nutrient requirements (Caldwell 2005; Allison et al. 2011, Burns et at. 2013). The availability of nutrients fluctuates over time, so the given nutrient supply does not necessarily correspond to the microbial or plant nutrient requirements. Thus, one of the main functions of most

extracellular enzymes is a better match to the nutrient supply (from complex chemical resources) with the required nutrient demand. When the supply of available resources is already matched to microbial and plant needs, enzyme production decreases (Allison et al. 2011). Extracellular enzymes are therefore only excreted when substrate is available or there is a need of a special substrate or nutrient for microorganisms and plant roots (Allison et al. 2011, Ottow 2011, Tian et al. 2019).

Soil microbial biomass (SMB) represents only a small fraction (1-5%) of the total content of organic carbon (C), nitrogen (N) and phosphorus (P) and various micronutrients in topsoils, but its turnover rate can be relatively high under optimal site conditions (Ottow 2011). The microbial activity in soils is determined by various factors, including availability and quality of soil organic carbon (SOC) and abiotic factors (e.g. temperature, soil water content). When biochemical processes are stimulated by continuous or short-term high input of organic substrates (e.g. dissolved organic matter) or nutrients (e.g. rhizodeposition), microbial hotspots may be formed (Schimmel and Weintraub 2003, Tian et al. 2019). These are characterized by a phased, very intensive but locally limited increase in microbial activity, often accompanied by a higher extracellular enzymes production leading to enhanced organic carbon and nutrient turnover (Schimmel and Weintraub 2003; Tian et al. 2019).

Soil organic matter (SOM) and nutrient content broadly vary according to climate, topography, vegetation cover, land use and erosion or other disturbances (Blume et al. 2010). The highest C, N and P contents are mostly found in the topsoil, but high spatially heterogeneities occur due to localized litter fall, root inputs or preferential flow path distribution or slope based erosional features (Ottow, 2011). Both climate and forest type had significant effects on soil enzyme activities and microbial communities with considerable interactive effects (Xu et al. 2018). Within a single vegetation type enzyme activity are mostly related to litter input, microbial environment and soil nutrient status (Xu et al. 2018). Remarkably, enzyme activities

were more varied between vegetation types than climate or root proximity effects (He et al. 2020). Slope inclination is an important factor influencing soil development and thickness of the topsoil. It does impact on the extent of soil redistribution known to control the spatial patterns of enzyme activity in erosion-dominated landscapes (Nie et al. 2015; Saraptka et la. 2018). Lower activity being observed in upper eroded sections, but higher enzyme activity occurred in lower slope section where eroded material accumulated. Exponential decrease of enzyme activities due to accelerated soil erosion are reported (Moreno-de las Heras, 2009). These are caused by constraints on development and spatial organization of physical structure and soil biological functionality preventing vegetation development and soil organic matter accumulation. Topographical controls on enzyme activities remained after widescale burning, with direct fire burn effects were most pronounced for the enzyme β-glucosidase at the surface, with moderating interactions of landscape position and depth on potential enzyme activities were observed throughout (Fairbanks et al. 2020). Enzyme activity primarily depended on soil organic matter quality and to a lesser extent on pH, but was not related to climate or land-use in study covering 79 European sites (Hendriksen et al. 2015). Globally, the potential of enzymes for labile C decomposition was related to substrate availability, soil pH and microbial nutrient stoichiometric demand, whereas for recalcitrant C it was most strongly related to soil pH (Sinsabaugh et al. 2008). Overall relationships of enzyme activity with mean annual temperature (MAT) and precipitation (MAP) are generally weak. Enzyme responses in a Mongolian grassland differed significantly under four precipitation treatments, i.e., -30%, normal (control), +30%, and +50% precipitation, with the highest values generally found in the +30% treatment (Akinyemi et al, 2020). The direction of enzyme responses to enhanced or full anaerobic soil conditions or climate related changes to a more oxic soil condition status remains less clear and may depend on soil type (Burns et al., 2013; Piotrowska-Długosz et al. 2022). Furthermore, the driving abiotic and biotic factors that spatially control enzyme

production have not yet been conclusively proven even after 100 years of research history (Allison et al. 2011). We therefore examined in a German small mountainous coniferous forest catchment how topography and location (river valleys vs slopes), slope orientation and angle (steeper Eastern vs gentler Western slopes) and soil type (Cambisols vs Gleysols) spatially influenced enzyme activity. We focused on four enzymes in our study which play a key role in soil C, N and P cycling (Table 1). β-glucosidase and β-cellobiosidase are cellulases and part of the hydrolytic, synergistically acting C enzymes, and produced by soil microorganisms for degradation of cellulose (Deng and Tabatabai 1994, Hendriksen et al. 2015, Ottow 2011). Leucine-aminopeptidase (associated with microbial N uptake) hydrolyses amino acids from polypeptides and provides proteins by splitting amino acids from the peptide chains (Hendriksen et al 2015, Jian et al 2016). Acid phosphatase cleaves PO_4 from P-containing organic compounds and represents the P-Cycle (Eivazi and Tabatabai, 1977). The aim of this study was the quantification of enzyme activities on a catchment scale, to provide information on spatial distribution and enzymatic "hot spots" localization. The following hypotheses were tested:

I. Extracellular enzyme activities are lower where (temporary) anaerobic site conditions occur, for example river valley soils.

II. Enzyme activities are lower in the more sloping parts of the catchment, i.e. Northern Eastern and also Eastern slopes, due to enhanced erosion of the organic horizon.

III. Enzyme activities are more varied on Northern and Western slopes due to the wider differences in overall soil nutrients, with both Cambisols and Gleysols being present.

Materials and methods

2.1 Site Description

142 This study was carried out in the Wüstebach catchment (50.504°N, 6.331°E) (Fig. S1), a small
143 sub catchment of the river Rur, in the south of the Eifel/Lower Rhine Valley observatory of the
144 German climate change research network TERENO (Terrestrial Environmental Observatories).
145 The Wüstebach catchment is located close to the Belgian border in the Eifel National Park,
146 covers 38.5 ha and varies in altitude from 595 m a.s.l. to 628 m a.s.l. (Bogena et al., 2015;
147 2018). The climate can be classified as warm temperate–humid. The average yearly
148 precipitation of the Wüstebach catchment is 1220 mm (1979-1999) (Bogena et al., 2010) and
149 average temperature is 7 °C (Zacharias et al., 2011). The vegetation is dominated by Norway
150 spruce (*Picea abies*) and Sitka spruce (*Picea sitchensis*), which were planted after 1945
151 (Etmann, 2009). In late summer/early autumn of 2013 spruce trees were removed from 9 ha
152 near the riparian zone, i.e., approximately 23% of the total catchment area, to create space to
153 regenerate the endemic beech forest (Bogena et al. 2015). However, the soil sampling took
154 place in June 2013 prior to the deforestation, so the whole catchment was still fully forested
155 (Fig. S1). The soils are formed on Devonian shale bed rock in a silty clay loam parent material
156 with a large fraction of coarser material (0.2 cm to several cm) (Graf et al., 2014; Rosenbaum
157 et al., 2012).
158 The Wüstebach site is located in a syncline valley, the average slope within the catchment is
159 3.6% and the maximum slope is 10.4% (Bogena et al. 2015). The steepest slopes occur near
160 the river valley (RV), with more gently slopes in Northern and Western part of the catchment.
161 Steeper slopes are more frequent in the Eastern than Western part of the catchment (Fig. 1).
162 The soils on the steeper hill slopes are mainly shallow, Cambisols (CM) and Planosols (PL)
163 (Inceptisols in the USDA classification). (Fig. 1, Fig. S1). Histosols (HS) and Gleysols (GL)
164 (Histosols and Inceptisols in the USDA classification) are mostly found in the riparian zone (Fig.
165 1, Fig. S1). Histosols (HS) are only found in very small areas and therefore not included in the
166 enzyme data set. In the western part of the catchment area Cambisols are the dominant the

soil type. In the Eastern area, as well as in direct proximity to Planosols and Gleysols , Cambisol/Planosols are predominant (Fig. 1, Fig. S1). Planosols are located on the river valley, whereas above the groundwater level, periodically dammed water influence occur. Gleysols are formed on the slopes of the river valley and in depressions in the vicinity of the stream.

2.2 Soil sampling

Soil sampling was done in June 2013 as part of a large sampling campaign of the TERENO Project. In total 145 soil samples were collected from a depth of 0cm to 60cm. After the removal of the organic overlay with a 40 x 40 x 30 cm metal frame, the mineral horizons A and B were extracted as drill cores. This resulted in the following subsamples: Two litter layer samples (L/Of, Oh) were collected within frames and subsequently five cores were taken. One with the litter layer, two cores containing only the mineral Ah and B horizons and two cores consisting of the deeper B horizons (Gottselig et al. 2017). For this study, Oh samples were used as for the organic soil and Ah as mineral soil. The samples are stored at -18°C in a cooling container. For the current study we analyzed 70 Oh and 75 Ah samples. There were less Oh than Ah samples as some profiles with Ah did not have a distinguishable Oh horizon.

2.3 Laboratory analysis

The soil samples were defrosted over 24 hours and then a subsample was selected for the enzyme activity measurement. Due to the long storage time after sampling and the freezing-thawing handling lysing of microbial cells might be induced which could impact on the absolute values of enzyme activity in comparison to freshly measured soil samples. We assume however, due to the fact that all treatments/soil samples are handled in the same way, the comparability is still given. The impact of sample storage conditions for enzyme activity measurement was analyzed by Peoples and Koide (2012) who showed that freezing has a lesser

192 impact on enzyme activity in comparison to drying to the basis of fresh material. Wallenius et
193 al. (2010) also compared storage procedures (frozen and dried) with fresh material and
194 detected that freezing lead to a lesser impact on extracellular enzyme activity in mineral soil
195 and organic layer, with an impact strength of less than 20% (Wallenius et al. 2010). Thus, the
196 storage impacts absolute activities but enables a realistic comparison of results due to equal
197 handling. The analysis of the soil enzyme activities (Table S1) involved within different energy
198 and nutrient cycles was performed with a microplate assay according to Marx et al. 2001. This
199 standardized multiplate assay investigates the activity of extracellular enzyme activity under
200 optimal conditions (substrate concentration, temperature, pH) (Marx et al. 2001, Ottow 2011).
201 Procedure in brief, 1g of fresh soil was weight into a sterilized beaker and filled up with 100 ml
202 of sterile water. The soil water solution was treated by an ultrasonic bar at 150 W to solve
203 sorbed enzymes from soil particles. 50 µl of the soil suspension was taken and pipetted with
204 50 µl of 0.1M MES buffer and 0.05M TRIZMA buffer solution and the respective substrate (100
205 µl) for enzyme activity detection into 96-well plates. The specific substrates for enzyme activity
206 measurements involved in the C-cycle were: 4-Methylumbelliferylß-D-Glucoside for β-
207 glucosidase (β-glu) and 4-Methylumbelliferyl-ß-D-Cellobioside for β-cellobiosidase (β-cello),
208 for N-cycling enzymes: L-Leucine-7-amido-4-methylcoumarin hydrochloride for Leucin-
209 aminopeptidase (l-leu), and for P-cycling: 4-Methylumbelliferyl phosphate disodium salt for
210 acid phosphatase (pho). The fluorescence was analyzed at excitation wavelength of 360 nm
211 and an emission wavelength of 440 nm (Marx et al. 2001). The samples were measured after
212 0, 60, 120 and 180 minutes with the Tecan Infinite 200pro microplate reader heated to 30°C
213 arranging optimal microbial enzyme activity conditions (Nannipieri et al. 2018, Tscherko et al.
214 2004, Stemmer et al. 2004). In order to convert the fluorescence values into concentrations a
215 standard series using 4-MUF or 7-AMC and respective buffer solutions were conducted in
216 accordance to Marx et al. 2001 with the concentrations of 0, 100, 200, 500, 800, and 1200

pmol well^{-1}. The soil parameters used in this study included: soil water content, pH-value, total C, N and P and plant available P (P_{cal}), K (K_{cal}), Fe and Mn. The data was downloaded from the links to the Tereno database for the Wüstebach catchment available via Gottselig et al. (2017) and Wu et al. (2017). Details on how these soil parameters were determined can also be found in these two publications.

2.4 Spatial characterization of the catchment

The separation of the research area was made with a height model DGM as open data WMS source in ArcGIS (Fig. S2). The included shading and illumination by a fictitious light source from north-western direction gives indications for the presence of terrain edges, slopes and depressions within the research area. In addition, a layer with contour lines of the ground was included to provide information about the characteristics of the slopes and to uncover possible plateaus within the research area. A final classification based on the slope alignment into five different subareas was made: northern slope (NS), north-eastern slope (NES), western slope (WS), eastern slope (ES) and river valley (RV) (Fig. S2). The river valley was established with the geoprocessing tool "Buffer" and is located around the actual stream. Within the whole catchment, the wetter, weaker sloping river valley area solely consists of Gleysols. The relatively steeper sloping north-eastern slope and eastern slopes encompass mainly Cambisols and Planisols/Cambisols. The latter soils are also predominant on the less steep Northern and Western slopes, but some Gleysols are found near RV. The area more to the western edge of the catchment is flatter than other parts of the Wüstebach catchment (Fig. S2). This subdivision is based on the exposure of the slopes and the orientation of the catena within the area. The spatial distribution of enzyme activities calculated by the geoprocessing tool "Interpolation", using the point-based interpolation method IDW (**I**nverse **D**istance **W**eighted), separated by organic layer (Oh) and mineral topsoil Ah horizon. IDW determines the values of the cells using

242 a linear combination of sample points by giving a large weight to the closest point, which
243 decreases with increasing distance. Consequently, the heavier the weight, the smaller the
244 influence of the points farthest from the measuring point (Pavão et al. 2012). If the average
245 distance has a value that is inferior to the average for a hypothetical aleatory distribution, the
246 distribution of the analyzed thickness points is considered as grouped. And if the average
247 distance is larger than the average for a hypothetical aleatory distribution, the characteristics
248 are dispersed (Pavão et al. 2012).
249
250 **2.5 Statistical analysis**
251 A Pearson correlation matrix was performed to show significant relationships between the
252 individual variables (Lohninger 2012). In advance, the data was tested for their normal
253 distribution and variance homogeneity. In addition, the data was evaluated using a single
254 factor ANOVA with post-hoc test (Tukey). This analysis of variance was used to check whether
255 there are random or recurring differences between the enzyme activities of the respective area
256 and horizons. The significance level was defined as $p < 0.05$.
257
258 **3. Results**
259 The spatial distribution of β-glu activity within both horizons showed localized higher activities
260 at the eastern (ES) sites, and in some areas closer to the Wüstebach stream in the western
261 (WS) slope area(Fig. 1a,b). Lower activity of β-glu was found within the Northern (NS) slope
262 (Fig. 1). For β-cello activity, larger hotspots occurred mainly in the ES and North-Eastern (NES)
263 parts, with smaller hotspots of activity distributed over the whole study area. Within the Oh
264 horizon, lower activity was measured at the western slope (WS), but in the Ah horizon the
265 activity was decreased in the south-eastern part of the western (WS) and northern slope (NS)
266 (Fig. 1cd). Acid phosphatase activity showed significant spatial differences between the two

sampled horizons. While the enzyme activity in the Oh horizon built hotspots in the central river valley (RV) and WS part of the catchment, within the Ah horizon these hotspots shifted mainly to the center area. Low pho activities in the Oh horizon were visible in the ES, southeast (SE) and additionally in WS (Fig 1ef). The activity of l-leu was highest in the Oh horizons on the ES and WS, whereas in NS location the N-cycling enzymes showed only low activities. The spatial distribution of l-leu within the Ah horizons seemed to be more homogeneous considering the total catchment site. There was no discernible spatial focus visible, except the trend towards higher activities in the NES and NS catchment area (Fig. 1g-h).

The activities of ß-glu, pho, l-leu and the sum of enzyme activities (Σenzymes) in Oh horizons were significantly higher than in Ah horizons (Table 1). However, ß-cello activity in the Oh and Ah horizon was similar (Table 1). For the topographic based (slope aspect) only a difference in enzyme activity was found for β-glucosidase, with significant higher activity at the Eastern slope and the lowest values occurring near the river valley (Table 1).

The Oh horizon was characterized by significantly higher total C, total N, total P, C:N, C:P, N:P, P_{cal}, K_{cal} and soil moisture content compared to the Ah horizon (Table 2). In contrast, pH value and Fe_{DPTA} content were significantly higher in the Ah when compared to Oh horizon (Table 2). Within the Oh horizon significant difference between the 'slope' grouping were found for total C, total N, C:P ratio, Fe_{DPTA} and Mn_{DPTA} (Table 2). The value of the first three parameters was the highest on the Western slope. For Fe it occurred in the river valley, whereas for Mn highest values were detected on the northeastern and northern slopes of the catchment (Table 2). The lowest total C and N were found at the northern slope, for C:P ratio and Fe_{DPTA} at the eastern slope, whereas the Mn_{DPTA} value was the smallest in the river valley (Table 2). For the Ah horizon significant differences between the 'slope' grouping were observed for water content and C:P ratio (Table 2). Like in the Oh horizon, C:P ratio was the highest on the Western

slopes. The soil moisture content in the Ah horizon was highest in the river valley and lowest contents were measured at the eastern and north-eastern slopes (Table 2).

The results of the Pearson correlation (Fig. 2) showed a significant correlation of all enzyme activities among each other, only β-cello was solely correlated to β-glu (Fig. 2). All soil parameters, except Mn, were mostly significantly correlated with individual and total enzyme activity, as well as with each other (Fig. 2). Interestingly, iron showed negative correlation to all enzyme activity parameter and to most of other soil properties within the whole dataset. Looking at region specific correlation patterns, NS and RV regions showed different correlation behaviors than the other sites, which were somewhat similar to the correlations pattern of the whole datasets (Fig. 3). Specific enzyme activities and the sum of enzyme activities showed only a few correlations to other soil parameters at the NS and RV region, respectively. At the NS region, pho and the sum of enzyme activities correlated with C/P-ratio; available K and N/P-ratio showed significant correlation to pho, β-glu and sum of enzyme activities (Fig. 3). At the RV, there were the lowest overall correlations detected between enzyme activity and soil parameters. Nevertheless, RV was the only site where Mn showed significant correlations to β-cello and the sum of enzyme activities (Fig. 3).

4. Discussion

4.1 Spatial distribution of enzyme activity in the catchment

The hotspots of C- and N-cycling enzyme activity were generally located on drier slopes within the catchment (Fig. 1 a-dgh). In contrast, the highest localized P enzyme activities were found in the wetter river valley (Fig. 1ef). This partly confirmed our soil moisture related spatial activity hypothesis H1; because pho did not follow our assumption, that enzyme activities would be lower where (temporary) wetter, anaerobic site conditions occur, as found for β-glu, β-cello and l-leu. Landscape position is known to be a strong driver of microbial biomass and

316 community composition due to its control over variations in water and nutrient flow from
317 planar to convergent zones (Brockett et al., 2012, Du et al., 2015, Nemergut et al., 2005).
318 Vertical and lateral redistributions of water and solutes lead to 'hotspot' variations in
319 vegetation type and cover, rates of biogeochemical processing, and various soil properties
320 (Bernhardt et al., 2017, Fairbanks et al. 2020, Lybrand and Rasmussen, 2015). Higher enzyme
321 activities, except for pho, were measured in the drier Cambisols and Cambisol/Planosols
322 compared to wetter Gleysols (Fig. 1). Gleysols are generally more nutrient rich than other soils
323 because they receive solutes via groundwater (Blume et al., 2010). Vegetation also affects soil
324 enzyme activities (He et al. 2020), within the Wüstebach catchment, the slopes are fully
325 forested, but the cover decreases toward the river Valley. Additionally, in Wüstebach areas
326 with lower soil water content, enzyme activity could be increased, as soils there (e.g.,
327 Cambisol) experience more frequently 'drought stress' (Zhou et al. 2014). However, we only
328 found topographically based (slope aspect) differences in enzyme activity for β-glu with higher
329 activity at the Eastern slope and the lower values near the river valley (Table 1). We therefore
330 could not confirm hypothesis II i.e., that enzyme activities were lower in the steeper sloping
331 Eastern and Northeastern parts of the catchment due to enhanced erosion of the organic
332 horizon. We also had to reject our hypothesis III that enzyme activities show higher spatial
333 heterogeneity on Northern and Western orientated slopes, due to the wider variations in
334 overall soil nutrients, because both Cambisols and Gleysols were present there.
335 Looking at the horizon specific differences, significantly higher enzyme activities were
336 measured in the Oh horizon compared to the corresponding Ah horizon (Fig. 1 and Table 2),
337 related to the regular input of fresh organic matter inputs via litter and associated which causes
338 a higher microbial biomass and activity (Allison et al. 2011, Burns et al. 2012, Ottow 2011, Xu
339 et al. 2018). However, evaluated more a geospatial basis, the lowest C contents were measured
340 in NS, ES and NES (Table 2), but associated here with elevated activities of the C-cycling

341 enzymes (β-glu and β-cello; Fig. 1). Still, Grandy et al. (2007) noted that significant correlations
342 between enzymes and substrates may not always occur, as soil organic matter chemistry
343 reflects the history of long-term decomposition processes, while enzyme dynamics fluctuate
344 more with current conditions. In line with other studies pointing to the well-known fact that C
345 'deficiency' increases C enzymes activity to meet the microbial energy and nutrient demand
346 (Allison et al. 2011, Bueis et al. 2017, Burns 1978).
347 The lowest P concentrations were found in NS, ES and NE in the Oh and RV for the Ah horizon
348 (Table 2) inducing an increased phosphatase activity on the RV site due to P deficiency or low
349 P availability (Allison and Vitousek 2005, Bueis et al. 2017, Burns 1978).
350 The l-leu activity was increased in the Oh horizon in the ES, NE, and WS (Table 1), where the
351 lowest nitrogen levels were found (Table 2). Within the Ah horizon, increased activities of l-
352 leu are found in all subareas toward the edge of the catchment. Low N contents are found
353 only in the North-Eastern slope (Table 2). Lower soil N and higher C:N ratio may enhance
354 cellulose degradation (e.g., β-glu, β-cello), subsequent higher C presence and implicit lower
355 C:P ratio may thus overcome P-deficiency (Allison et al. 2011 Weand et al. 2010). β-glu had
356 high activities in both Oh and Ah horizons, and β-cello in the Ah horizon in ES. However,
357 although the highest total N was measured within the Oh horizon in the ES, for the A horizon
358 the highest value was measured in RV. Since the highest (ES) and lowest (RV) activities of the
359 extracellular enzymes were measured there, it is difficult to assess the role of soil N content
360 on the spatial enzyme activity in our study. One assumption for the low enzyme activities in
361 the near stream area would be that the soil C, N and P present are already bioavailable and
362 non-limiting. Therefore, microbes would not benefit economically from investing in enhanced
363 enzyme production in this part of the catchment (Weand et al. 2010). It is also possible that
364 nitrogen was transported downhill into the river valley with the leachate or surface runoff.

365 The decrease of l-leu activity with increasing N-availability may indicate an achieved N-
366 limitation of the microorganisms (Andersson et al. 2004).
367
368 **4.2 Enzyme activity and soil parameter relationships**
369 The enzyme activity was, with the exception of β-cello, significantly correlated with most soil
370 parameters (Fig. 2). The β-glu, pho and l-leu were significantly positively correlated with the
371 humus content, i.e. soil enzyme activity did increase with higher soil organic carbon content.
372 This was also shown in various other studies (Allison et al. 2011, Blume et al. 2010, Ottow
373 2011). Higher organic C contents were measured in the Oh horizon compared to the Ah
374 horizon. For the Oh horizon, the highest SOC contents were generally found around the RV, but
375 for the Ah horizon they occurred at the WS of the catchment. The organic C content in the
376 Wüstebach soil is known to show a high horizontal variance and a pronounced decrease with
377 depth (Gottselig et al. 2017). Total C and soil moisture were also significantly correlated (Fig.
378 2), likely because under anaerobic conditions the degradation of organic C is prevented, which
379 leads to topsoil C accumulation (Gottselig et al. 2017, Kalbitz et al. 2000). In contrast, the
380 drying-out of soil leads to osmotic stress and this may impact enzyme activity (Burns et al.
381 2012). The inverse relationship between inorganic P availability and pho activity has been
382 observed, depending on actual labile/bioavailable P (Weintraub and Schimel 2005; DeForest
383 et al., 2012). Similar to pho, the activities of N enzymes such as peptidases are stimulated by
384 low N-availability but inhibited by high concentrations of inorganic N in many systems (e.g.,
385 Allison et al. 2011). Within conifers forest, like those in the Wüstebach, a large amount of N is
386 bound in SOC and immobilized (Korhonen and al. 2013).
387 Available iron (Fe_{DPTA}) had a significant negative correlation with all enzymes, except ß-cello,
388 hence enzyme activity decreased with increasing soil Fe contents (Fig. 2). Heavy metals can
389 become mobile in soils with low pH or redox values (Blume et al. 2010) like the Gleysols present

390 the Wüstebach. However, the fact that β-cello was not significantly affected by soil Fe content
391 it is in line with Niemi and Vepsäläinen (2004), who noted that the activity of ß-cello was
392 highest in the forest samples with high heavy metal concentrations. Although none of the
393 enzyme activity data were correlated to Mn within the whole dataset, Mn showed significant
394 correlation with β-cello ($p < 0.01$) and the sum of all enzyme activities ($p < 0.05$) at RV site (Fig.
395 3). This site is located in the lowest parts of the catchment which is directly impacted by the
396 river and associated soil water fluctuations (Bogena et al. 2014; 2018). This might be the
397 reason for higher Mn demand due to building up Mn peroxidase by microorganisms to
398 decompose organic matter at this site (Hemkemeyer et al. 2021). The influence of higher soil
399 moisture on increasing Mn peroxidase activity was also described by Baldrian et al (2010a)
400 who detected a strong dependency of soil moisture and microbial biomass regulating specific
401 enzyme production. The excretion of Mn peroxidases for organic matter break down is
402 dominated by fungi (Baldrian et al. 2010b, Hofrichter 2002, Wong 2009), which may indicate a
403 stronger contribution of fungi within the microbial community at the RV site (Baldrian et al.
404 2010b).
405 Available K (K_{cal}) in our study was significantly positively correlated with ß-glu, pho and l-leu
406 (Fig. 2). Blume et al. (2010) noted that potassium (here K_{cal}) is a key nutrient in terms of enzyme
407 activation and had a strong regulatory effect on osmotic stress balance within microbial cells
408 (Csonka 1989). The presence of a certain amount of K in the soil stimulates enzyme activity,
409 especially pho activity, to ensure an improved plant nutrient supply. Symanowicz et al. (2018)
410 did also show that fertilizing the soil with different K concentrations always led to an increase
411 in (acidic) pho activity. Although high K levels were found at the river valley (Oh horizon) and
412 the Northern slope, where ß-glu, pho and l-leu had low activities, especially within the river
413 valley (RV) (Fig. 3). However, in the Ah horizon of NS, indeed the highest pho activity was

414 measured. Consequently, high soil available K (K_{cal}) does not necessarily imply a high pho, ß-
415 glu, or l-leu activity and vice versa for the investigated Wüstebach area (Fig 2; 3).
416
417 5. **Conclusions**
418 The large scale Wüstebach field campaign shed light on the spatial distribution of enzyme
419 activities across different parts of the catchment. Our research thereby showed that β-glu and
420 l-leu activity was distinctly affected by water content with the lowest values in the river valley
421 (RV). Neither enhanced Oh horizon erosion on steeper Eastern (ES) and Northeastern (NES)
422 slopes, nor larger spatial soil nutrients heterogeneity on Northern (NS) and Western (WS)
423 slopes, did significantly affect enzyme activity. Catchment enzyme activities were overall,
424 except for β-cello, significantly higher in the Oh than Ah horizon. The site-specific variation in
425 C-cycling enzymes (β-glu and β-cello) was most marked at the drier ES, NES and NS slopes.
426 While N-cycling (l-leu) enzyme activity was much more homogeneously distributed over the
427 whole catchment, the P-cycling (pho) enzyme activity was the highest within the central wetter
428 river valley. Enzyme activities were strongly correlated to soil properties (especially soil
429 moisture and organic carbon), but locations NS and RV showed less site-specific correlations
430 due to nutrient heterogeneity and anaerobic conditions, respectively.
431

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602 **Table 1** *Catchment and topography-based soil enzyme activity of the Oh and Ah horizon*

Enzyme Activity *nmol g^{-1} h^{-1}*	***Horizon***	***Whole Catchment (all slopes)***	***Eastern Slope (ES)***	***North-Eastern Slope (NES)***	***Northern Slope (NS)***	***Western Slope (WS)***	***River Valley (RV)***
β-glucosidase	Oh [(+)]	**610**[*]	**781**[**]	**535**	**420**	**656**	**395**
		(467)	(385)	(221)	(211)	(609)	(145)
	Ah	**300**	**478**[a] [***]	**228**[b]	**248**[b]	**288**[ab]	**209**[b]
		(253)	(438)	(124)	(115)	(174)	(63)
β-cellobiosidase	Oh	**74**	**80**	**85**	**64**	**73**	**53**
		(45)	(37)	(53)	(34)	(48)	(25)
	Ah	**68**	**75**	**66**	**67**	**69**	**43**
		(24)	(25)	(22)	(28)	(20)	(6)
Phosphatase (acid)	Oh [(+)]	**8626**	**9671**	**8347**	**7335**	**8672**	**8481**
		(3183)	(3438)	(2940)	(1849)	(3504)	(747)
	Ah	**5000**	**5146**	**4639**	**5320**	**5008**	**5038**
		(2117)	(2451)	(2062)	(2020)	(1959)	(1159)
leucine-aminopeptidase	Oh [(+)]	**267**	**333**	**262**	**218**	**264**	**194**
		(120)	(134)	(102)	(92)	(117)	(81)
	Ah	**177**	**188**	**182**	**172**	**172**	**156**
		(97)	(72)	(128)	(85)	(89)	(40)

603 [(+)] Overall catchment enzyme activity in Oh significantly higher than Ah horizon (p <0.001)
604 [*] Mean values in bold and standard deviation in brackets
605 [**]maximum and minimum value for each class are double and single underlined, respectively
606 [***] indicate significant difference between the 'slope' groups in enzyme activity
607
608 The whole catchment samples numbers were Oh (n=70) and Ah-horizon (n = 75). For Oh horizon ES
609 (n=14), NES (n=13), NS (n=9), RV (n=5) and WS (n=29). For Ah horizon ES (n=15), NES (n=20), NS (n=15),
610 RV (n=2) and WS (n=23)

611 **Table 2** *Catchment and topography-based soil parameters of the Oh and Ah horizon*

Horizon + Area	Total C	Total N	Total P	C:N	C:P	N:P	P_{CAL}	K_{CAL}	Fe_{DPTA}	Mn_{DPTA}	pH	Moisture
	%dw	%dw	%dw				mg P kg soil^{-1}	mg K kg soil^{-1}	mg Fe kg soil^{-1}	mg Mn kg soil^{-1}	Value	%[grav.]
Oh	**25.7**^{A}	**1.25**^{A}	**0.086**^{A}	**20.4**^{A}	**299**^{A}	**14.6**^{A}	**234**^{A}	**63.6**^{A}	**985**^{B}	**37.5**	**3.07**^{A}	**61.2**^{A}
	(8.7)	(0.37)	(0.014)	(2.5)	(107)	(4.5)	(111)	(44.9)	(249)	(32.7)	(0.22)	(8.5)
Ah	**10.2**^{B}	**0.56**^{B}	**0.066**^{B}	**18.0**^{B}	**155**^{B}	**8.5**^{B}	**72**^{B}	**8.6**^{B}	**1278**^{A}	**30.8**	**3.27**^{B}	**42.6**^{B}
	(5.4)	(0.28)	(0.019)	(2.9)	(68)	(2.8)	(42)	(10.9)	(259)	(50.2)	(0.26)	(9.8)
Oh-ES	**23.0*****	**1.16*****	**0.090**	**19.7**	**254*****	**12.8**	**216**	**53.3**	**894*****	**43.2*****	**3.14**	**61.3**
	(7.3)	(0.33)	(0.013)	(2.5)	(72)	(3.0)	(86)	(23.6)	(243)	(19.4)	(0.16)	(9.5)
Oh-NES	**22.7**	**1.10**	**0.085**	**20.4**	**275**	**13.2**	**233**	**67.4**	**1015**	**67.5**	**3.01**	**59.5**
	(7.4)	(0.30)	(0.011)	(1.7)	(112)	(4.4)	(62)	(34.9)	(287)	(43.4)	(0.21)	(8.1)
Oh-NS	**20.9**	**0.98**	**0.074**	**19.8**	**263**	**12.3**	**145**	**43.0**	**954**	**30.3**	**2.80**	**53.9**
	(7.9)	(0.38)	(0.022)	(6.3)	(88)	(4.0)	(50)	(35.2)	(410)	(27.3)	(0.80)	(16.0)
Oh-RV	**24.9**	**1.33**	**0.086**	**18.6**	**297**	**16.0**	**206**	**78.1**	**1030**	**14.4**	**3.18**	**67.5**
	(7.0)	(0.31)	(0.017)	(1.1)	(80)	(4.6)	(51)	(33.6)	(121)	(6.0)	(0.32)	(7.9)
Oh-WS	**29.9**	**1.43**	**0.088**	**20.8**	**342**	**16.4**	**278**	**75.0**	**960**	**25.9**	**3.08**	**62.4**
	(8.7)	(0.3)	(0.016)	(2.2)	(115)	(4.7)	(138)	(57.0)	(233)	(22.7)	(0.23)	(7.2)
Ah-ES	**9.7**	**0.55**	**0.070**	**17.6**	**137*****	**7.7**	**70**	**10.0**	**1206**	**39.5**	**3.37**	**40.8*****
	(5.4)	(0.32)	(0.017)	(1.9)	(51)	(2.6)	(35)	(10.0)	(282)	(35.9)	(0.32)	(9.7)
Ah-NES	**8.9**	**0.48**	**0.066**	**18.1**	**136**	**7.4**	**69**	**6.7**	**1214**	**39.6**	**3.29**	**40.4**
	(3.9)	(0.16)	(0.009)	(3.6)	(57)	(2.5)	(24)	(7.3)	(274)	(45.1)	(0.27)	(8.7)
Ah-NS	**10.4**	**0.60**	**0.070**	**17.3**	**148**	**8.4**	**90**	**12.0**	**1331**	**49.9**	**3.22**	**41.1**
	(5.5)	(0.31)	(0.026)	(2.6)	(52)	(2.1)	(57)	(13.5)	(219)	(86.1)	(0.15)	(8.2)
Ah-RV	**12.7**	**0.85**	**0.077**	**14.9**	**167**	**11.2**	**65**	**9.5**	**736**	**3.0**	**3.24**	**65.0**
	(1.0)	(0.07)	(0.005)	(0.0)	(24)	(1.6)	(3)	(1.7)	(343)	(0.8)	(0.42)	(0.1)
Ah-WS	**11.2**	**0.58**	**0.059**	**18.9**	**187**	**9.7**	**66**	**7.0**	**1348**	**10.6**	**3.23**	**44.6**
	(6.3)	(0.29)	(0.021)	(2.6)	(83)	(3.0)	(46)	(11.9)	(256)	(5.9)	(0.20)	(9.2)

612 * Mean values in bold with standard deviation in brackets, **maximum and minimum value for each class are double and single underlined, respectively, ***
613 indicate significant difference between the' slope' groups in soil parameters, A vs. B indicate significant difference between horizons in soil parameters
614 The whole catchment samples numbers were Oh (n=70) and Ah-horizon (n = 75). For Oh horizon ES (n=14), NES (n=13), NS (n=9), RV (n=5) and WS (n=29).
615 For Ah horizon ES (n=15), NES (n=20), NS (n=15), RV (n=2) and WS (n=23)

Figure legend

Fig. 1 Spatial distribution of enzyme activities (ß-glucosidase, ß-cellobiosidase, acid phosphatase, leucine-aminopeptidase) within the research area, areal calculation with ArcGIS. Left: Oh horizon, right: A-horizon. Geoprocessing-Tool: IDW Interpolation. (Own presentation, calculation with ArcGIS, 2020).

Supplementary figure legend

Fig. S1 Wüstebach and reference stream in 2013 (Adapted from Bogena et al., 2018) and its most important soil types and instrumentation (Adapted from Bogena et al., 2021)

Fig. S2 Study area, subdivided by slope exposure. Insert relative contribution of each soil type within each catchment slope area (Own presentation with ArcGIS, Geobasis NRW 2019)

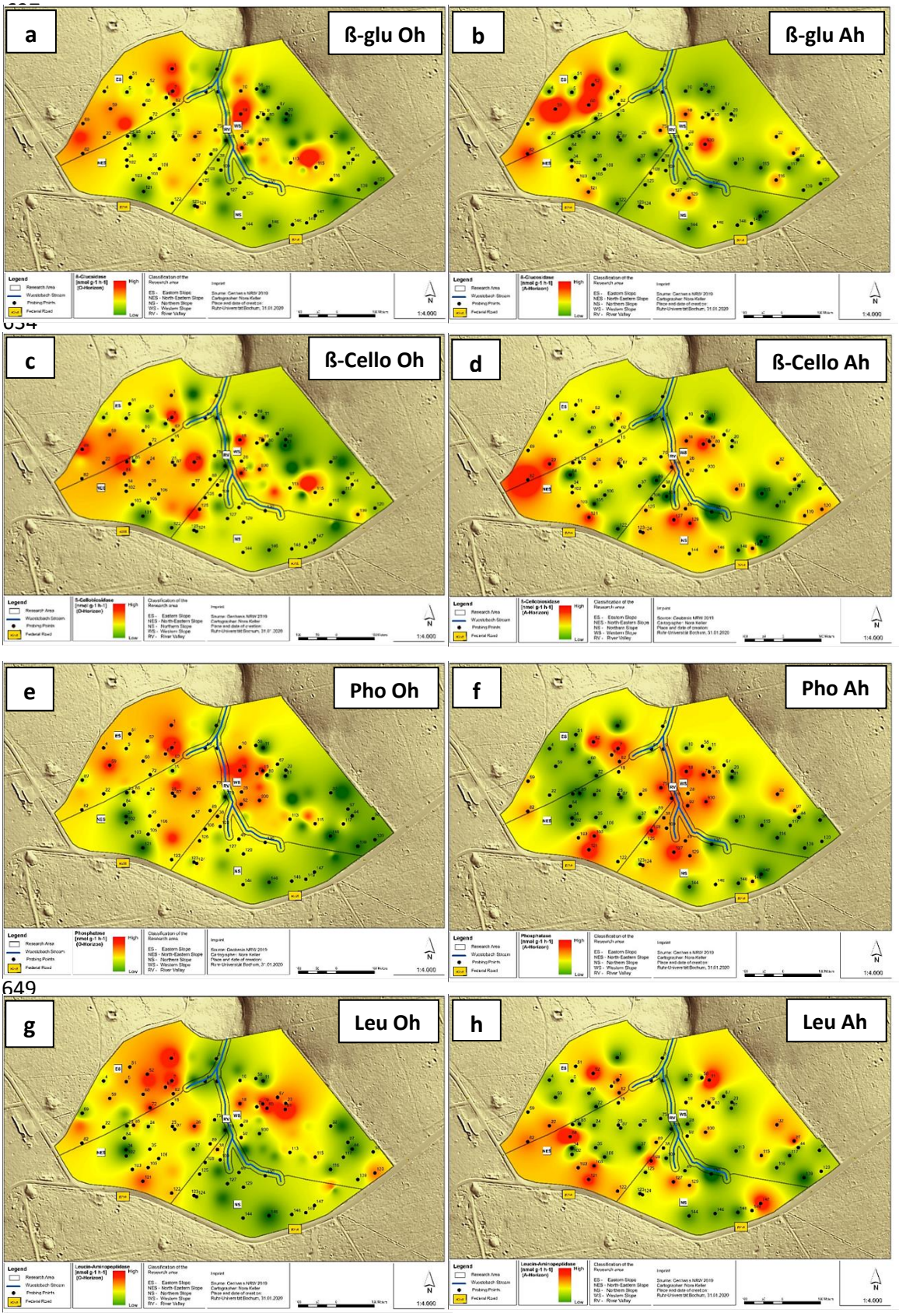
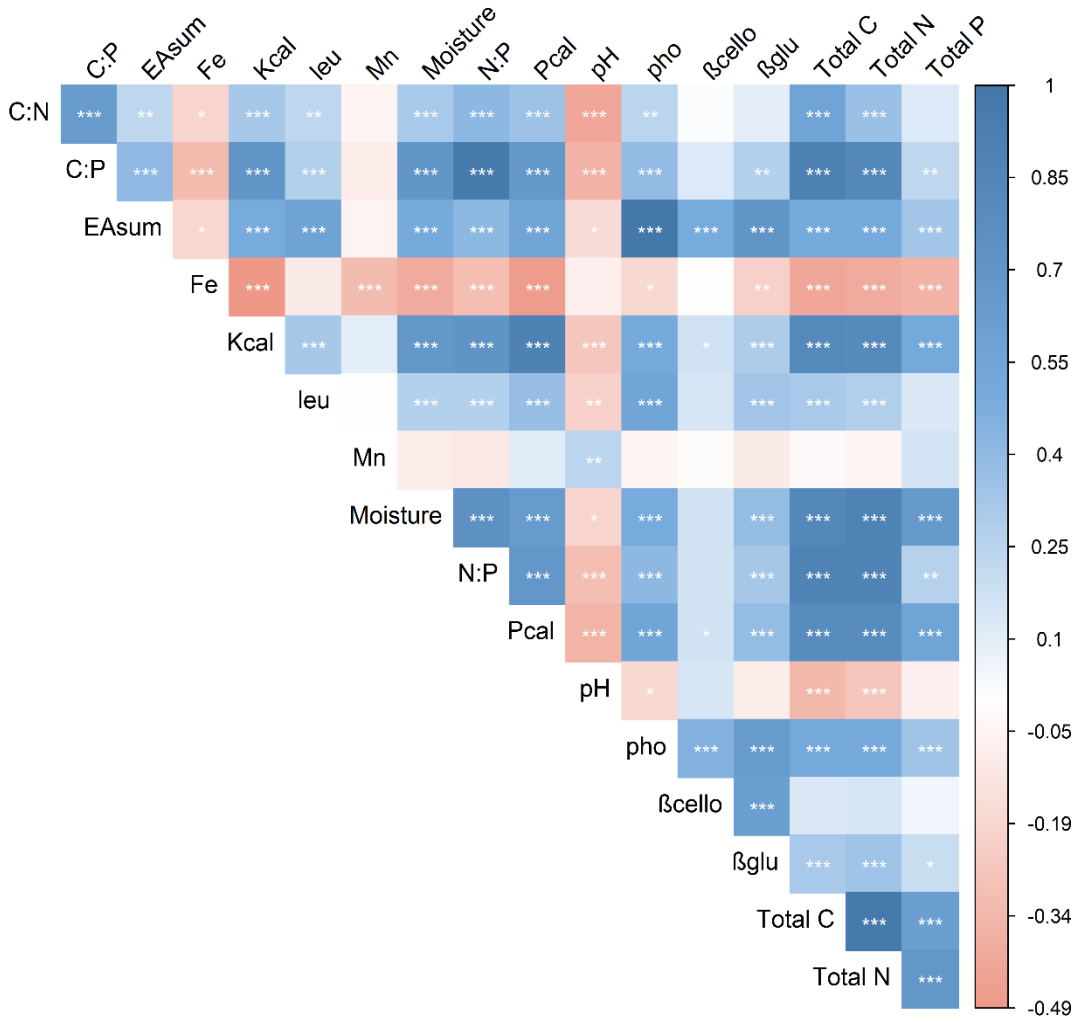


Fig. 1: *Spatial distribution of enzyme activities - ß-glucosidase (1a, 1b), ß-cellobiosidase (1c, 1d), acid phosphatase (1e, 1f), leucine-aminopeptidase(1g, 1h) within the whole catchment research area, areal calculation with ArcGIS. Left: Oh horizon, right: Ah horizon. Geoprocessing-Tool: IDW Interpolation. (Own presentation, calculation with ArcGIS, 2020).*

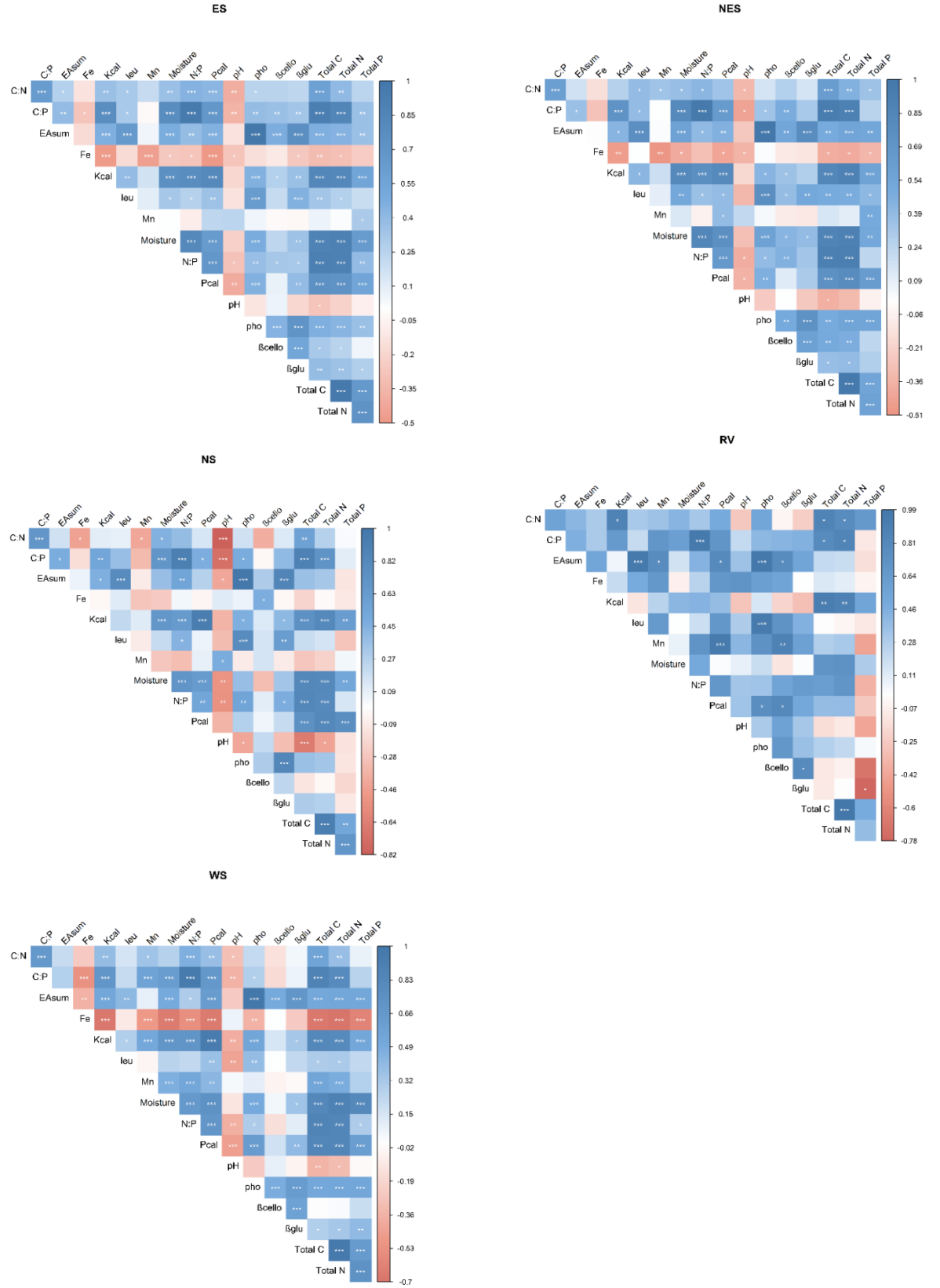
661



662

663 Fig. 2: Pearson correlation of the entire dataset (*p <0.05; **p <0.01, ***p <0.001)

Fig. 3. Pearson correlations of soil parameters and enzyme activities separated for each site (Eastern Slope (ES), Northeastern Slope (NES), Northern Slope (NS), River Valley (RV), Western Slope (*p <0.05; **p<0.01, ***p<0.001)



Supplementary Figures

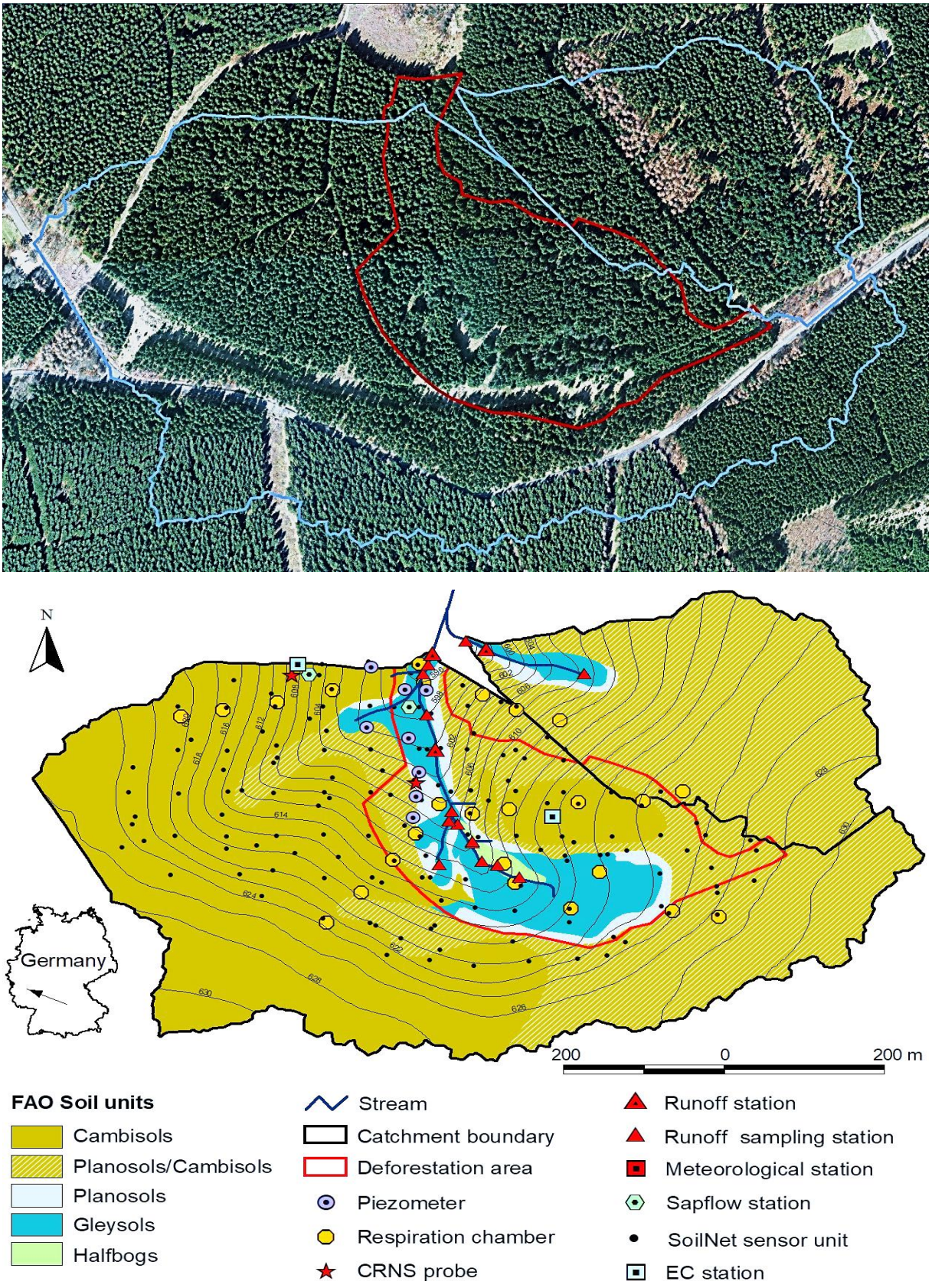


Fig. S1: Top: Wüstebach and reference stream in 2013 (Adapted from Bogena et al., 2018). Bottom: Its most important soil types and instrumentation (Adapted from Bogena et al., 2021). The black dots mean probing points.

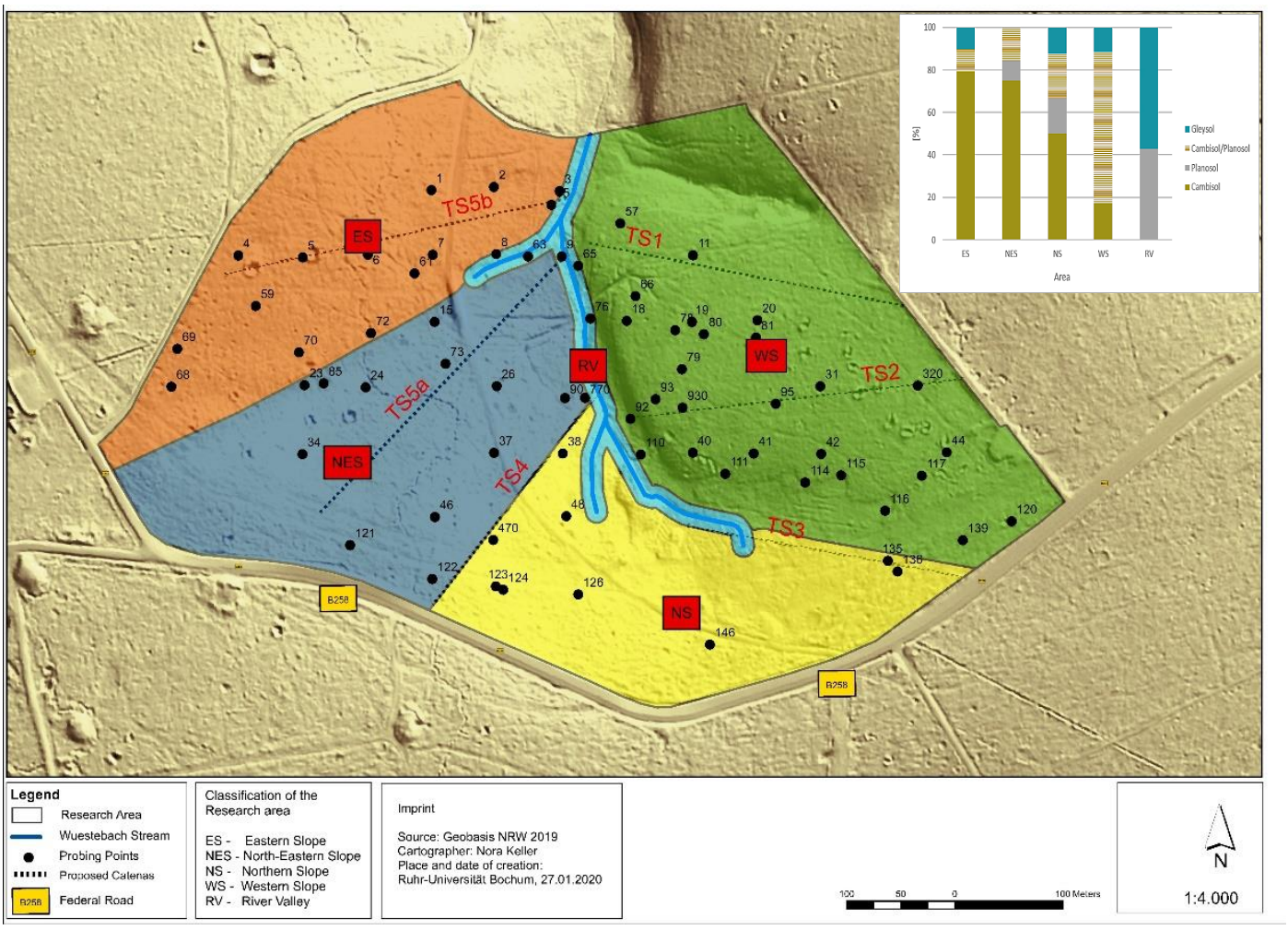


Fig. S2 Study area, subdivided by slope exposure. Eastern slope in orange, North Eastern slope in blue-gray, North Western slope in yellow, Western slope in green and River Valley in light blue. Insert relative contribution of each soil type within each catchment slope area (Own presentation with ArcGIS, Geobasis NRW 2019).

699 ***Table S1.*** *Overview of function and reaction of selected soil enzymes*

Enzyme Acronym Classification	Function Cycle	Reaction	Products
1,4-ß-glucosidase ß-glu EC 3.2.1.21	Cellulose degradation, energy provision (Glucose) Carbon cycle	Hydrolysis of cellobiose to glucose and terminal, non-reducing beta-D-glucosyl residues with release of beta-D-glucose	Glucose
1,4-ß-cellobiosidase ß-cello EC 3.2.1.91	Cellulose degradation Carbon cycle	Hydrolysis of (1->4) cellobiose dimers from the non-reducing ends of cellulose molecules	Cellobiose
phosphatase (acid) pho EC 3.1.3.2	Phosphate mobilisation Phosphorus cycle	phosphomonoesters hydrolysis releasing phosphate	ROH + Phosphate
leucine-aminopeptidase l-leu EC 3.4.11.1	Protein degradation Nitrogen cycle	Hydrolysis of hydrophobic amino acids from the N terminus of polypeptides and splitting of amino acids from peptides (proteins)	Amino acids, Proteins

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