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Soil physicochemical and microbial characteristics of contrasting land-use types along soil depth gradients

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ABSTRACT

Soil physicochemical properties can be regarded as an important tool to assess soil health, which further form a base for biological activity in soil. These soil physicochemical properties are comparable in identical land-uses and so reflect similar soil microbial properties. However, the changes in land-use types and their effects on soil physicochemical and microbial properties are largely debated and rather unclear.

The aim of this study is to assess the impact of land-use types and soil depth on physicochemical properties (Organic C, total N, C/N ratio, available phosphorus, bulk density, pH and electrical conductivity), nitrogen forms (Nitrate-N, ammonium-N, organic N, mineralizable N, microbial biomass N and extractable organic N) as well as microbial indices (basal respiration, respiratory quotient, microbial quotient, microbial biomass). Land-use types - farmland, orchard, grassland and abandoned land served as horizontal factors while soils at 0–10 cm, 10–30 cm and 30–60 cm depth were used as vertical factors for accessing the physicochemical and microbial properties. Discriminant analyses (DA) indicated that soil microbial properties were affected by both land-use types and soil depths than nitrogen and physicochemical properties in our study. We found that the overall trend of percentage of discriminant function 1 (DF1) was highest for microbial indices (~90%) > nitrogen (~80%) > physicochemical properties (~70%). All investigated soil properties differed with higher significance by land-use types than by soil depths. The results further indicated that among all investigated soil properties in different land-use types, electrical conductivity, mineralizable nitrogen and microbial biomass carbon served as best discriminating indices. Regarding soil depths, total organic carbon followed by mineralizable nitrogen and basal respirations were found to be the decisive indicators of soil conditions.

Overall, our results demonstrate the sensitivity of various soil properties and their differential provenience along horizontal and/or vertical gradients. These outcomes suggest that differences in land-use types are reflected in soil physicochemical properties that are actual drivers of soil microbial properties in this region. Thus they are promising guideline tools for further studies related to soil quality, soil management and sustainability in long run.

1. Introduction

As a dynamic biological entity within a continuously changing environment (García-Ruiz et al., 2008), the quality of soil is defined by its physicochemical and/or biological conditions. Land-use change is directly associated with soil quality variation. This change is normally reflected by the shifts of a set of soil physicochemical properties and microbial indices (Aon and Colaneri, 2001). Soil physicochemical properties are relatively stable as it takes decades to detect their changes even after years of land use transformations (Arévalo-Gardini et al., 2015; Parr and Papendick, 1997). Compared with physicochemical changes, soil nitrogen (N) pool is more sensitive to aboveground plants/land-use variations. For instance, extractable organic nitrogen (EON represented in the form of peptides and amino acids) can be directly assimilated by plants (Nordin et al., 2001; Weigelt et al., 2003, 2005). On the other hand, individual plant species differ and are limited in their capacity to readily use a range of chemical forms of N (Miller and Bowman, 2002, 2003). The differential extent of competition between soil microbes and plant roots for soil N along soil depths has always been debatable. However, soil microbes have established

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their advancement by enhancing microbial competition for acquisition of soil N through stimulation and increase in microbial biomass (Dunn et al., 2006; Zeller et al., 2001). Soil microbes-related indices such as biomass, respiration and enzyme activities are both directly and indirectly regulated through soil nutrient and physicochemical conditions such as pH and moisture (Sinsabaugh, 1994) or due to shifts in environmental conditions such as temperature and precipitation (Acosta-Martinez et al., 2007; Caravaca et al., 2002; Guo et al., 2009).

Among soil microbial indices, microbial biomass carbon (MBC), MBC to soil organic carbon (SOC) ratio (also referred as microbial quotient; MQ), microbial respiration to MBC ratio (referred as respiratory quotient (RQ))have generally served as sensitive indices for quantitative and qualitative changes in microbial communities caused by changes in land-use types/management systems (Almagro et al., 2013; Dlamini et al., 2016; Nsabimana et al., 2004; Raiesi and Beheshti, 2015; Stevenson et al., 2016). In addition, soil microbial communities vary substantially along soil depths closely relating to horizontal gradients (such as changes in land-use types) in soil properties (Allison et al., 2007; Blume et al., 2002; Fierer et al., 2003; Steven et al., 2013). Therefore, it is imperative to examine the changes in the aforementioned indices (physicochemical-, nitrogen related- and microbial) with strong consideration at both land-use types and soil depth gradients.

The Chinese Loess Plateau is recognized as one of the most severely eroded areas in the word (Wang et al., 2006). The Government of China has enacted and recuperated "shift from farmland to forest or grassland" policy since the late 1990s in order to reduce erosion and protect the fragile ecosystems in Loess Plateau. Hence, typical land-use types in this region are re-vegetated grassland and/or forestland, still-existing farmland and the abandoned land as remnants of previous cropland/farmlands. In recent years, in the context of the Chinese land-use transformations, numerous studies have been conducted to compare the impact of land-use shifts on soil nutrients (Dang et al., 2014; Gong et al., 2006), nitrogen forms and its mineralization (Bu et al., 2015), microbial communities (Zhang et al., 2013) as well as soil respiration (Deng et al., 2010; Yu et al., 2015). However, very few studies have focused specifically on assessing whether vertical (soil depth) or horizontal (land-use type) factors impose stronger impact on soil properties in this region. In our study, we thus emphasized on the interactions between these two factors, and how soil variables vary along soil depth gradients in changing land-use types.

To this end, this study was designed to assess the changes in soil physicochemical, nitrogen and microbial indices along vertical gradients (0–10, 10–30 and 30–60 cm soil depths, classified on the base of spatial variability of SOC density, as described earlier Xue et al. (2015) and across contrasting land-use types refereeing them as horizontal gradients. Discriminant analysis (DA) is used as powerful statistical tool in soil research to assess biodiversity among various habitats (Feret et al., 2011) and also to determine differences in soil chemistry and microbial structure and function (Gelsomino and Azzellino, 2011; Xu et al., 2006). This potential tool was thus employed in our study to achieve the following objectives: a) to assess whether the investigated soil properties have discriminating features along land-use types and soil depths b) to determine which parameters contributed most to these variations and c) to establish whether discriminations in microbial indices were stronger than in physicochemical and nitrogen properties. We thus hypothesized that: i) the selected soil properties would be significantly affected by the interactions of both land-use types and soil depths; ii) soil C-related indices would be more important in discriminating these variations; and iii) the microbial indices would be more clearly discriminated than physicochemical properties by further exploiting Discriminant Analysis as statistical test.

2. Material and methods

2.1. Study area description

The study area is located in the Zhifanggou watershed, Ansai Research Station of Soil and Water Conservation of the Chinese Academy of Science (CAS) in the northern Shaanxi Province of China (108°5′–109°26′E, 36°30′–37°39′N) ranging from 1010 to 1400 m above sea level (m asl). The mean annual air temperature of the area was 8.8 °C and the mean annual precipitation was 513 mm (1980–2010). According to the soil classification system of the Food and Agriculture Organization of the United Nations (FAO), soil in this region is classified as Calcic Cambisols (IUSS Working group WRB, 2014) silty loam texture. The vegetation type is of the forest-grassland belt variety, which represents a transitional environment between the warm, temperate deciduous broadleaved forest and the dry grassland belt (Xu et al., 2009). In the Zhifanggou Watershed prior to approximate period of 1950, large parts of land resources were transformed to farmland as a strategy to manage resources for increasing population. However, since the 1970s, vegetation restorations have been carried out extensively. In the year 1999, the Government of China implemented the "Grain for Green" project where the substantial amount of croplands were shifted into grassland and artificial forestland (Fu et al., 2009). Currently, the main land-use types in this region are grassland (*Medicago sativa* L.), shrubland (*Caragana korshinskii* Kom.; *Hippophae rhamnoides* L.), forestland (*Robinia pseudoacacia* L.) as well as some remained abandoned, crop and orchard land. In the studied area, the grassland is dominated by two types of Herbaceous vegetation – *Artemisia sacrorum* and *Heteropappus altaicus* (Table 1); the abandoned land was previously cultivated with corn (*Zea mays* L.) and wheat (*Triticum aestivuml.*); the orchard cultivate typical apple trees (*Malus pumila Mill.*) with the planting age of around 10–15 years; the main crop on the farmland is monoculture with maize (*Zea mays* L.), without any rotation applied in the cropping system. The mean annual application of organic (manure based) and inorganic (nitrogen-, phosphate-, and potash-based) fertilizers in the orchard and farmland were investigated through questionnaire method to understand fertilization status of these two managed soil types. The organic fertilizer applied ranged from about 30 kg hm^{-2} and 60 kg hm^{-2} whereas inorganic fertilizer accounted for about 275 kg hm^{-2} and 320 kg hm^{-2} (∼9 and ∼5 times more as compared with organic fertilizers) in orchard and farmland respectively.

Table 1
The general geographical and vegetative characteristics of the investigated sites.

Sites	Altitude (m asl)	Geomorphological position	Slope aspect	Slope orientation	Latitude (N)	Longitude (E)	Undergrowth vegetation
Grassland	1361	Mid-slope	Slight	South	36°43.880′	109°14.237′	*Artemisia sacrorum* *Heteropappus altaicus*
Abandoned	1420	Convex creep slope	Slight	Top	36°43.491′	109°14.290′	*Artemisia capillaris* *Buddleja lindleyana*
Orchard	1384	Mid-slope	Slight	South	36°43.696′	109°15.251′	*Apple tree* (*Malus pumila Mill.*)
Farmland	1035	Mid-slope	Terrace	South	36°46.234′	109°16.102′	Corn (*Zea mays* L.) Wheat (*Triticum aestivuml.*)

2.2. Soil sampling and experimental design

Four typical land-use types were selected as representatives of land-use change: previously existed cropland and orchard (unconverted), abandoned land (for natural recovery) and grassland (naturally recovered). These land-uses are located in the same area within the Zhifanggou catchment, with similar geomorphological positions and slope aspects. In July 2011, an area of 100 m × 100 m were selected in each of land-use types. The geographical and vegetation characteristics of the investigated sites are as given in Table 1. In each site, three replicate subplots were randomly selected with an area of 10 m × 10 m and five replicate soil subsamples were collected form each subplots, at the soil depths of 0–10 cm, 10–30 cm and 30–60 cm using auger boring with a drill size of 80 cm length and 5 cm diameter. Three scales of 10, 30 and 60 cm were marked on the drill before sampling. 5 subsamples of identical soil depths were mixed to form a composite sample of approximate 500 g. The same process was repeated for each soil depth separately to minimize the spatial variability of soil. The fresh soil samples were then sealed in plastic bags and transported to the laboratory in iceboxes. Collected soil samples were then sieved (< 2 mm) to remove discernible roots, stones and macro-fauna and were air dried to measure the soil physicochemical properties. Aliquots of the samples were stored at 4 °C for the microbial biomass and basal respiration analyses.

2.3. Soil sample analysis methods

2.3.1. Soil physicochemical properties analysis

Soil water content was measured gravimetrically by oven drying moist soil samples (105 °C, 24 h). The bulk density was determined using cutting ring by the standard methods recommended by the soil agricultural and chemical analysis (Nu, 1999). Soil pH and electrical conductivity were determined in a soil-to-water (1:2.5, *W/V*) mixtures of dry soil and distilled water using a Delta 320 pH meter (Mettler-Toledo Instruments (Shanghai, China) Co., Ltd. Soil organic carbon (SOC) was determined via wet oxidation using dichromate in an acid medium followed by the $FeSO_4$ titration method (Bao, 2007). Available P (AP) content was measured by lixiviating-molybdenum blue colorimetry after extraction with 0.5 M $NaHCO_3$ (pH 8.5) for 30 min (Nu, 1999). Total nitrogen (TN) was measured with Kjeldahl digestion and distillation azotometry. Nitrate-N (NN) and ammonium-N (AN) were determined by extraction with 1 M KCl at a ratio of 1:5 (*W/V*) using an automated Continuous-Flow Auto Analyzer (Bran Luebbe AA3, Germany) (Nu, 1999). Mineralizable nitrogen (MN) was determined by the aerobic culture method (Nu, 1999) and was calculated by subtracting the initial soil ammonium-N from the final soil ammonium-N after an incubation at 40 °C for 7 days. Organic N was calculated by subtracting the content of inorganic N (NH_4^+ and NO_3^-) from total N. Extractable organic N was determined by extracting with K_2SO_4 as described by Wu and Brookes (2005). The K_2SO_4-organicN extracts were oxidized by alkaline persulphate to determine nitrate using ultraviolet spectrophotometry analysis at 220 nm and 275 nm in a Hitachi UV2300 spectrophotometer.

2.3.2. Soil basal respiration (BR) and microbial biomass

Soil basal respiration (BR) was determined by incubating soil samples for 24 h at 25 ± 1 °C, trapping the evolved CO_2 in an alkaline solution (1 M NaOH) and finally measuring the amount of CO_2 evolved by HCl titration (Nu, 1999). BR was expressed as μg CO_2 day^{-1} g^{-1} soil.

Microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP) were measured by the chloroform fumigation–extraction method (Brookes et al., 1982; Ross, 1990). The amount of K_2SO_4-extracted C was determined using a total organic carbon analyzer (Phoenix 8000). A K_{EC} factor of 0.45 (Wu and Brookes, 2005) was used for converting the extractable-C (the difference in the TOC between the fumigated and non-fumigated samples) to the microbial biomass C. Microbial respiratory quotient (RQ) was calculated by dividing the basal respiration by the microbial biomass C (MBC) (Anderson and Domsch, 1990). Microbial quotient (MQ) was calculated as the ratio of soil MBC to total organic C (that ratio of MBC/TOC; Anderson and Domsch, 1989). Soil total N content, in fumigated and non-fumigated samples was determined by extraction using 0.5 M K_2SO_4 at a 1:4 soil to extractant ratio. Further, the amount of K_2SO_4-extracted total N was analyzed using modified method of persulfate oxidation -ultraviolet spectrophotometry (Cabrera and Beare, 1993). The MBN was measured as the difference in total N between fumigated and non-fumigated samples using a converting factor $K_{EN} = 0.54$ (Wu and Brookes, 2005). The inorganic P (from the fumigated and non-fumigated samples) was extracted using 0.5 M $NaHCO_3$ (in a ratio of 1:20) extracts, the amount of extracted phosphate was analyzed through a colorimetric analysis at 882 nm. The MBP was calculated as the difference in inorganic P between fumigated and non-fumigated samples using a converting factor $K_{EP} = 0.4$ (Brookes et al., 1982).

2.4. Data analysis

Two-way ANOVA was performed (with PASW Statistics 18) to evaluate the influence of land-use types, soil depths and their interaction (land-use ∗ depths) on soil physicochemical, nitrogen and microbial indices. In addition, simple main effects were analyzed (also with PASW Statistics 18) for soil properties that have statistically significant interactions. For this purpose, we developed a one-way ANOVA to assess significant differences in terms of soil depth and land use. Differences among means were assessed with *Turkey* HSD post hoc texts ($P < 0.05$) Discriminant functions were calculated using PASW Statistics 18. Soil physicochemical (Organic C, total N, C/N ratio, available phosphorus, bulk density, pH and electrical conductivity), nitrogen forms (Nitrate-N, ammonium-N, organic N, mineralizable N, microbial biomass N and extractable organic N) and microbial properties (basal respiration, respiratory quotient, microorganism quotient, microbial biomass carbon (MBC) and microbial biomass phosphorus (MBP) were taken for calculations of discriminant functions of land-use types and soil depths (0–10 cm, 10–30 cm and 30–60 cm) separately. In order to identify the significant/dominant characteristics as drivers of discrimination/separation of the various soil parameters, two discriminant functions - discriminant function 1 (DF 1) and discriminant function 2 (DF 2) were calculated with the detailed discriminant functional coefficients (for each soil parameter).

3. Results

3.1. Physicochemical properties

Soil physicochemical properties were all significantly influenced by land-use types, soil depths and their interactions (Table 2, $P < 0.05$). With soil depths as main factor, chemical properties including TOC, total N and AP showed a stronger decrease along depth gradients ($P < 0.05$) as compared to physical properties (Table 2). The analyzed soil samples were all typically alkaline (pH > 8) loess soils. Soil pH of grassland and orchard showed an increase along depths (Table 2). All soils were neutral in salinity (EC < 2000 μs/cm) with EC values ranging from 74 (0.4) to 164 (1.3) μs/cm. A reverse trend indicating decrease in changes of EC with soil depths for grassland and abandoned land-use type soils was noted in comparison to an increase in case of orchard and farmland-use (Table 2) types. Soil BD slightly varied among the four sites ranging from 1.1 to 1.4 g/cm. The mean values (three soil depths) of the content of TOC were higher in the orchard and farmland (6.5–7.6 g kg^{-1}) than other land-use types (3.5–4.1 g kg^{-1}) (Table 2). Contents of soil total N, decreased in the order of grassland (0.60 g kg^{-1}) > farmland (0.46 g kg^{-1}) > orchard (0.26 g kg^{-1}) > abandoned land (0.13 g kg^{-1}) (Table 3). Soil C/N ratios were

Table 2
Physicochemical properties of soils from four land uses and the two-way ANOVA (soil depth and land use).

Land use type	Depth	TOC (g kg^{-1})	TN (g kg^{-1})	C/N ratio	AP (mg kg^{-1})	BD (g cm^{-3})	pH	EC (μs/cm)
Grassland	0–10	6.1 (0.0) b_A	0.7 (0.1) a_A	8.6 (0.7) c_A	9.1 (0.0) b_A	1.2 (0.0) b_A	8.4 (0.0) d_C	106.5 (4.1) b_A
	10–30	4.9 (0.8) b_B	0.7 (0.1) a_A	7.0 (0.5) d_A	8.8 (0.0) ab_B	1.2 (0.1) b_A	8.4 (0.2) b_B	93.3 (0.4) b_B
	30–60	1.1 (0.2) c_C	0.4 (0.1) b_B	3.1 (0.1) c_B	8.7 (0.0) a_C	1.2 (0.1) c_A	8.6 (0.2) a_A	88.0 (0.3) b_C
Abandoned	0–10	2.5 (0.2) c_A	0.2 (0.0) d_A	15.4 (0.3) b_C	6.1 (0.1) d_B	1.3 (0.0) a_A	8.7 (0.0) b_A	84.2 (0.4) c_A
	10–30	4.3 (0.2) c_B	0.1 (0.0) d_B	33.1 (3.5) a_A	6.8 (0.1) b_A	1.3 (0.1) a_A	8.7 (0.0) a_A	81.6 (0.2) c_B
	30–60	3.7 (0.7) b_C	0.1 (0.0) d_B	26.2 (3.7) a_B	5.3 (0.1) c_C	1.3 (0.0) b_A	8.7 (0.1) a_A	74.4 (0.4) d_C
Orchard	0–10	6.8 (0.5) a_A	0.3 (0.0) c_A	17.6 (1.5) a_C	7.5 (0.4) c_A	1.2 (0.0) c_B	8.7 (0.0) c_C	75.6 (0.2) d_C
	10–30	6.8 (0.3) a_B	0.3 (0.0) c_B	21.8 (0.3) b_B	6.2 (0.3) b_B	1.1 (0.0) c_C	8.7 (0.2) a_B	81.7 (0.1) c_B
	30–60	5.9 (0.3) a_C	0.2 (0.0) c_C	27.8 (2.1) a_A	4.8 (0.0) d_C	1.2 (0.1) b_A	8.8 (0.3) a_A	84.5 (0.1) c_A
Farmland	0–10	7.8 (0.5) a_A	0.5 (0.0) b_A	15.4 (0.5) b_B	26.6 (0.5) a_A	1.3 (0.0) b_C	8.7 (0.0) a_A	115.3 (2.2) a_C
	10–30	9.0 (2.1) a_B	0.5 (0.0) b_B	18.3 (2.8) c_A	16.1 (6.2) a_A	1.3 (0.0) a_B	8.7 (0.0) a_B	137.4 (1.8) a_B
	30–60	6.0 (0.3) a_C	0.4 (0.1) a_C	15.2 (2.4) b_B	5.8 (0.1) b_C	1.4 (0.1) a_A	8.7 (0.3) a_B	164.0 (1.3) a_A
Two-way ANOVA	Land-use	$F = 66$	$F = 220$	$F = 151$	$F = 62$	$F = 107$	$F = 145$	$F = 7855$
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	Depth	$F = 28$	$F = 60$	$F = 25$	$F = 35$	$F = 27$	$F = 24$	$F = 186$
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	Interaction	$F = 10$	$F = 16$	$F = 20$	$F = 22$	$F = 16$	$F = 16$	$F = 773$
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Notes: Values shown are means of three replicates with standard deviation (SD) in brackets. Soil variables are as follows: TOC, total organic C; TN, total N; C/N ratio, total organic C to total organic N ratio; AP, available P; BD, bulk density; EC, electrical conductivity. By soil depth, values of same land-use follow the various upper-case letters indicate significant difference. By land use, values of same soil depth follow the various lower-case letters differ significantly.

higher in the abandoned land (averaged 24.7) and orchard (averaged 22.3) than farmland (averaged 16.3) and the grassland (averaged 6.2) (Table 2) respectively.

3.2. Different forms of N

Six measured soil N forms were all significantly affected by the interactions of land-use types and soil depths ($P < 0.05$; Table 3). Soil N forms showed a significant difference among land-use types (Table 3). However, the concentration of nitrate-N (NN) and extractable organic N (EON) did not differ significantly across soil depths ($P > 0.05$; Table 3). Almost half of the concentrations of soil N indices (including Organic N (ON), mineralizable N (MN) and microbial biomass N (MBN)) decreased along soil depths (Table 3).

Specifically, nitrate-N concentration was highest in farmland (25.5 mg kg^{-1}; Table 3). At the 0–10 cm and 10–30 cm soil depths, the concentrations of ammonium N (AN), ON, and EON were approximately 2 times higher in the grassland (averaged at 2.5 mg kg^{-1} (AN), 701 mg kg^{-1} (ON), and 50 mg kg^{-1} (EON), respectively) than in other land-uses (Table 3). Concentrations of MN were similar under grassland and farmland types (~26.3 mg kg^{-1}) but higher than abandoned land (~2 mg kg^{-1}) and orchard (~7.5 mg kg^{-1}) (Table 3) at 0–30 cm soil depths. Orchard and farmland had higher MBN concentrations (> 100 mg kg^{-1}) as compared to grassland (~60 mg kg^{-1}) and abandoned (~10 mg kg^{-1}) land types (Table 3).

3.3. Microbial indices

The land-use type ($P < 0.001$) and soil depths ($P < 0.001$) as well as their interactions ($P < 0.001$) had significant effects on six soil microbial indices (Table 4). Soil respiration quotient (RQ) increased with soil depths, whereas other microbial variables decreased (Table 4). With regard to the land-use types, the amounts of microbial biomass C

Table 3
Nitrogen contents of soils from four land uses and the two-way ANOVA (soil depth and land use).

Land use type	Depth	NN (mg kg^{-1})	AN (mg kg^{-1})	ON (mg kg^{-1})	MN (mg kg^{-1})	MBN (mg kg^{-1})	EON (mg kg^{-1})
Grassland	0–10	1.9 (0.1) b	2.4 (0.2) a	702.5 (60.1) a_A	25.3 (1.2) a_A	114.8 (20.6) b_A	52.2 (4.8) a
	10–30	2.4 (0.5) c	2.4 (0.4) a	699.1 (61.2) a_A	26.6 (1.1) a_A	53.8 (3.2) c_B	48.7 (3.4) a
	30–60	1.6 (0.1) c	1.5 (0.1) b	347.1 (69.5) a_B	1.5 (1.2) a_B	34.8 (7.1) c_C	35.5 (2.9) b
Abandoned	0–10	2.1 (1.0) b	1.5 (0.2) b	162.9 (8.1) d_A	2.9 (1.5) c_A	19.6 (1.9) c_A	22.5 (2.8) b
	10–30	1.8 (0.2) c	1.7 (0.0) bc	129.3 (8.9) d_A	1.0 (0.3) c_A	6.6 (1.1) d_B	21.3 (1.3) c
	30–60	1.1 (0.1) d	1.7 (0.1) a	138.9 (4.8) c_A	0.4 (0.2) b_B	3.7 (0.3) d_C	21.0 (0.2) c
Orchard	0–10	3.5 (0.7) b	1.4 (0.3) c	384.3 (6.8) c_A	10.4 (2.3) b_A	173.9 (11.2) a_A	14.6 (0.7) c
	10–30	5.5 (1.5) b	1.5 (0.1) c	307.4 (11.9) c_B	4.6 (1.1) c_AB	117.7 (5.5) b_B	7.6 (0.6) d
	30–60	3.3 (0.4) b	1.5 (0.1) b	210.1 (6.9) b_C	1.0 (0.6) b_B	89.3 (3.4) b_C	17.3 (3.5) d
Farmland	0–10	10.0 (6.5) a	0.8 (0.0) d	493.5 (10.9) b_A	18.4(3.5) a_B	131.1 (2.8) a_A	19.0 (4.5) b
	10–30	32.4 (2.4) a	1.3 (0.4) b	453.1 (18.1) b_AB	36.2 (2.1) a_C	145.0 (13.7) a_B	26.5 (8.5) b
	30–60	33.1 (3.7) a	1.2 (0.5) a	371.3 (48) a_B	44.2 (5.1) a_A	161.7 (14.7) a_B	41.8 (9.5) a
Two-way ANOVA	Land-use	$F = 25$	$F = 22$	$F = 249$	$F = 229$	$F = 381$	$F = 60$
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	Depth	$F = 3$	$F = 4$	$F = 73$	$F = 38$	$F = 52$	$F = 1$
		$P = 0.075$	$P = 0.045$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.42$
	Interaction	$F = 3$	$F = 5$	$F = 17$	$F = 9$	$F = 26$	$F = 8$
		$P = 0.034$	$P = 0.003$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Notes: Values shown are means of three replicates with standard deviation (SD) in brackets. Soil variables are as follows: NN, nitrate N; AN, ammonium N; ON, organic N; MN, mineralizable N; MBN, microbial biomass N; EON, extractable organic N. By soil depth, values of same land-use follow the various upper-case letters indicate significant difference. By land use, values of same soil depth follow the various lower-case letters differ significantly.

Table 4
Microbial indices of soils from four land uses and the two-way ANOVA (soil depth and land use).

Land use type	Depth	MBC ($mg\ kg^{-1}$)	MBN ($mg\ kg^{-1}$)	MBP ($mg\ kg^{-1}$)	BR (μg CO2 $day^{-1}\ g^{-1}$soil)	RQ ($mg(CO_2)_q\ (biomass\text{-}C)^{-1}\ h^{-1}$)	MQ (%)
Grassland	0–10	328.4 (8.9) a_A	114.8 (20.6) b_A	21.3 (0.1) b_A	75.4 (2.4) c_C	9.5 (0.3) d_C	5.3 (0.0) a_A
	10–30	121.7 (9.6) a_B	53.8 (3.2) c_B	17.5 (0.1) a_B	82.7 (4.2) c_B	28.6 (2.2) c_B	2.4 (0.1) a_C
	30–60	46.0 (17.1) a_C	34.8 (7.1) c_C	4.5 (0.1) c_C	102.7 (8.8) b_A	111.2 (49.2) b_A	1.9 (1.2) a_B
Abandoned	0–10	88.9 (3.0) c_A	19.6 (1.9) c_A	1.1 (0.2) d_B	87.1 (7.6) b_B	41.0 (4.9) c_C	3.4 (0.2) b_A
	10–30	16.6 (0.5) c_B	6.6 (1.1) d_B	2.1 (0.1) d_A	103.7 (2.5) b_A	260.0 (0.7) a_B	0.4 (0.0) d_B
	30–60	9.4 (1.4) c_B	3.7 (0.3) d_C	1.8 (0.0) d_A	86.6 (5.5) b_B	386.2 (34.5) a_A	0.3 (0.0) b_B
Orchard	0–10	138.9 (9.1) b_A	173.9 (11.2) a_A	1.4 (0.8) c_C	99.2 (6.7) c_C	276.2 (39.9) a_B	2.1 (0.4) b_A
	10–30	112.9 (5.4) a_A	117.7 (5.5) b_B	4.2 (0.0) c_B	242.7 (0.6) c_B	89.7 (4.5) c_B	1.6 (0.6) b_A
	30–60	28.0 (6.8) b_B	89.3 (3.4) b_C	6.0 (0.5) b_A	96.0 (3.6) b_A	148.8 (363.8) b_A	0.5 (0.1) b_B
Farmland	0–10	115.9 (5.1) b_A	131.1 (2.8) a_A	27.4 (0.3) a_A	235.7 (15.6) a_A	84.5 (1.9) b_C	1.5 (0.2) c_A
	10–30	54.9 (2.1) b_B	145.0 (13.7) a_B	15.2 (0.4) b_B	180.5 (35.4) a_C	138.1 (32.2) b_B	0.6 (0.1) c_B
	30–60	25.2 (7.4) b_C	161.7 (14.7) a_B	9.2 (0.2) a_C	200.8 (30.3) a_B	346.1 (51.5) a_A	0.4 (0.1) b_C
Two-way ANOVA	Land-use	F = 281	F = 381	F = 5490	F = 3351	F = 45	F = 70
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	Depth	F = 640	F = 52	F = 1708	F = 556	F = 74	F = 124
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	Interaction	F = 87	F = 26	F = 1168	F = 506	F = 28	F = 12
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Notes: Values shown are means of three replicates with standard deviation (SD) in brackets. Soil variables are as follows: MBC, microbial biomass C, MBN, microbial biomass N (the value of MBN was also included in Table 3 as one of important soil N indicators); MBP, microbial biomass P; BR, basic respiration; RQ, respiration quotient; MQ, microbial quotient (ratio of MBC/TOC). By soil depth, values of same land-use follow the various upper-case letters indicate significant difference. By land use, values of same soil depth follow the various lower-case letters differ significantly.

(MBC), microbial biomass N (MBN) and microbial biomass P (MBP) were lowest in the abandoned land (~30 $mg\ kg^{-1}$, 10 $mg\ kg^{-1}$ and 1.7 $mg\ kg^{-1}$, respectively) (Table 4). The grassland soil had the highest values of MBC (~160 $mg\ kg^{-1}$) and microbial quotient (MQ) while those of MBN were highest under the orchard (~127 $mg\ kg^{-1}$) and farmland (~146 $mg\ kg^{-1}$). Simple main effect revealed that microbial biomass carbon (MBC) was significantly higher in grassland top (0–10 cm, $P < 0.05$) and deeper (30–60 cm, $P < 0.05$) soil depths than other land-use types. At the middle soil depth (10–30 cm), MBC content even though higher in grassland was not significantly different from orchard ($P > 0.05$) (Table 4). Soil basal respiration (BR) was highest in farmland (~200 μg CO_2 $day^{-1}\ g^{-1}$ soil) compared to other land-use types. RQ showed soil depths dependent variations whereas among land-use types the value was highest in 0–10 cm depths in orchard (276.2 mg (CO_2)q $(biomass\text{-}C)^{-1}\ h^{-1}$) form of land-use. Further it was highest under abandoned land (~320 mg (CO_2)q $(biomass\text{-}C)^{-1}\ h^{-1}$) at 10–30 cm and 30–60 cm depths respectively (Table 4).

3.4. Discriminant analysis of soil properties

In the present study, discriminant analysis (DA) was applied to differentiate soil physicochemical, nitrogen and microbial parameters at different soil depths of multiple land-use types and also to test the significance of the differences in these parameters. Discriminant functions showed that the three soil parameters were clearly separated by land-use types (Fig. 1A, B and C) than by soil depth gradients (Fig. 1D, E and F).

Among the investigated four different land-use types, 3 discriminating functions (DF) were generated using discriminant analysis. We chose the sum of the first two significant ($P < 0.001$) DF1 and DF2 as an indicator to assess the sensitivity of soil properties. As revealed by a gradual decrease in the summed percentage of DF1 and DF2 (Fig. 1), soil microbial indices (98.6%) were more sensitive than nitrogen (97.9%) and physicochemical properties (93.4%). At the three soil depths, two DF's were generated from the discriminant analysis. From these we used the first significant ($P < 0.001$) DF1 as an indicator to assess the sensitivity of soil properties along soil depth profiles. The results showed that the sensitivity decreased from microbial indices (87.8%) to nitrogen (73.1%) to physicochemical properties (70.5%) even along soil depth gradients. In comparison to soil depths, soil properties varied more along land-use types. In our analysis, this was indicated by clear separation and the relatively higher DF percentages (Fig. 1).

The discriminant functional coefficients (Table 5) revealed that soil physicochemical, nitrogen forms as well as microbial properties have a notable and selectively different influence on the discriminant functions (features that can be distinguished from land-use types and soil depths). Precisely, EC, MN and MBC are regarded as most important parameters/predictors responsible for major distinction among land-use types for DF 1 (as indicated by highest absolute values of coefficients) and TOC, MBN and MBC as most essential indices for DF 2. Regarding soil depths induced variations; TOC, MN and BR were the strongest predictors for DF 1 while C/N ratio, NN and MQ strongly predicted DF 2 (Table 5).

4. Discussion

4.1. Soil physicochemical properties

In line to our first hypothesis, we found that the seven investigated soil physicochemical properties were all significantly affected by the interactions of land use types and soil depths (Table 2). Based on the discriminant analysis (DA), soil electrical conductivity (EC) can be regarded as the most important soil property responsible for major distinction among land-use types (Table 5). EC significantly decreased with soil depths ($P < 0.05$) under the natural recovering land-use types (grassland and abandoned land) where as in the human intervened land-use types (orchard and farmland) EC significantly ($P < 0.05$) increased along the soil depths. Higher EC at the top soil depth of the abandoned land could be attributed to increased capillary action of water resulting from absence or removal of vegetation cover. This further increased the salt accumulation in top soils due to reduced precipitation and accompanied increase in evaporation from deeper soil depths from such land-use types (Seguel et al., 2013). In case of grassland top soil, higher EC would be due to top to depth distribution of grassland roots. Secretions from these roots increase the amount of soil ions (Kodesova et al., 2011) favoring higher EC. A contrasting trend of variations in EC and other soil properties under managed soil types (orchard and farmland) can be attributed to the disproportionate mean annual application of organic and inorganic fertilizers in these two

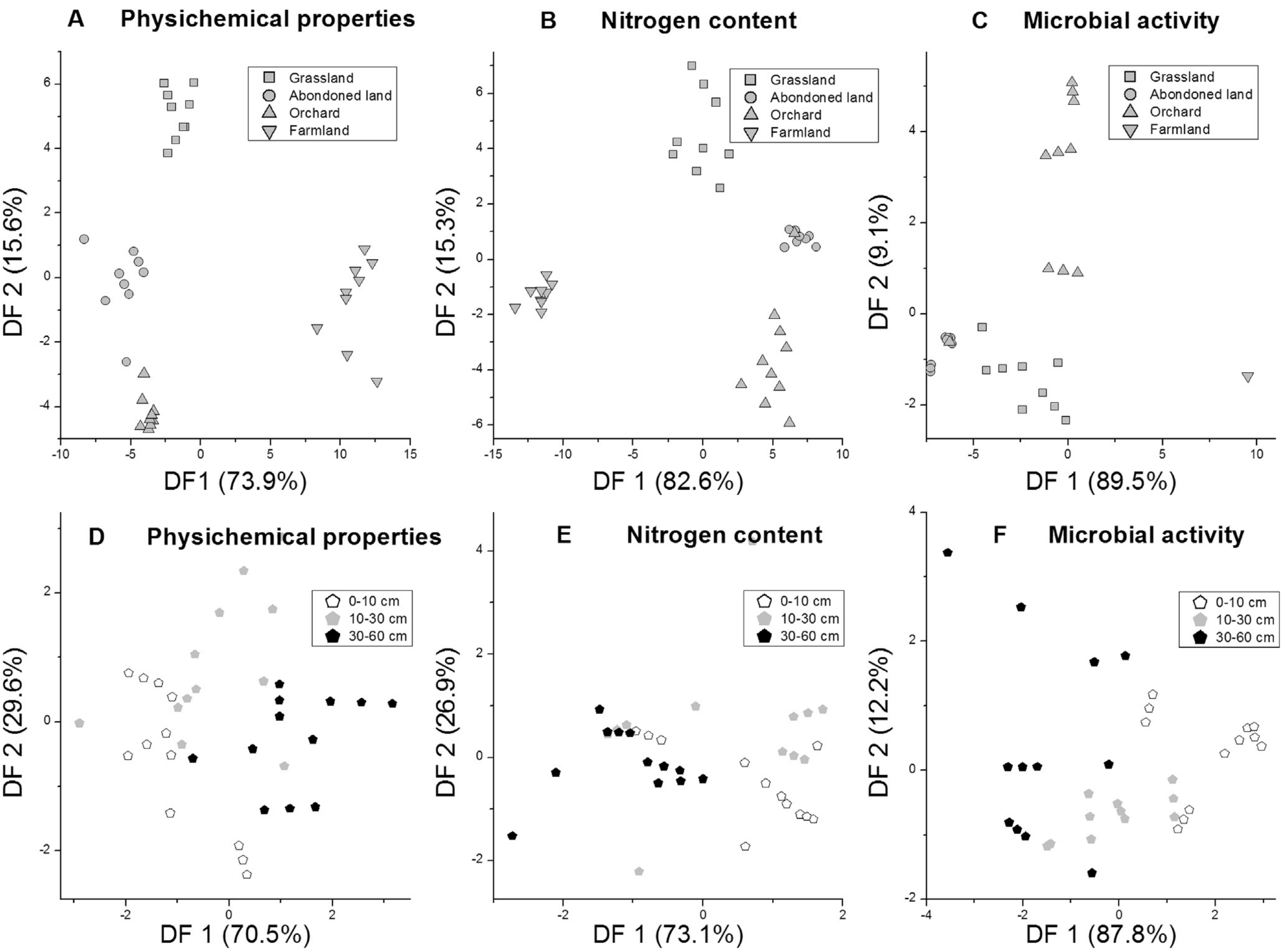

Fig. 1. Discriminant function analyses based on soil physiochemical properties (A and D), various N forms (B and E) and microbial indicators (C and F). Data used are from four different land use types (Abandoned land-triangle, Farmland-inverted triangle, Orchard-circle and Grassland-square) at three soil depths (five-pointed star with colors of light-0-10 cm, grey-10-30 cm and black-30-60 cm). Above part of the figure (A, B and C) is land use and below (D, E and F) is soil depth.

Table 5
Standardized discriminant function coefficients based on soil physiochemical properties, various N forms and microbial indicators, respectively.

	Soil parameters	Land-use types		Soil depths	
		Discriminant function		Discriminant function	
		1	2	1	2
Physicochemical properties	Total organic C (TOC)	−0.221	**2.416**	**−1.484**	−1.131
	Total N (TN)	0.613	−1.139	1.182	1.155
	C/N ratio	−0.297	−0.905	0.774	**2.339**
	Available P (AP)	1.503	−0.981	−0.733	0.04
	Bulk density (BD)	−0.102	−0.067	−0.324	−1.259
	pH	0.973	1.007	1.294	−0.194
	Electrical conductivity (EC)	**1.99**	−0.254	1.145	1.371
Different N forms	Nitrate-N (NN)	1.509	−0.097	0.62	**1.559**
	Ammonium-N (AN)	0.776	−0.141	−0.549	−0.006
	Organic N (ON)	−2.797	1.042	1.17	0.383
	Mineralizable N (MN)	**3.197**	−0.219	**1.308**	0.789
	Microbial biomass N (MBN)	0.114	**−1.274**	−0.062	−1.009
	Extractable organic N (EON)	−0.219	0.923	−0.973	−0.519
Microbial indices	Microbial biomass C (MBC)	**−3.244**	**0.88**	0.261	−0.694
	Microbial biomass N (MBN)	2.322	0.119	−0.425	0.329
	Microbial biomass P (MBP)	1.777	−0.65	0.484	−0.023
	Basal respiration (BR)	−0.59	0.847	**1.467**	0.11
	Respiration quotient (RQ)	0.172	−0.006	−1.223	0.809
	Microbial quotient (MQ)	0.587	−0.611	0.455	**1.179**

Note: The strongest factors of each function are marked in bold.

land-use types. Artificial land management practices such as fertilizer application (in orchard) and tillage intensities (in farmland) contribute the substantial amount of soluble ions seeping into the deeper soil horizons (30–60 cm) resulting in increased EC.

The EC variation among land-uses was well supported by our discriminant analysis indicated by the highest discriminate functional coefficients (Table 5). Compared to EC, soil pH and bulk density (BD) showed minor differences along soil depths in each land use type (Table 2). This result is in line with the previous studies conducted at the similar regions (An et al., 2009; Huang et al., 2015; Xu et al., 2016; Xue et al., 2015) owing to common soil parent material and soil type of the Loess Plateau in China. In support of our second hypothesis, soil organic carbon (SOC) was the most discriminating variables across soil depths (Table 5). Simple main effect revealed that i) at the 0–10 cm soil depths of grassland, SOC was significantly higher compared with abandoned land-use type ($P = 0.03$). Whereas no significant differences were observed when compared with orchard ($P = 0.44$) and farmland ($P = 0.62$). This clearly indicates that without aboveground litter accumulation, SOC content at top soils in abandoned land could be substantially decreased. Interestingly, at 10–30 cm soil depths, SOC content was not significantly different among any of the four land-use types. However at the deeper soil depths of 30–60 cm, SOC was found to be significantly lower in grassland type ($P > 0.05$). These results evidently show that the C pool in middle soil depths (10–30 cm) is relatively stable regardless of differences in land-use type of this region. At deeper depths (30–60 cm), decrease in C content and C pool in grassland type is relatively due to absence of grass roots as indicated by studies related grassland root distribution patterns (Olupot et al., 2010). The high SOC content in abandoned land can be explained by the legacy effect of land-use history, where this abandoned land was once a useful farmland type. The existing C in SOC pool in this land use type is thus due to accumulation of C contents in deeper soil horizons (Schulp and Verburg, 2009; Stevens and Van Wesemael, 2008; Wilson and Lonergan, 2013).

4.2. Different N forms

Changes in land-use patterns induced changes in above- and belowground environment. Among other soil properties, this is also a cause for alterations in soil N forms and contents due to differentially expressed plant and microbial capabilities towards uptake of various soil N forms (Weigelt et al., 2005). We found that mineralizable N (MN) discriminated more profoundly the effects of changes in land-use types and soil depths than other N forms (Table 3). These results are in agreement with previous studies using canonical correlation analysis (CCA) indicating soil mineralizable N as sensitive indicator reflecting changes in soil N stocks (Bremer and Kuikman, 1997; Jin et al., 2013; Murphy et al., 1998). Benintende et al. (2015) in their study have shown MN as suitable distinguishing factor among agricultural land-uses types further considering it as a potential biological index of N availability. However, information on variations in soil N properties along various soil depths in changing land-use types is still limited. Hence this study will greatly help to better our understanding in soil N properties in both soil depths and land-uses. We found that MN contents significantly decreased along soil depths (Table 3) of abandoned land and orchard land-use types. Further suggesting a substantial decrease of plant available N along soil depth gradients under these two land-use forms. Additionally, we also found decreasing trend of microbial biomass N (MBN) along the soil depth gradients. Variations in these two N forms could be well attributed to i) an important role played by MBN in maintaining soil nitrogen supply (Kooijman et al., 2009) and ii) the close relationship of MN and MBN where MBN shows rapid turnover capacity and larger mineralization potential contributing to the amount of traceable MN contents in soil (Iyyemperumal et al., 2007). In grassland, the MN content was 2–8 times higher than that of in the orchard and abandoned land at top and middle depth (0–10 cm and 10–30 cm, respectively) (Table 3). This implies the higher N supply ability in grassland at the top and middle soil depths, while for the grassland itself, there were no significant difference between top and middle soil depth. This would be closely related to the similar content of grassland soil total organic N as well as the inorganic N (nitrate-N and ammonium-N) (Table 3). The reason of none significant difference for the above-mentioned four N forms can be explained by the assumption that the simultaneously biological process lie in the inorganic N production and the releasing of plant available N (Sharifi et al., 2007; St. Luce et al., 2011).

Considering soil depths as main response variable, simple main effect showed significantly higher MN in grassland top ($P = 0.003$) and farmland middle and deeper soil depths ($P = 0.015$) when compared to other land-use types. Land management practices like crop rotation in farmland lead to overall changes in soil nutrient contents. N contents such as MBN and MN are related to biological variables (microbes and plant roots) which are sensitive to these practices (Marinari et al., 2006; Monokrousos et al., 2006). This is one reason for observing higher MN contents at deeper soil depths in farmland use in our study where such management practices are routinely followed.

4.3. Microbial properties

At the various soil microenvironments influenced by land-use types, changes in microbial indices such as respiratory capacities, microbial biomass and microbial quotients enable us to monitor alterations in microbial community structure and function (Lauber et al., 2013; Nannipieri et al., 2003; Steenwerth et al., 2003; Wright and Reddy, 2001). In general, MBC was consistently higher in grassland at all soil depths. This higher MBC at top and middle soil depths in grassland is due to higher plant diversity observed in comparison to other land-use types (Table 1) of our study. Grass root exudates and other C-containing compounds from soil get incorporated into microbial biomass leading to higher MBC (Millard and Singh, 2010; Thakur et al., 2015). Higher MBC at the deeper soil depths of grassland could be the result of leading processes that ultimately lead to vertical transport, transformation and accumulation of dissolved organic carbon compounds (via decomposition of grass root and/or other detritus soil material) from top to lower soil depths (Uselman et al., 2007). Discriminant analysis indicates MBC to be the most important index responsible for major distinction among land-use types (Table 5) justifying the second hypothesis of our investigation. In addition, MBC is easier to analyze when compared with microbial biomass nitrogen and phosphorus (MBN and MBP) rendering it as a potential tool for other future practical analyses.

Previously reports suggest that in most cases, the overall rate of soil respiration was controlled strongly by the combined effects of temperature, moisture availability and substrate properties than by vegetation type alone (Raich and Tufekcioglu, 2000). Our results also indicate that soil substrate-induced respiration (basal respiration, BR) was the most discriminating variables for soil quality, across soil depths rather than land-use types (Table 5). Additionally along soil depth gradients we found that, BR was the most significantly decisive parameter in comparison to other microbial indices (Table 5). This was in accordance with the changes in TOC at different soil depths (Table 2). As demonstrated by Gelsomino and Azzellino (2011) earlier demonstrated that TOC primarily influence the metabolic activities of soil microbes. Thus changes in TOC at different soil depths lead to changes in BR intensities in our study.

Highest BR in farmland top soil was probably due to the decomposition of crop residues such as straw which plays important roles in soil C storage and respiration (Fan et al., 2015). In orchard, the highest BR observed in the middle soil depths can be related to the production practices of mucking the field with organic fertilizers that accumulate at middle soil layers further serving as substrates BR (Liu et al., 2013; Zhao et al., 2014). It significantly affects soil microbial activities and efflux of CO_2 from this soil layer. Studies from European continent have already demonstrated sensitivity of soil respiration and microbial biomass C towards various soil management practices and thus has been included as a prominent indices in soil monitoring programs (Nielsen and Winding, 2002). Our results potentially indicate the suitability of these two microbial indices in evaluating soil quality changes from Chinese Loess plateau.

In accordance to our third hypothesis, these results demonstrate the higher sensitivity of soil microbial than soil physicochemical indices (Fig. 1) towards changes in soil conditions. Soil inhabiting microbes from complex interactions are vital part of soil ecosystems. They are further under continuous influence of soil ecological process and are affected by variations in land-use forms and the environmental fluctuations such as soil moisture and temperature regimes together with soil types (Blume et al., 2002; Zhang et al., 2016). In our study we established an understanding of the strength with which these soil properties vary along variations in horizontal and/or vertical soil gradients.

5. Conclusion

Our results demonstrate the differential effects of soil depths and land-use types on important soil properties further exhibiting dissimilar features among these observed effects. We here by propose strong consideration of soil electrical conductivity, mineralizable nitrogen and microbial biomass carbon in land-use relevant studies. Research activities focusing on soil properties along vertical soil profiles must consider soil organic carbon, mineralizable nitrogen and basal respiration as prominent properties among others for better understanding of soil quality and health. From our study we found discriminant analysis as a powerful statistical tool to discriminate the routinely measured soil properties at different levels such as horizontal and/or vertical gradients. Changes in land-use types lead to changes in soil chemical properties even at soil depth gradients. These together significantly affected the microbial indices such as basal respiration, microbial biomass C and mineralizable nitrogen forms. These variations in microbial indices can be regarded as potential indicator of soil health in response to variations in land-use types and below ground properties that are strongly interlinked to each other.

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