



# Small-Scale Spatial Variability of Soil Chemical and Biochemical Properties in a Rewetted Degraded Peatland

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There is indication in the literature that degradation of natural peatlands reduced spatial variability of soil chemical and biochemical properties. However, we lack empirical data on the impact of rewetting peatland on the spatial variability of these properties. We investigated the spatial variability of the soil properties of a peatland that has been used for extensive and intensive grazing from 1400 to 1970. The peatland has been rewetted since 1970, and we collected 50 soil samples from 50 grid cells of 0–10, and 10–20 cm soil depths in October 2001. We measured 33 important soil chemical and biochemical properties and evaluated the data with descriptive and geospatial statistical analyses. The concentrations of most plant available nutrients were low with high coefficients of variation (CV) that ranged from 15 to 117%, whereas the CV of most of the total and oxalate extracted elements was  $\leq 15\%$  CV. The degree of phosphorus (P) saturation (DPS) and P saturation ratio (PSR) were 11% and 0.05, which were low as compared to the threshold levels of 25% DPS and 0.11 PSR for mineral and wetland soils. The microbial biomass C and N ranged from 389 to 2,463 mg kg<sup>-1</sup> and 32 to 215 mg kg<sup>-1</sup> at the depth of 0–10 cm and from 343 to 1570 mg kg<sup>-1</sup> and 14 to 160 mg kg<sup>-1</sup> at the depth of 10–20 cm, respectively. Similarly, the dehydrogenase and  $\beta$ -glucosidase activities were lower by 76 and 61% at the soil depth of 10–20 cm compared to the upper 10 cm. The geospatial statistical analysis revealed that 87% of the soil chemical properties were spatially correlated and 85% of the spatial correlation was strong with  $< 0.20$  nugget to sill ratio at 5 to 12 m ranges. Similarly, 86 and 71% of the biochemical properties were strongly spatially correlated at the depth of 0–10, and 10–20 cm, respectively, with  $\leq 0.16$  nugget to sill ratio at the short ranges (4 to 6 m). The strong spatial correlation of most of the soil chemical and biochemical properties at short ranges indicate the high variability of the rewetted peatland.

**Keywords:** biochemical properties, oxalate extractable soil nutrients, soil variability, spatial correlation, nugget

## INTRODUCTION

Soil chemical and biochemical properties may vary strongly from small scale to large scale that influence services and functions obtained from peatland ecosystems (Jenerette and Wu, 2004). The heterogeneity is apparent in peatlands at landscape, habitat, and microscales (Larkin, 2016). A previous study showed that spatial heterogeneity of soil chemical and biochemical properties can be

reduced after degradation of natural peatlands (Brooks et al., 2005). However, rewetting degraded peatlands could create spatially heterogeneous soil properties (Gallardo, 2003). For instance, alternation of water loads to peatlands can alter soil chemical and biochemical properties (Kercher and Zedler, 2004) and plant community composition (Mentzer et al., 2006) thereby nutrient transformations and release not only at the landscape scale but also at small scale. The success of restoration of degraded peatland by rewetting can depend on the type of peatland, intensity of initial degradation, peat characteristic, biota community, and climatic condition (Joosten and Clarke, 2002; Höper, 2007). These important biotic and abiotic factors can cause spatial variability that could influence the biogeochemistry of natural and rewetted peatland ecosystems (Whiting and Chanton, 2001; Bubier et al., 2003).

Most European countries, including Germany, have degraded more than 85% of their original peatlands (Joosten, 1997; Lamers et al., 2015; Joosten et al., 2017). It has been estimated that 930 000 ha of peatlands have been drained to increase the area available for agriculture in Germany and about one-third of the degraded peatlands is located in Mecklenburg–West Pomerania, northern Germany (Förster, 2009). However, considerable efforts have been undertaken to restore the degraded peatlands since 2000. Between 2000 and 2008, about 10% of the degraded peatlands was rewetted (Förster, 2009). Although the biogeochemistry of peatlands has been researched intensively (Reddy and DeLaune, 2008; Strack, 2008; Landry and Rochefort, 2012), no information is available on the influence of rewetting degraded peatlands on spatial variability of soil chemical and biochemical properties.

A number of studies have been conducted to understand the effects of peatland restoration on chemical properties and greenhouse gas emissions (Höper et al., 2008; Couwenberg, 2009; Haapalehto et al., 2011; Krüger et al., 2015; Karki et al., 2016; Herzsprung et al., 2017), bioavailable nutrients (Dietrich and MacKenzie, 2018), water table depth and vegetation composition (Bantilan-Smith et al., 2009; Haapalehto et al., 2011; Görn and Fischer, 2015). Most previous studies investigated chemical and biochemical properties in contrasting peatland management systems (Groffman et al., 1996; Bruland et al., 2006; Dick and Gilliam, 2007; Nkheloane et al., 2012), microbial colonization, and activities in constructed wetlands (Hunt et al., 1997; Truu et al., 2009), rewetted (Baum et al., 2003; Andersen et al., 2006), and natural peatlands (Gutknecht et al., 2006). Studies conducted in rewetted peatlands also mostly focused on methane, nitrous oxide, and carbon dioxide emissions (e.g., Waddington and Roulet, 1996; Dasselaar et al., 1998; Krohn et al., 2017). A few studies also investigated the phosphorus status in degraded peatland and wetland soils (Litaor et al., 2003; Zak et al., 2008; Nair, 2014; Emsens et al., 2017).

Soil spatial variability can develop from uneven litter decomposition, vegetation composition, soil moisture content, topographic position, and historical land use, and soil management practice (Baldrian, 2014). These factors could influence chemical and biochemical processes differently in different peatlands. For example, dehydrogenase enzyme mostly operates under anaerobic soil conditions (Wolinska and Stepniewska, 2012), whereas protease and acid phosphatase

activities decline as soils become more anaerobic (Reddy and DeLaune, 2008). Furthermore, the rate of synthesis, release and stability of phosphatase depends on soil pH, and soil organic matter (SOM) (Tabatabai, 1994; Baldrian, 2014). The sensitivity of a soil enzyme to pH changes is variable from one soil enzyme to another (Acosta-Martínez and Tabatabai, 2000). A few studies conducted on soils in mountain forest indicated that acid phosphatase and  $\beta$ -glucosidase activities were spatially correlated at the range of 3 m (Yang et al., 2018), whereas, microbial biomass showed spatial variability within 6 m (Štursová et al., 2016). Similarly, soil pH and total N showed strong spatial correlation in the range of  $\sim$ 0.4 m to a few meters because of variations in soil moisture, vegetation, soil, and management practices (Baldrian, 2014).

Vegetation composition and distribution can significantly influence soil chemical and biochemical properties, and thereby restoration of rewetted degraded peatlands (Borga et al., 1994; Boon et al., 1996; Dick and Gilliam, 2007; Wiedermann et al., 2017). For instance, restoration of a degraded peatland depends on the recolonization of the original flora, and fauna that play a leading role in peat accumulation and nutrient cycle. Understanding the plant community composition and distribution is particularly important in a rewetted peatland for monitoring a success of a degraded peatland restoration process (Zerbe et al., 2013).

Employing conventional soil sampling and statistical analysis such as composite sampling with the assumptions of sample independent, normal sample distribution, and a randomized experimental design have less power in detecting spatial variability of soil properties than geospatial analysis (Rossi et al., 1992; Kravchenko et al., 2006; Nkheloane et al., 2012). Suitable geospatial analysis can help detect spatial variability of soil chemical and biochemical properties. The geospatial analysis has been also used successfully in detecting spatial distributions of SOM (Kravchenko et al., 2006; Kumar, 2015; Liu et al., 2015). The results of many studies indicated that correlation, linear regression and regression kriging captured spatial variability of mineral soils (Kumar et al., 2012; Wang et al., 2013). However, only a few studies used such a statistical tool to understand spatial correlation of soil chemical and biochemical properties in peatlands (Nkheloane et al., 2012; Marton et al., 2015).

The scale at which samples are collected to study spatial variability of soil chemical and biochemical properties can influence interpretations of biogeochemical processes in peatlands (Hunt et al., 1997; Gutknecht et al., 2006). To understand spatial variability in rewetted peatland, small-scale soil sampling is required as the soil environments could be heterogeneous depending on management practices and historical land uses (Klironomos et al., 1999; Truu et al., 2009). However, little information is available on spatial variability of soil chemical, and chemical properties at small scale in rewetted peatlands. Therefore, the objective of the present study was to investigate the spatial variability of selected soil chemical and biochemical properties and vegetation composition of a rewetted degraded fen peatland.

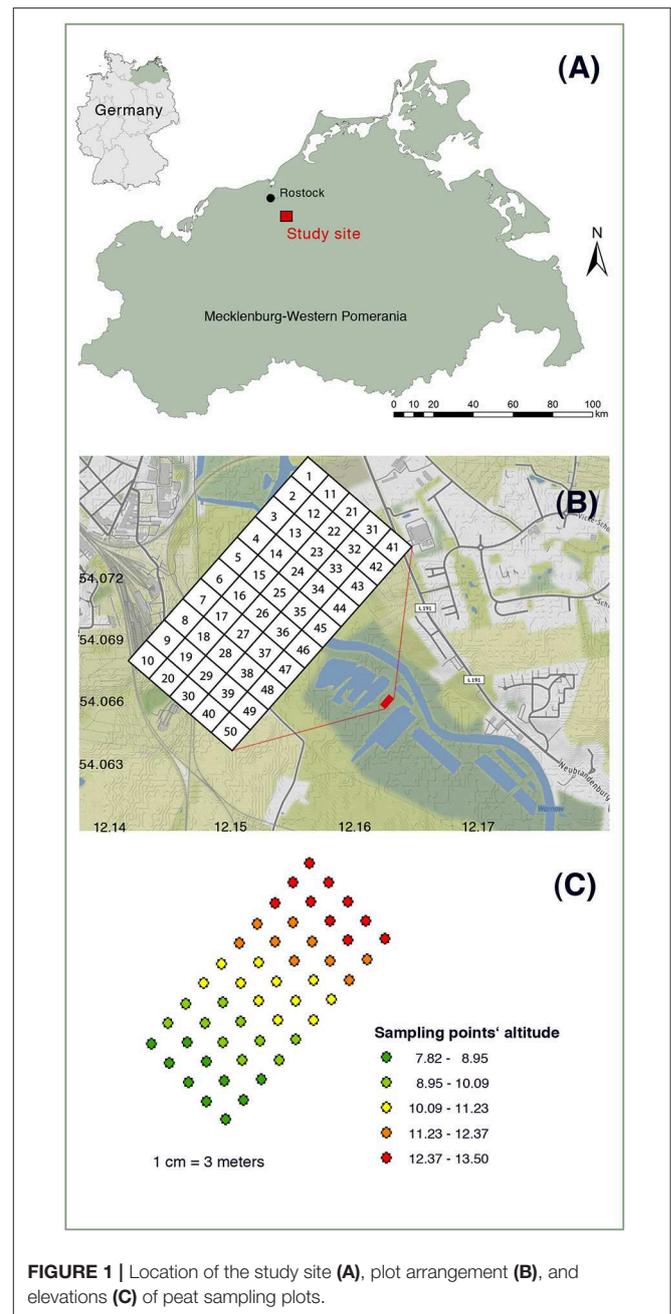
## MATERIALS AND METHODS

### Site Description and Soil Sampling

The study site is located in a telmatic peatland of Warnow Valley, Northern Germany (Figure 1A). The site is part of about 250,000 hectares of peatlands in the Federal State of Mecklenburg-Western Pomerania, Northeast Germany. Historically, the first references to any agricultural use of these fens near Rostock date back to 1471, and the use has been very extensive for more than 400 years (Hanschke, 1996). Nearby the sampling site, there are ponds originating from peat excavation after the mid-Nineteenth century; however, the most intensive of the peatland has been started immediately after the WW2 (1945–59). Lowering of groundwater level to enable peat excavation concurrently allowed a stepwise intensification of the previously only occasional agricultural use of the peatland, which however remained only moderately intensive until the 1960s. In almost all similar fen peatlands in Northern Germany, drainage, and agricultural use have been intensified from the early 1970s on by digging deeper drainage ditches that enabled heavy machinery for compacting meadows, fertilizer application, and grass harvest to travel at the fen peatland. Two to three cuts for silage and intermitted mineral fertilizer applications became the common management regime, which essentially led to peat subsidence and mineralization, nutrient transfers into waterways (Warnow River). The intensive uses degraded the site so severely and the meadows became unproductive, cleaning of drainage ditches was given up (end 1950s). Finally, the areas were taken out of a regular agricultural use (end 1970s) and gradually rewetted passively because the drainage ditches get closed by sediment accumulation (Hanschke, 1996). The degradation of the fenland probably resulted in a more heterogeneous soil structure because of stamping through from the undulated mineral ground beneath the peat. The passive rewetting of the degraded fenland likely reduced further peat oxidation although the surface soil of the peatland usually dries for at least 2 months during summer season (June to August).

The climate is characterized by annual means of 592 mm precipitation and 8.4°C temperature. The thickness of the peat layer is about 2.7 m and classified as Eutri-Ombic Histosol and Hemic Haplofibrist according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015), and Soil Survey Staff (1999), respectively.

The soil samples were collected from the upper 0–10 and 10–20 cm depths after 20 years of rewetting the degraded peatland in October 2001. Samples from both depths were used for the analyses of pH, water content, and biochemical properties. Since the contents of SOM, plant available, total and oxalate-extracted elements were similar in both depths, we used only the data generated from the 0 to 10 cm depths for these soil variables. The soil samples were collected from 50 grid cells with an individual grid cell size of 3 m × 3 m (Figure 1B). A soil sample was collected from each grid cell using a Macauley sampler. After the sampling, the plant roots were removed, and the samples were homogenized. Soil water content at sampling was determined by gravimetry, whereas SOM was determined by loss-on-ignition method. Field moist samples were used for the biochemical



**FIGURE 1** | Location of the study site (A), plot arrangement (B), and elevations (C) of peat sampling plots.

analysis immediately after sampling or stored in a freezer until required for analysis. Separate subsamples were air dried at 35°C for the determination of selected soil chemical properties.

### Chemical and Biochemical Analyses

The soil pH was determined by suspending the samples in 0.01 M CaCl<sub>2</sub>-solution with 1:2 ratio of sample to solution using a glass electrode and pH-meter. The Al, Fe, Mn, and P associated with non-crystalline minerals were extracted by acid ammonium oxalate (Schwertmann, 1964; Courchesne et al., 2008), whereas the P was extracted by sodium hypobromide (P<sub>hyp</sub>), and microwave assisted acid digestion (total P) (Dick

and Tabatabai, 1977). The concentration of oxalate extracted elements and total P were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Jobin Yvon 238 Ultrace, Instruments S.A. GmbH, D-85630 Grasbrunn, Germany). Furthermore, the degree of P saturation (DPS) was calculated using the following formula as suggested for wetland soils by Nair and Reddy (2013):

$$DPS = \frac{P_{ox}}{\alpha [Fe_{ox} + Al_{ox}]}$$

where  $P_{ox}$ ,  $Fe_{ox}$ , and  $Al_{ox}$  were oxalate extracted P, Fe, and Al in molar concentrations.  $\alpha$  is an empirical factor to account for the fraction of Fe and Al responsible for P sorption and researchers usually use 0.5 (Litaor et al., 2003; Nair, 2014). When the corrective factor  $\alpha$  is omitted, the P saturation ratio (PSR) can be used which is a simple ratio of molar  $P_{ox}$  to molar  $(Fe_{ox} + Al_{ox})$  (Nair, 2014).

The amounts of  $C_{org}$  and total N were determined by dry combustion using a CNS analyzer (Vario EL, Elementar Analysensysteme GmbH, D-63452 Hanau, Germany). The plant

**TABLE 1** | Gravimetric water (GW) content and pH in the surface and subsurface soil of a rewetted peatland.

0–10 cm soil depth	Mean	Median	Minimum	Maximum	Skewness	Kurtosis	SD	CV%
GW (%)	78	79	66	83	−1.18	0.55	4	6
pH <sub>H2O</sub>	6.54	6.52	6.06	7.20	0.00	0.00	0.26	4
pH <sub>CaCl2</sub>	5.98	5.97	5.60	6.74	1.05	1.80	0.24	4
10–20 cm SOIL DEPTH								
GW (%)	72	73	63	79	−0.79	0.42	4	5
pH <sub>H2O</sub>	6.22	6.20	5.77	6.75	0.00	0.00	0.22	4
pH <sub>CaCl2</sub>	5.82	5.81	5.56	6.17	0.00	0.00	0.13	2

**TABLE 2** | Selected plant available nutrients (mg kg<sup>−1</sup> dry soil) in the surface soil of a rewetted peatland.

	Mean	Median	Minimum	Maximum	Skewness	Kurtosis	SD	CV%
P	1.3	1.33	0.4	2	−0.1	0.5	0.4	28
Mg	17	171	113	234	0.1	0.0	26	15
S	113	109	55	229	0.9	0.9	37	33
Fe	1.7	1.38	0.5	5	1.5	2.4	0.9	55
Mn	5.6	2.50	0.6	27	1.6	2.2	7	117

**TABLE 3** | Soil organic matter, total, and ammonium oxalate extractable elements concentrations in the surface soil of a rewetted peatland.

(g kg <sup>−1</sup> dry soil)	Mean	Median	Minimum	Maximum	Skewness	Kurtosis	SD	CV%
SOM	640	660	490	760	−0.52	−0.68	70.0	11
$C_{org}$	320	330	250	360	−0.52	−0.46	30.0	9
N	30	30	20	30	−0.67	−0.30	2.0	9
S	6	6	5	8	0.23	−0.80	1.0	12
P	1.5	1.5	1.4	1.8	−0.37	0.04	0.1	10
$P_{hypo}$	1.28	1.28	0.98	1.52	−0.20	−0.26	0.13	10
$P_{ox}$	0.44	0.44	0.32	0.58	0.11	−0.73	0.07	15
K	0.50	0.49	0.32	0.65	0.23	0.32	0.07	14
Ca	25	25	17	29	−0.63	−0.44	3.0	13
Mg	1.1	1.1	1.0	1.3	−0.27	0.07	0.1	7
Fe	16	16	11	20	−0.12	0.84	1.7	11
$Fe_{ox}$	13	13	9	16	−0.63	0.05	1.7	14
Al	8	8	6	10	−0.43	0.69	0.8	11
$Al_{ox}$	1.14	1.17	0.85	1.53	0.20	1.43	0.12	10
Mn	0.6	0.6	0.3	0.8	−0.20	−0.54	0.1	22
$Mn_{ox}$	0.4	0.4	0.2	0.7	−0.04	−0.52	0.1	24
DSP	10.57	10.75	7.47	13.16	−0.28	0.06	1.17	11
PSR	0.05	0.05	0.04	0.07	0.09	−0.03	0.01	13

*hypo*, hypobromite extractable P; *ox*, oxalate extractable P; Fe, Al, and Mn; DPS, degree of P saturation in percent; and PSR, P saturation ratio; SOM, soil organic matter.

available nutrients were extracted by 0.01 M CaCl<sub>2</sub> and measured by ICP-OES. The total K, Ca, Mg, Al, Fe, and Mn were extracted by aqua regia digestion after calcination of the soil samples and ICP-OES was used to determine their concentrations.

The microbial biomass carbon and nitrogen were extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub> solution after fumigation following the method recommended by Vance et al. (1987) and modified by Joergensen (1995). The biomass carbon was estimated using the following formula:

$$\text{Microbial biomass C} = \text{EC} * \text{KEC} \quad (1)$$

$$\text{Microbial biomass N} = \text{EN} * \text{KEN} \quad (2)$$

Where EC is the organic C extracted from fumigated soil sample minus the organic C extracted from non-fumigated soil sample, KEC = 0.45 is the proportionality factor to convert EC to microbial biomass C. EN: the flush of NH<sub>4</sub><sup>+</sup>-N due to fumigation, KEN = 0.57 was the proportionality factor to convert EN to microbial biomass N (Jenkinson, 1988). The dehydrogenase, acid phosphatase and β-glucosidase activities were determined following the procedures outlined by Tabatabai (1994), whereas the protease activity by the method of Ladd and Buttler (1972).

## Vegetation Analysis

We recorded species compositions of each of grid cell in August 2008, and the percent of the vegetation cover of each 50 grid cell was estimated by the “vegan” version 2.4-5 (Oksanen et al., 2017) using the R environment version 3.3.2. (R Development Core Team 2016). The vegetation data were analyzed for species diversity using the Shannon and the Simpson indices and for grid cell vegetation composition’s dissimilarity by the Bray-Curtis and the Gower indices. The multivariate ordination methods “detrended correspondence analysis” (DCA) and “non-metric multidimensional scaling” (NMDS) were used to explore compositional gradients in vegetation patterns across

the grid cells. Soil chemical and biological parameters were fitted to NMDS ordination to analyze soil-vegetation-relationships. Goodness of fit was tested by permutation ( $n = 999$ ). Only traits with a marked correlation to the ordination matrix at the  $p < 0.1$  level were plotted as vectors into the ordination frame.

## Statistical Analysis

The descriptive statistics, correlation matrix and principal component analysis of the gravimetric water content, chemical, and biochemical properties such pH, SOM, plant available nutrients, oxalate extractable, and total elements, soil microbial biomass, and enzymes activities were analyzed using SAS version 9.4 (SAS Institute Inc., 2013). However, the spatial correlation was analyzed by Geostatistical software GS<sup>+</sup>™ version 7 (Gamma Design Software, Plainwell, Michigan, USA). We run a semivariogram analysis to evaluate the spatial correlation of soil chemical and biochemical properties. A typical semivariogram can be described by nugget effect, range and sill. Nugget represents micro-scale variation at  $h = 0$ , range is the distance at which data are no longer correlated, and sill is the plateau where the semivariogram reaches at the range (Berry, 2005). The soil chemical and biochemical properties were fitted to linear, spherical, exponential, and Gaussian models to obtain best fit based on the lowest residual sum of squares (RSS). The semivariogram was interpreted based on nugget: sill ratio. The nugget to sill ratio indicates what percent of the overall variance is found at a distance smaller than the smallest lag interval, and it gives a sense of how much variance was accounted for in the model. The nugget: sill ratio can be interpreted as strong spatial correlation (<0.25), and moderate (0.25–0.75), and no spatial correlation (>0.75) (Cambardella et al., 1994). The low nugget: sill ratio (<0.25) indicates a large part of the variance is introduced spatially that implying a strong spatial dependence and vice versa for the large nugget: sill ratio.

**TABLE 4 |** Soil microbial biomass and enzyme activities in surface and subsurface soil of a rewetted peatland.

0–10 cm soil depth	Mean	Median	Min.	Max.	Skewness	Kurtosis	SD	CV%
Biomass C (mg kg <sup>-1</sup> )	1,277	1,210	389	2,463	0.68	0.90	402	31
Biomass N (mg kg <sup>-1</sup> )	120	122	32	215	-0.08	-0.72	45	37
MBC: MBN	11	10	4	23	1.30	1.54	4	36
Dehydrogenase (μg TPF g soil <sup>-1</sup> )	3,044	2,876	513	7,910	0.84	1.49	1,483	49
APA (μg p-Nitrophenol g soil <sup>-1</sup> h <sup>-1</sup> )	851	862	539	1,106	-0.59	-0.26	138	16
β-Glu (μg p-Nitrophenol g soil <sup>-1</sup> h <sup>-1</sup> )	3,533	3,670	1,405	5,747	0.03	-0.16	993	28
Protease (μg Amino-N g soil <sup>-1</sup> 15 h <sup>-1</sup> )	301	295	130	462	0.06	-0.32	76	25
10–20 cm SOIL DEPTH								
Microbial biomass C (mg kg <sup>-1</sup> )	689	640	343	1,570	1.60	3.66	240	35
Microbial biomass N (mg kg <sup>-1</sup> )	54	47	14	160	1.52	4.14	26	48
MBC: MBN	14	13	6	30	1.34	2.06	5	36
Dehydrogenase (μg TPF g soil <sup>-1</sup> )	717	633	211	2,016	1.24	1.19	415	58
APA (μg p-Nitrophenol g soil <sup>-1</sup> h <sup>-1</sup> )	652	662	476	911	0.33	-0.25	105	16
β-Glu (μg p-Nitrophenol g soil <sup>-1</sup> h <sup>-1</sup> )	1,379	1,354	639	2,294	0.52	1.49	286	21
Protease (μg Amino-N g soil <sup>-1</sup> 15 h <sup>-1</sup> )	283	266	147	501	0.56	0.04	79	28

MBC, microbial biomass C; MBN, microbial biomass N; APA, acid phosphatase; β-Glu, β-glucosidase; Min, minimum; Max, maximum. All concentrations were kg<sup>-1</sup> dry soil.

## RESULTS

### Gravimetric Water Content, and Plant Nutrient Elements

The gravimetric water content and pH were slightly higher at the soil depth of 0–10 than at the 10–20 cm (Table 1), and the water content was in the range of 66 to 83% for the 0–10 cm depth, and 63 to 79% for the 10–20 cm soil depth. However, depths did not change  $\text{pH}_{\text{H}_2\text{O}}$  and  $\text{pH}_{\text{CaCl}_2}$ , but the  $\text{pH}_{\text{H}_2\text{O}}$  was slightly higher than  $\text{pH}_{\text{CaCl}_2}$  at both depths. The coefficient of variation (CV) of the water content at the 0–10 cm soil depth was higher than at the 10–20 cm soil depth. Concentration of the selected plant available

nutrients were low except for sulfur (S) and magnesium (Mg) (Table 2). Furthermore, the CV of most plant available nutrients were high and ranged from 15 to 117%. The CV of plant available Mn was particularly very high (117%) followed by plant available Fe (55%).

### SOM, Oxalate Extracted, and Total Elements

The concentrations of SOM, oxalate-extracted, and total elements are presented in Table 3. The SOM,  $C_{\text{org}}$  and total N were in the range of 490 to 760, 250 to 360, and 20 to 30  $\text{g kg}^{-1}$ , respectively, with CV in the range of 9 to 11%. Similarly, the concentration of total P and S were in the range of 1.4 to 1.8, and 5 to 8  $\text{g kg}^{-1}$ , respectively, and their CVs were in the range of 10 and 12%. The mean mass ratios of C: N, C: P, and C: S calculated from the Table 3 were 11, 213 and 53, respectively. Similarly, the N: P, and N: S ratios were 20 and 5, respectively. The hypobromide-extracted P ( $P_{\text{hyp}}$ ) was 85% of the total P extracted by aqua regia, whereas the  $P_{\text{ox}}$  was about 29% of the total P (Table 3). The concentration of Ca was the highest followed by total Fe and Al excluding  $C_{\text{org}}$  and total N, whereas the concentrations of total K and Mn were the lowest.

The oxalate-extracted elements were in the ranges of 0.32 to 0.58  $\text{g kg}^{-1}$  for  $P_{\text{ox}}$ , 9 to 16  $\text{g kg}^{-1}$  for  $\text{Fe}_{\text{ox}}$ , 0.85 to 1.53  $\text{g kg}^{-1}$  for  $\text{Al}_{\text{ox}}$ , and 0.2 to 0.7  $\text{g kg}^{-1}$  for  $\text{Mn}_{\text{ox}}$  (Table 3). These were 29, 81, 14, and 67% of the total concentrations of P, Fe, Al, and Mn, respectively. The CV of the oxalate extracted elements were in the range of 10 to 24%, and the CV of  $\text{Al}_{\text{ox}}$  was the lowest, whereas the CV of  $\text{Mn}_{\text{ox}}$  was the highest. Furthermore, the DPS, and PSR were in the range of 7.47 to 13.16% and 0.04 to 0.07, respectively

**TABLE 5 |** Pearson correlation coefficient among biochemical activities and plant available nutrients in surface of a rewetted peatland.

	$C_{\text{mic}}$	$N_{\text{mic}}$	DHA	APA	Mg	Mn
$N_{\text{mic}}$	0.75***					
APA	0.34*	0.48**	0.28*			
GLU	0.33*	0.23	0.28*	0.36**		
PTA	−0.09	−0.08	−0.27	−0.39		
Fe	0.17	0.13	0.14	0.11		
Mg	0.13	0.23	−0.41	−0.03		
Mn	0.17	0.22	−0.03	0.02	0.42*	
P	0.11	0.33*	−0.05	0.05	0.50***	0.45**

Number of observations: 50, \*, \*\*, and \*\*\* indicate significant correlation coefficient at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively.  $C_{\text{mic}}$ , microbial biomass C;  $N_{\text{mic}}$ , Microbial biomass N; DHA, Dehydrogenase; APA, Acid phosphatase; GLU,  $\beta$ -Glucosidase.

**TABLE 6 |** Pearson correlation coefficients among soil organic matter, selected available plant nutrients and total element concentrations in surface soil of a rewetted peatland.

	$S_t$	$N_t$	$C_{\text{org}}$	SOM	$P_t$	$P_{\text{pyro}}$	$P_{\text{ox}}$	$\text{Mn}_t$	$\text{Mn}_{\text{ox}}$	$\text{Mg}_t$	$\text{Fe}_t$	$\text{Fe}_{\text{ox}}$	$\text{Ca}_t$	$\text{Al}_t$
$N_t$	0.74***													
$C_{\text{org}}$	0.77***	0.93***												
SOM	0.72***	0.93**	0.94***											
$P_t$	0.30	0.68***	0.59***	0.73***										
$P_{\text{pyro}}$	0.75***	0.85***	0.85***	−0.74***	0.50***									
$P_{\text{ox}}$	−0.86***	−0.85***	−0.85***	0.99***	0.01	−0.79***								
$\text{Mn}_t$	−0.1	0.51***	0.40**	0.53***	0.73**	0.39**	−0.03							
$\text{Mn}_{\text{ox}}$	−0.20	0.07	0.00	0.18	0.55***	0.18	0.13	0.76***						
$\text{Fe}_t$	−0.1	0.25	0.24	0.28	0.38**	0.26	−0.10	0.57***	0.58***	0.12				
$\text{Fe}_{\text{ox}}$	0.08	0.29*	0.24	0.06	0.57***	0.29*	−0.01	0.71***	0.72***	−0.22	0.82***			
$\text{Ca}_t$	0.47***	0.85***	0.80***	0.90***	0.83***	0.32*	0.11	0.77***	0.60***	−0.11	0.46***	0.69***		
$\text{Al}_t$	0.00	0.36**	0.35*	0.40**	0.48***	0.25	−0.01	0.64***	0.66***	0.03	0.95***	0.89***	0.55***	
$\text{Al}_{\text{ox}}$	−0.02	−0.02	0.00	−0.09	−0.10	0.00	−0.06	−0.28	0.01	0.14	−0.03	−0.18	−0.27	−0.13
$S_a$	0.52***	0.11	0.1	0.12	−0.14	−0.22	0.23	−0.36**	−0.30*	0.11	−0.24	−0.11	−0.05	−0.16
$P_a$	0.45***	0.42**	0.40**	0.45***	0.21	−0.14	0.23	0.02	−0.16	0.19	−0.01	0.13	0.30*	0.05
$\text{Mn}_a$	0.36*	0.16	0.19	0.09	−0.1	−0.23	0.19	−0.27	−0.24	0.42**	−0.03	−0.11	−0.05	−0.05
$\text{Mg}_a$	0.81***	0.66***	0.73***	0.70***	0.23	−0.13	0.29*	−0.07	−0.10	0.34*	−0.09	0.13	0.46***	0.00
$\text{Fe}_a$	0.12	0.01	−0.05	−0.02	0.04	−0.12	0.18	−0.12	−0.11	0.05	−0.34*	−0.29*	−0.01	−0.35

Number of observations: 50, \*, \*\*, and \*\*\* indicate significant correlation coefficient at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively; t, total element; a, plant available nutrient; ox, oxalate extracted, hypo, hypobromite extractable P; See Tables 2, 3 for the unit of each parameter.

(Table 3). The CV of DPS and PSR were similar to the CV of total and oxalate extracted elements.

## Biochemical Properties

The microbial biomass C ranged from 389 to 2,463 mg kg<sup>-1</sup> (0–10 cm) and 343 to 1,570 mg kg<sup>-1</sup> (10–20 cm). Furthermore, the microbial biomass N was in the ranges of 32 to 215 mg kg<sup>-1</sup> (0–10 cm) and 14 to 160 mg kg<sup>-1</sup> (10–20 cm). This resulted in microbial biomass C: N ratios of 11 (0–10 cm) and 14 (10–20 cm) (Table 4). The microbial biomass C and N were low by 46 and 55% at the 10–20 cm soil depth, respectively, as compared to that of the 0–10 cm soil depth. Similarly, the dehydrogenase and  $\beta$ -glucosidase activities was low by 76 and 61%, and the acid phosphatase and protease activities were low by about 23 and 6% at the depth of 10–20 cm as compared to that of the 0–10 cm soil depth, respectively.

## Relationships of Chemical and Biochemical Properties

The soil microbial biomass C was significantly ( $P < 0.05$ ) correlated with the microbial biomass N,  $\beta$ -glucosidase, and acid phosphatase activities, whereas microbial biomass N was significantly correlated with the acid phosphatase activity (Table 5). Similarly,  $\beta$ -glucosidase activity was significantly correlated with dehydrogenase and acid phosphatase activities. However, there were no significant correlations between the biochemical activities and plant available nutrients except for the available P and microbial biomass N. We also observed a few significant correlations among the plant available elements. For instance, the plant available Mg was significantly correlated with plant available Mn and P.

The correlation of biochemical properties with the concentration of SOM, total and oxalate extracted elements were not significant ( $P > 0.05$ ) except for the dehydrogenase activity which was significantly negatively correlated with total Ca, SOM, C<sub>org</sub>, total N, and S (data not shown). However, the correlations among the SOM, total N, total S, total P, P<sub>hyp</sub>, and P<sub>ox</sub> and total Mn were highly significant ( $P < 0.001$ ) (Table 6). These parameters were also strongly correlated with concentrations of most of the plant available nutrients. Similarly, the concentration of total Ca was strongly correlated with the concentration of SOM and most of the total elements. Furthermore, the concentration of total P was highly significantly correlated ( $P < 0.001$ ) with P<sub>hyp</sub>, Mn<sub>ox</sub>, and Fe<sub>ox</sub>, total Al, Ca, and total Fe. The concentration of total Mn was also strongly correlated with Mn<sub>ox</sub>, total Fe, Fe<sub>ox</sub>, total Ca, and total Al. The correlation among the concentration of some plant available nutrients and total elements were positive; however, the P<sub>ox</sub> was significantly negatively correlated with the C<sub>org</sub>, total S, total N, and P<sub>hyp</sub>.

principal component analysis separated the biochemical properties in two depths in which the biochemical properties at the 0–10 cm depths mostly contributed to PC1 and those at the 10–20 cm mostly contributed to the PC2 (Figure 2). The PC1 explained about 23% of the total variance with the positive loading of microbial biomass C and N, acid phosphatase,  $\beta$ -glucosidase, dehydrogenase, and with the negative loading

of protease of the 0–10 cm depth and  $\beta$ -glucosidase of 10–20 cm depth. On the other hand, PC2 explained 20% of the total variance with the positive loading of microbial biomass C and N, acid phosphatase,  $\beta$ -glucosidase, dehydrogenase, and negative loading of the protease at the depth of 10–20 cm. Furthermore, the principal component analysis separated the chemical properties into four main groups depending on their contribution to the total variance with positive and negative loadings where the PC1 explained 51 % and PC2 14% of the total variance (Figure 3). The high positive loading came from C<sub>org</sub>, total N, DSP, hypobromide extracted P and water content, whereas total S, PSR, SOM, P<sub>ox</sub>, Al<sub>ox</sub>, Mn<sub>ox</sub>, and pH-CaCl<sub>2</sub> contributed to the high negative loading.

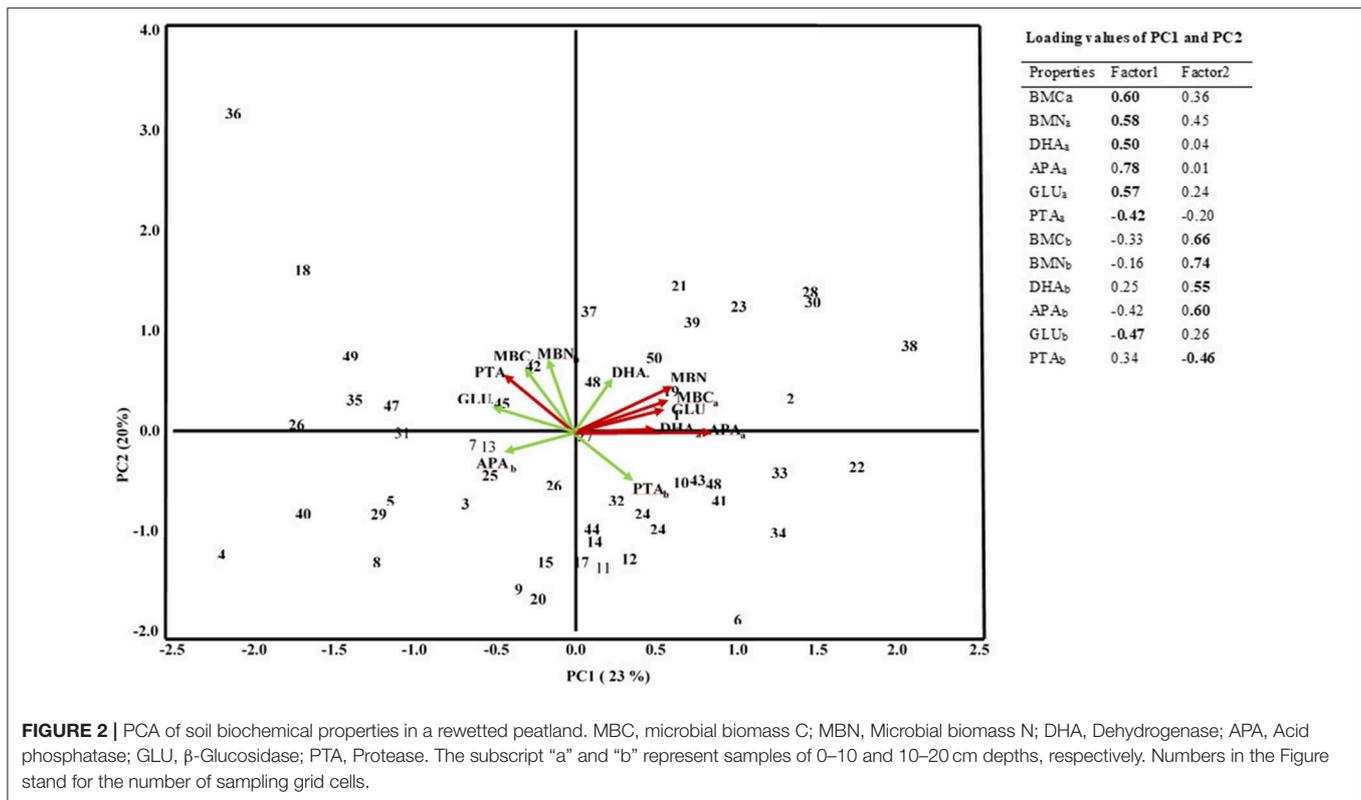
## Spatial Correlation of Chemical and Biochemical Properties

The nugget to sill ratio of soil pH<sub>H2O</sub> and pH<sub>CaCl2</sub> were 0.07 and 0.16 at 6 and 10 m ranges, respectively, at the 0–10 cm soil depth (Table 7). All of the biochemical properties showed strong spatial correlation at the depths of 0–10 cm except for protease activity (Table 7 and Figure 4). The strong spatial correlation was revealed by the lowest nugget to sill ratio, which was  $\leq 0.07$  except for microbial biomass C where the nugget to sill ratio was 0.27. At 10–20 cm peat depth, pH<sub>H2O</sub>, pH<sub>CaCl2</sub>, microbial biomass C had no spatial correlation since they were fitted best to the linear model. However, the microbial biomass and enzymes activities were strongly spatially correlated at the soil depth of 10–20 cm except for the microbial biomass C and dehydrogenase activity. The biochemical properties were not only spatially correlated but also the spatial correlation appeared at the shortest range of 4 to 9 m (Table 7). Overall, about 86, and 71% of the biochemical properties were strongly spatial correlated at the depth of 0–10 cm, and 10–20 cm, respectively.

The gravimetric water content, plant available nutrients, total and oxalate extracted elements showed strong spatial correlations (Table 8). In general, the nugget to sill ratio of 15 of the 24 soil properties were  $\leq 0.1$  at the ranges of 5 to 10 m which indicated the plant available and total elements were strongly spatially correlated at the short distance interval. Similarly, the DPS and PSR were strongly spatially correlated with the nugget to sill ratio of 0.17 and 0.16 at the range of 8 and 5 m, respectively. Among the 23 soil chemical properties, only Fe<sub>ox</sub>, total Al, and Fe lacked spatial correlation, whereas plant available Mn and S, and total K showed moderate spatial correlation with the nugget to sill ratio of 0.32, 0.32, and 0.41, respectively (Table 8).

## Vegetation Composition

The vegetation composition indicated the transition from wet grassland to extensive reed (*Phragmites australis*) dominated vegetation (Figure 4). Turquoise colored dots in the DCA-plot indicate the plot positions as arranged according to their similarity in plant composition. The position of the single species in the ordination space is given by the brown colored BCI-name, an abbreviation of their Latin names. Five plots differed markedly in their composition of the vegetation from the conglomerated rest. NMDS was based on a Bray & Curtis



**FIGURE 2 |** PCA of soil biochemical properties in a rewetted peatland. MBC, microbial biomass C; MBN, Microbial biomass N; DHA, Dehydrogenase; APA, Acid phosphatase; GLU,  $\beta$ -Glucosidase; PTA, Protease. The subscript "a" and "b" represent samples of 0–10 and 10–20 cm depths, respectively. Numbers in the Figure stand for the number of sampling grid cells.

dissimilarity matrix and allows a gradient analysis to explore possible soil-vegetation-relationships. Fitting of all analyzed soil-chemical and soil-biological traits as environmental variables and use them for gradient analyses was successful for  $N_{mic}$ ,  $Al_{ox}$ , and  $Fe_{ox}$  (Figure 4).

## DISCUSSION

### Chemical and Biochemical Properties

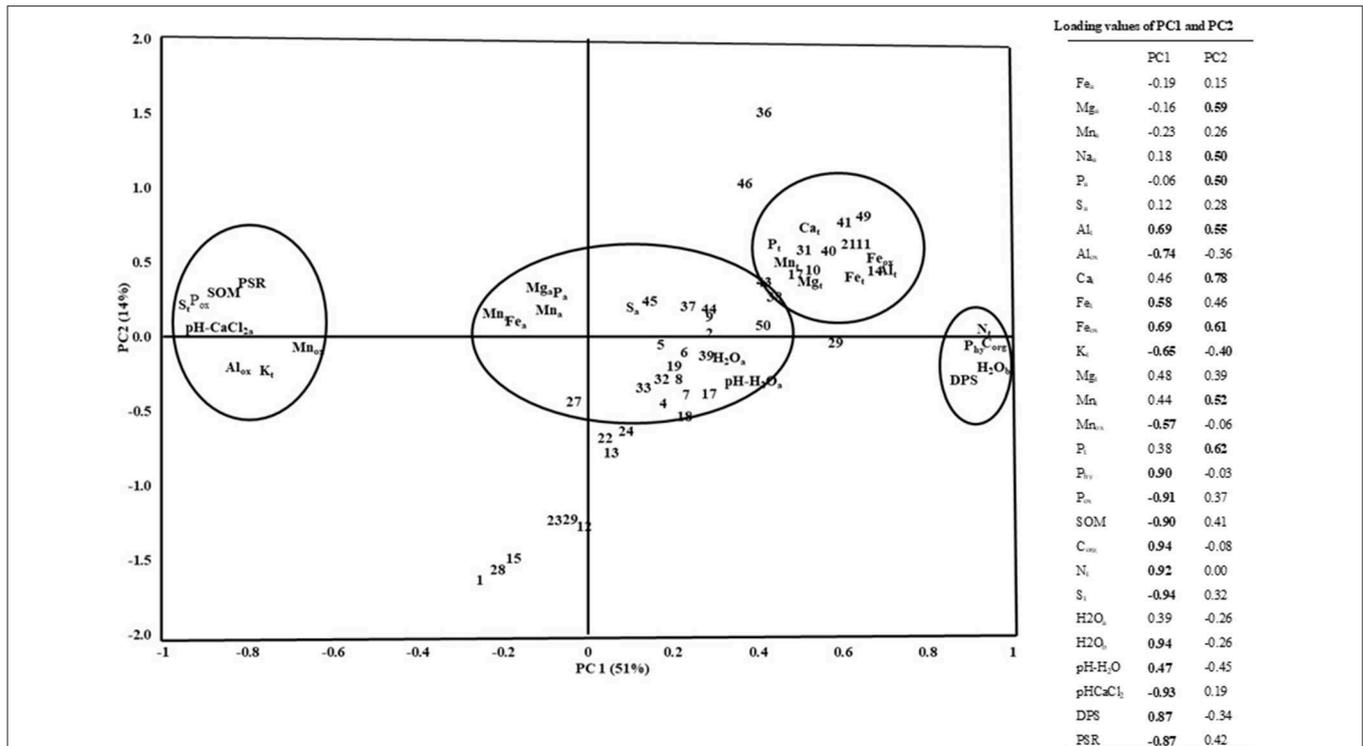
The pH-H<sub>2</sub>O and pH-CaCl<sub>2</sub> remained constant around 6 which can be attributed to the buffering capacity of CO<sub>2</sub> in the saturated peat soil (Reddy and DeLaune, 2008). Furthermore, the low CV of pH (Table 1) can be attributed to pH is the log transformed H<sup>+</sup> concentration. Different studies also reported low CV for pH from the tropical wetlands (Nkheloane et al., 2012) and temperate bog and fens (Ulanowski and Branfireun, 2013; Barrett and Watmough, 2015).

The low concentrations of some plant available nutrients agreed with the results of previous study that indicated 0.01 M CaCl<sub>2</sub> recovered the lowest plant available P among 14 soil P extraction methods (Wuenschel et al., 2015). This is also true for the proportion of plant available P, Fe, and Mn to their respective concentrations of total elements that were in the range of 0.01 to 1% (Tables 2, 3). We explain the low concentrations and proportions of 0.01 M CaCl<sub>2</sub> extracted plant available nutrients by the weak extractant and/or existence of the elements in stable organic and inorganic complex forms (Table 2). Such low concentrations of plant available nutrients could jeopardize

the growth and development of plant communities adapted to the drained peatlands, although plants grown in anoxic soil conditions have their own mechanisms to adapt to low plant available nutrients (Elzenga and van Veen, 2010). The highest CV of the plant available Mn followed by Fe indicated the highest variability of these plant available nutrient elements in the studied peatland.

In contrast to the plant available nutrients, the CV of the total concentrations of most elements and SOM were low (Table 3) which indicates that the rewetting and drying cycles of peats did not influence the variability of these soil constituents. Similarly, low CV was reported for SOM from restored temperate peatland and tropical wetlands (Bruland et al., 2006; Nkheloane et al., 2012).

The  $Fe_{ox}$  was 81% of the total Fe that indicated the major proportion of total Fe existed in poorly crystalline mineral form (Table 3) (Courchesne et al., 2008). The higher concentration of  $Fe_{ox}$  than  $Al_{ox}$  and  $Mn_{ox}$  also showed the non-crystalline Fe in the rewetted peatland could control P solubility (Nair, 2014). However, the mean of (DPS 10.6%) and PSR (0.05) in the present study were very low as compared to the threshold level of P leaching which are 25% DPS in degraded peatland (Litaor et al., 2003) and 0.11 PSR for wetland soil (Nair and Reddy, 2013). This implies that potential loss of P to the ground and drainage water could be low. Although oxalate could recover some P associated with SOM, the proportion of  $P_{ox}$ , 29% of the total P, was comparable to the proportion of sum of inorganic P fractions in peat soils of northern Germany



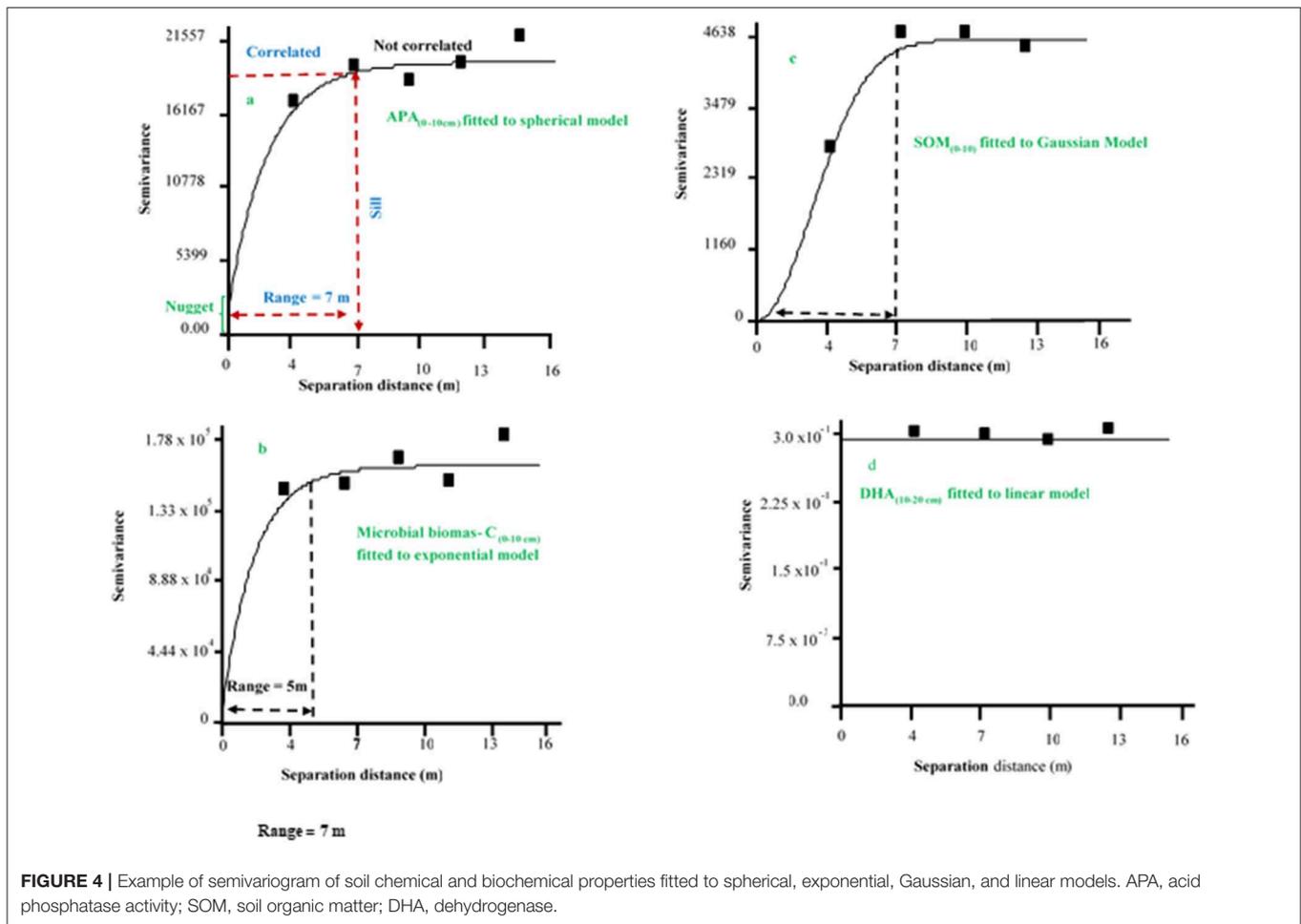
**FIGURE 3 |** PCA of soil chemical properties in a rewetted peatland. Subscript: “a”: plant available elements, “t”: total element, ox, oxalate extracted elements; Phy, hypobromide extracted P; H2Oa, water content of 0–10 cm soil depth; H2Ob, water content of 10–20 cm soil depth; DPS, degree of P saturation; PSR, P saturation ratio, numbers in the Figure stand for the number of sampling grid cells.

**TABLE 7 |** Spatial correlation of soil pH and biochemical properties in the surface and subsurface soil of a rewetted peatland.

0–10 cm soil depth	Model	Nugget	Sill	Range (m)	Nugget: Sill ratio	RSS	R <sup>2</sup>
pH <sub>H2O</sub>	Spherical	0.0056	0.0755	6	0.07	7.60 × 10 <sup>-5</sup>	0.66
pH <sub>CaCl2</sub>	Exponential	0.011	0.0682	10	0.16	1.94 × 10 <sup>-5</sup>	0.92
Microbial C	Exponential	200	10,800	5	0.05	5.65 × 10 <sup>8</sup>	0.33
Microbial N	Spherical	662	2,429	13	0.27	3.50 × 10 <sup>3</sup>	0.99
Mic-C : Mic-N	Exponential	0.63	14.32	6	0.04	2.49	0.64
Dehydrogenase	Spherical	52,000	2,085,000	5	0.02	1.25 × 10 <sup>11</sup>	0.39
Acid phosphatase	Spherical	310	19,070	5	0.02	8.23 × 10 <sup>5</sup>	0.83
β- Glucosidase	Spherical	47,000	1,035,000	5	0.05	3.06 × 10 <sup>9</sup>	0.79
Protease	Linear						
<b>10–20 cm SOIL DEPTH</b>							
pH <sub>H2O</sub>	Linear						
pH <sub>CaCl2</sub>	Linear						
Microbial C	Linear						
Microbial N	Exponential	92	742	4	0.12	6.03 × 10 <sup>2</sup>	0.53
Mic-C : Mic-N	Exponential	0.015	0.099	10	0.15	5.37 × 10 <sup>-4</sup>	0.44
Dehydrogenase	Linear						
Acid phosphatase	Exponential	1,040	11,390	4	0.09	1.91 × 10 <sup>6</sup>	0.2
β-Glucosidase	Spherical	6,800	88,510	4	0.05	3.91 × 10 <sup>7</sup>	0.00
Protease	Spherical	10	5,712	4	0.0018	6.60 × 10 <sup>5</sup>	0.00

(Schlichting et al., 2002). According to these authors, 75–80% of the total P were organic P in the peat soils. Since more than 75% of the total P existed in organic P form, maintaining

and/or improving the SOM content of peatlands by rewetting not only sequesters C but also stores P in unavailable organic P form.



The higher biochemical activities at the soil depths of 0–10 cm than at the soil depth of 10–20 cm (Table 4) indicated the presence of enough aeration particularly during the summer season and rooting which together can increase microbial activities and transformations of organic matter (Tokarz and Urban, 2015). On the other hand, the highest dehydrogenase activity confirmed the significance of oxidoreductases under anaerobic soil conditions (Salazar et al., 2011; Wolinska and Stepniewska, 2012). Furthermore, the  $C_{org}$ , microbial biomass C and N of the present study were considerably lower than that reported for a rewetted peatland of Trebel valley, northern Germany (Baum et al., 2003). Similarly, the acid phosphatase activity in the current study was less than that of the Trebel valley by 3 to 4-folds. Such a great variation in enzyme activity can be attributed to seasonal and site-specific effects rewetting degraded peatland. Dehydrogenases are intracellular enzymes with indicator value for the soil microbial activity, but also for fine root activity (Zhang et al., 2010). The discrepancy of high dehydrogenase activity but low protease and acid phosphatase activities at both peat depths (0–10 and 10–20 cm) can be attributed to low aeration, since the activities of these enzymes are favored in aerobic soil conditions (Kang et al., 2005; Reddy and DeLaune, 2008; Romanowicz et al., 2015).

## Correlations Among Soil Properties

The significant positive correlation between soil microbial biomass C and N (Table 5) proved the well-established facts of their strong association. Similarly, the significant positive correlation between microbial biomass C and acid phosphatase activity, and microbial biomass C and  $\beta$ -glucosidase activity indicated that SOM and hydrology regulate the microbial biomass and enzyme activity in peat soils (Groffman et al., 1996). However, none of the content of plant available nutrients was correlated with the microbial biomass and enzyme activity, which can be attributed to less decomposition of peats because of high gravimetric water content at sampling (Table 1). The significant positive correlation of plant available P with the plant available Mg and Mn could be attributed to the pH and gravimetric water content that influenced the plant availability of these nutrient elements in the same direction.

The significant positive correlations among the SOM,  $C_{org}$ , total S, total N, total P and  $P_{hyp}$ , and most plant available elements (Table 6) indicated the major source of the elements was the SOM in peat soil. However, the lack of significant correlation among microbial biomass C and  $C_{org}$ , microbial biomass N and total N (data not shown), can be explained by high variations between



the concentration of microbial biomass and the concentration of  $C_{org}$  and total N. A previous study also reported that the linearity of  $C_{org}$  to microbial biomass C association was achieved when  $C_{org}$  was below 2.5% (Anderson and Domsch, 1989). However, the  $C_{org}$  concentration in the present study was about 14 times higher than the critical level of  $C_{org}$  (2.5%) to detect a significant correlation between  $C_{org}$  and  $C_{mic}$ . Furthermore, high concentration of dissolved organic carbon and permanent flooding can contribute to lack of significant correlation between microbial biomass C with  $C_{org}$ , and microbial biomass N with total N (Reddy and DeLaune, 2008).

## Spatial Correlation

The nugget to sill ratio is a good indicator whether a given soil variable is strongly spatially correlated ( $<0.25$ ), moderately spatially correlated (0.25–0.75) or weakly spatially correlated ( $>0.75$ ) (Cambardella et al., 1994; Iqbal et al., 2005; Ruffo et al., 2005). Accordingly, most of the biochemical properties were not only strongly spatially correlated, but also the spatial correlation was apparent at the shortest ranges (Table 7 and Figure 4). The shortest range indicated a strong spatial variability of the soil variable under consideration. The highest spatial correlation particularly of the dehydrogenase activity was a good indicator of the overall microbial diversity in the rewetted peatland. Previous study also indicated that soil microbial activities showed strong spatial correlation below 0.25 m separation distance (Stark et al., 2004). This unequivocally indicated that a small-scale sampling is required to study biochemical properties in the rewetted degraded peatland. However, the lack of spatial correlation for the protease at the 0–10 cm soil depth and for the pH, microbial biomass C, and dehydrogenase activity at the depth of 10–20 cm can be attributed to the wet-dry cycles that could enhance variations from point to point in the rewetted degraded peatland. When a soil parameter lacks spatial correlation, reducing the sampling scale of the current study could exhibit spatial correlation (Cambardella et al., 1994) or a classic randomization block design could be used to handle the spatial variability (Kravchenko et al., 2006). The strong spatial correlation of most of the soil chemical properties could indicate the soil variables may be controlled by intrinsic soil variation (Cambardella and Karlen, 1999). Furthermore, extrinsic factors such as drainage, rewetting, historical land use and similar vegetation composition can contribute to strong spatial correlation. The vegetation composition is linked to the soil microbial colonization via rhizosphere effects and litter quality (Eisenhauer et al., 2010), and this is confirmed in our data set by the significant correlation with  $N_{mic}$  (Figure 5). The vegetation composition was assumed to represent a relative stable “summary” of the effects of multiple drivers, like the soil chemical properties, over time and, thus, a good predictor of the soil microbial community (Mitchell et al., 2010).

In conclusion, the combined use of the descriptive and geospatial statistical analyses have paramount importance

in disclosing the spatial variability pattern of soil chemical and biochemical properties in a rewetted degraded peatland at the small scale. Among 33 soil chemical and biochemical properties investigated in the present study, the CV of plant available nutrients were the highest. Similarly, soil biochemical activities were higher by many folds at the depth of 0–10 cm than 10–20 cm because of aeration during dry periods. Furthermore, about 88% of the soil chemical and biochemical properties were spatially correlated and 83% of the spatial correlation was strong at the  $\leq 8$  m range. The strong spatial correlation at such short range clearly indicated a small-scale spatial variability of the rewetted peatland. Those soil chemical and biochemical properties lacking spatial correlation were also spatially heterogeneously distributed because they varied from point to point. The main causes of such spatial variability can be attributed to slight variations in topography (Figure 1C) which are enhanced by peat degradation and subsidence. This essentially influence the soil moisture distribution and almost all biogeochemical processes that are related to moisture and redox conditions. Furthermore, direct anthropogenic influences like uneven distributions of formerly applied fertilizers have added to the spatial variability of the soil properties. Thus, small-scale sampling is required to understand the influence of rewetting degraded peatland on biogeochemistry and restoration processes thereby avoiding undesirable effects on environment.

## DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

## AUTHOR CONTRIBUTIONS

WN statistical data analysis and writing the manuscript. CB and AS design of the study and editing the manuscript. PL conception of the research and editing the manuscript. JM collected vegetation composition data, data analysis, and editing the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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