Journal of Animal & Plant Sciences, 35(2): 2025, Page: 531-545
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.2.0044

DISENTANGLING CLIMATIC AND INTRINSIC PHYSICOCHEMICAL CONTROLS ON ORGANIC CARBON POOLS OF AGRICULTURAL SOILS ALONG A CLIMATE GRADIENT

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ABSTRACT

Recently, physicochemical properties of soils are emerging as an equally important control on soil organic carbon (SOC) pools as climatic factors. However, the same needs to be established across biomes and soil types to generalize the emerging paradigm. We sampled 21 agricultural soils from a climate gradient of humid to arid covering ~1600 km distance between 28.02°-35.8° N in Pakistan. An array of soils' physicochemical characteristics was determined in addition to various SOC pools. Backward stepwise regression, correlation matrix, and principal component analysis were performed to link climatic and soils' physicochemical variables with SOC pools. When controlled for soil's physicochemical properties, climatic variables did not show any significant relationship with any of the SOC pools. However, physicochemical properties of soil like clay content, calcium (Ca^{2+}) and magnesium (Mg^{+2}) ions, and total reserve in base cations (TRB) were found in a strong positive relationship with SOC content and stocks as well as stable SOC fractions. Moreover, as the SOC content of a soil increased, living part of the SOC i.e., microbial biomass also increased while its mineralizable fraction decreased. On the other hand, an increasing pH resulted in higher SOC mineralization and lower SOC content and stocks. Overall, these results indicate that the clay particles, and their component base minerals protect the SOC through aggregation and by making organo-mineral complexes. In conclusion, we show that climatic variables mean annual temperature (MAT) and mean annual precipitation (MAP) were not found strongly linked with any of the SOC pools across a large gradient of soil's intrinsic physicochemical properties. However, across a large gradient of climatic variables, soil's physicochemical properties were found to strongly control SOC and its pools. Specifically, clay particles and total reserve in base cations were found to be key determinants of SOC.

Key words: Soil organic carbon (SOC), SOC fractionation, mean annual temperature, mean annual precipitation, microbial biomass, soil respiration

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INTRODUCTION

Soil inhabits over 1.55 × 10^{12} t of global carbon and is the largest pool of carbon in terrestrial ecosystems (Lal, 2008; Liu *et al.*, 2023). All the definitions of soil from ecological and agricultural perspective are based on soil's functions i.e. soil quality, soil fertility, soil health, soil security. All these function-based definitions of soil have SOC as a pivot (Lehmann *et al.*, 2020). The SOC holds such an important position vis-à-vis defining soils' functions because it can substantially modulate physicochemical and biological properties of a soil (Lal, 2006; Zahid *et al.*, 2025). Moreover, CO_2 emissions from mineralization of only 10% of SOC stock is equal to 30 years of anthropogenic CO_2 emissions (Kirschbaum, 2000). This estimation further underlines the importance of SOC. Consequently, the critical potential of the SOC to serve as a reservoir or source of CO_2 in atmosphere warrants research on understanding the mechanisms of stabilization of SOC. The physicochemical properties of soil, climate, management practices and land use interact in complex ways to determine the fate of SOC (Davidson and Janssens, 2006; Schmidt *et al.*, 2011; Keuper *et al.*, 2020).

Rate of SOC accretion or depletion in different localities or even within similar geographic locations varies widely. It is controlled by the critical equilibrium between organic carbon input and loss as a result of complex interplay between various abiotic and biotic controls (Balesdent *et al.*, 2018; Chenu *et al.*, 2018). These controls may be categorized into three groups. Firstly, climatic variables like temperature and precipitation are important determinants of organic carbon pools in soil (Davidson and Janssens, 2006; Alvarez *et al.*, 2018; Keuper *et al.*, 2020). Secondly, landscape properties, including land use type, soil management regimes, and vegetation density and quality are also crucial in determining the balance of organic carbon inputs and outputs from soil (Shahzad, 2012; Shahzad *et al.*, 2012; Ausseil *et al.*, 2015; Kaneez-e-Batool *et al.*, 2016; Wiesmeier *et al.*, 2019). Thirdly, soils' physicochemical properties like texture, structure, aggregation, cation exchange capacity, pH, mineral composition etc., are also important determinants of size and turnover of SOC (Dungait *et al.*, 2012; Doetterl *et al.*, 2018; Shahzad *et al.*, 2019). The relative value of these sets of controls is a moot point and may vary in different environmental and geographical settings.

Historically, climatic variables, particularly precipitation and temperature have been regarded as primary controls over the SOC (Davidson *et al.*, 2006; Paré *et al.*, 2006; Conant *et al.*, 2011). Generally, warming has been shown to increase the mineralization of SOC, though SOC mineralization often acclimated to soil warming on ecosystem scale (Davidson *et al.*, 2006; Conant *et al.*, 2008; Hopkins *et al.*, 2014). However, warming in polar regions has started exposing frost-protected SOC for microbial mineralization thereby leading to potential climate warming feedback (Keuper *et al.*, 2020). Despite plenty of research on this question, there is ambiguity about magnitude and direction of the changes induced in SOC by climate. The caveat may lie in the soil's intrinsic properties which are often overlooked in such studies. In recent years, physicochemical properties of soil have been gaining traction as strong determinants of SOC dynamics and stocks (Dungait *et al.*, 2012; Doetterl *et al.*, 2015b; Shahzad *et al.*, 2018). Soil's mineral component help stabilize SOC in two ways. The organic matter may be occluded within aggregates formed by fine soil particles like silt and clay (Lützow *et al.*, 2006; Dungait *et al.*, 2012). It can also form organo-mineral complexes with charged clay particles (Lützow *et al.*, 2006; Shahzad *et al.*, 2018). Both these mechanisms, collectively known as physical protection by soil particles, preclude microbial access to SOC thereby keeping a check on its decomposition (Dungait *et al.*, 2012). The strength of these two mechanisms varies with soil type, i.e., particle distribution as well as composition of the minerals, which may then account for up to 95% of the total SOC (Luo *et al.*, 2017).

Due to strong tie-ins between climatic factors and soils' physicochemical properties, isolated analysis of each set of factors may limit valuable prediction of SOC dynamics. Additionally, the estimated role of any individual factor might be a combined effect of its direct and indirect effects on SOC dynamics. For example, climatic controls are regarded as major controls on SOC according to various ecosystem C cycle and earth system models, but many uncertainties remain in their predictions (Bradford *et al.*, 2016). For instance, projections of five SOC models based on 147 field experiments showed that warming increased SOC mineralization and decreased SOC stocks. However, one third of these experiments showed a decrease in SOC mineralization and nearly half of the experiments showed an increase in SOC stocks under warming (Sulman *et al.*, 2018). Consequently, recent modeling and experimental studies propose to assess climatic control of SOC in perspective of physical protection of SOM through its interaction with soil properties and land use type (Gershenson *et al.*, 2009; Tang and Riley, 2015; Moinet *et al.*, 2019; Qin *et al.*, 2019).

Here, we investigated multifaceted controls on SOC content, fractions of the SOC varying in their recalcitrance, and SOC stocks in arable soils found in different climatic zones of Pakistan. For this, we collected soil from a latitudinal and longitudinal gradient of 28.02°-35.8° N and 68.59°-74.98° E respectively (~650 KM from East to West, and ~1600 KM from North to South). Most of Pakistan is located in semiarid and arid climatic regions and covers huge variation in natural ecological and land use settings making them a good candidate for the objectives of this study. The soil of these areas has been formed by the alluvial processes carried out by the five major rivers of the land over centuries. Moreover, the alkaline and calcareous soils of most of the agricultural land are low in SOC content (Khan and Lal, 2007).

The key objectives of this research were: (i) to determine major controls on SOC dynamics in soils sampled from various climatic zones and varying in physicochemical properties, ii) to assess a quantitative relationship between primary determinants of SOC in diverse types of soils belonging to various climates.

MATERIALS AND METHODS

Soil sampling: Soil was sampled from 21 sites spanning across the latitude between 28.02°-35.8° N and longitude 68. 59°-74.98° E (~650 KM from East to West, and ~1600 KM from North to South) of Pakistan covering almost all geoclimatic zones of the country as classified by Koppen climate classification (Figure 1). These latitudinal and longitudinal gradients provide a large variation in terms of climatic variables as well as soil's physicochemical properties. Thirty-year (1981-2011) data of mean annual temperature (MAT) and mean annual precipitation (MAP) data of the selected sites was taken from Pakistan Metrological Department (https://www.pmd.gov.pk/en/). From each site, soil was mixed from five to six 20cm deep randomized cores to form a composite sample. Visible rocks and fauna, roots, and clippings were removed by handpicking followed by sieving through 2mm mesh. Approximately, three kilograms soil from each site was transferred to laboratory on ice in coolers and stored at 4℃ in laboratory until analysis. To avoid storage effects, microbial biomass and potential respiration were determined as soon as the samples reached in the laboratory.

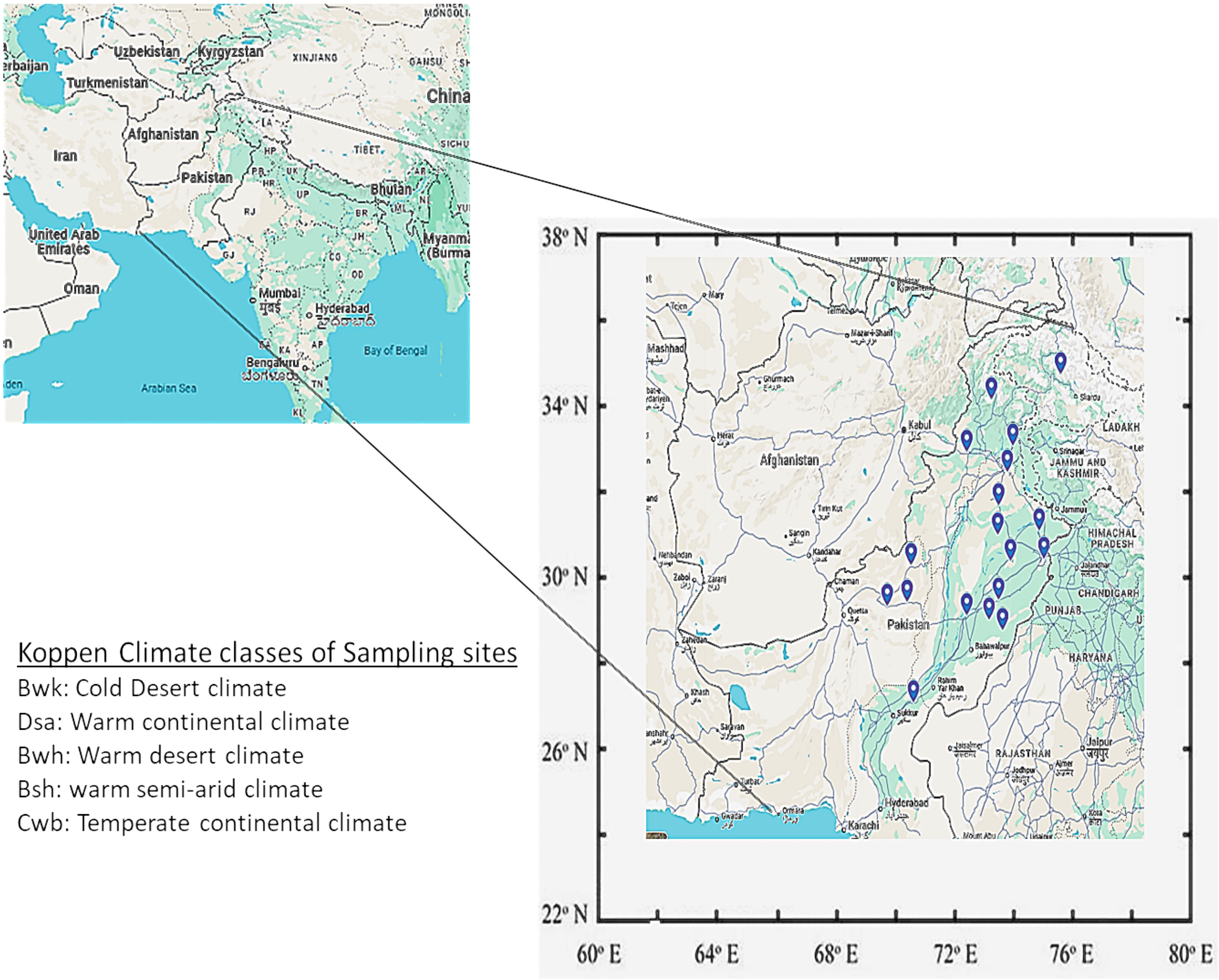


Fig 1. Map of Pakistan climate classification based on Köppen climate classification system showing the location and sampling points of the study area.

Determination of soils' physicochemical properties: Soil pH and EC were measured in soil: water slurry prepared by mixing soil and water in 1:5 (w/v). The pH meter Hanna model 210 and EC meter Hanna model HI 8733 were used to measure pH and EC respectively. Water holding capacity was determined with protocol of Jarrell *et al*., (1999). Briefly, 50 g of field soil was weighed in plastic cylinders (internal diameter 3 cm) whose one end was closed with a fine mesh. These cylinders were then placed in a tray containing water at a level as high as soil columns in the cylinder. After one hour, when the soil was drenched in water through capillary action, cylinders were placed onto the already prepared sand bath. After three hours, when excess water was sucked out of the cylinders via fine mesh under the influence of gravity, the saturated soil in cylinders was weighed. The soil then dried to constant weight at 105 °C. The difference of weight between soil weight before and after oven drying is considered as water holding capacity.

The base cations i.e. Ca^{2+}, Mg^{2+}, N^{+} and K^{+} contents were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Total reserve in `base' cations (TRB, c mole (+)/kg) was determined by taking sum of total Ca^{2+}, Mg^{2+}, N^{+} and K^{+} concentrations. Soil texture (sand, silt, clay and textural class) was determined using Bouyoucos hydrometer method (Bouyoucos, 1962).

Table 1: Geographical location and sampling site characteristics.

ID	City	Coordinates	Dominant crops	Annual production estimates '000' tone	Koppen climate class	MAT °C	MAP mm
P	Peshawar	34° 0' N / 71° 52' E	Wheat, Maize	86/30	Bsh	22	761
C	Chichawatni	30° 53' N / 72° 69' E	Wheat, Maize	186/12	Bwh	27	471
VF	Vehari	30° 0' N / 72° 34' E	Fallow, Wheat	752	Bwh	27	182
V	Vehari	30° 15' N / 72° 69' E	Wheat, Cotton	752/691	Bwh	27	182
LW	Lahore	31° 5' N / 74° 35' E	Rice, Maize	83.4/13.8	Bsh	27	761
M	Multan	30° 15' N / 71° 52' E	Wheat, Cotton	590/670	Bwh	27	182
GR	Gujranwala	32° 18' N / 74° 19' E	Rice, Fodder	495	Bsh	22	761
G	Gujranwala	32° 7' N / 74° 24' E	Rice, Maize	495/4.5	Bsh	22	761
ZW	Zhob	31° 34' N / 69° 46' E	Maize, Mashbean	0.3	Bsh	22	182
D	Dir	35° 19' N / 71° 8' E	Wheat, Rice	17.9/14.3	Dsa	15	1340
KW	Kalar Kahar	32° 77' N / 72° 70' E	Wheat, Fallow	198	Bsh	23	1050
CHW	Chishtian	29° 79' N / 72° 86' E	Wheat, Cotton	1.5/820	Bwh	27	182
RW	Rawalpindi	33° 56' N / 73° 01' E	Wheat, Fodder	207	Bsh	21	1050
B	Baltistan	35° 8' N / 74° 98'E	Wheat	3.8	Bwk	15	182
A	Abbotabad	34° 16' N/ 73° 22 ' E	Wheat, Maize	27/22	Cwb	15	1290
S	Sargodha	32° 07' N / 72° 68' E	Wheat, Maize	596/79	Bsh	21	471
MW	Mirpur Mathelo	28° 02' N / 69° 56' E	Wheat, Cotton	363/279	Bsh	27	182
SW	Swat	35° 22' N / 72° 42' E	Wheat, Maize	71/101	Bwk	15	1416
F	Faisalabad	31° 45' N / 73° 13' E	Wheat, Maize	846/125	Bwh	21	471
LL	Loralai	30° 38' N / 68° 59' E	Wheat, Maize	3.3/3.7	Bsh	27	182
MKW	Mekhter	30° 47' N / 69° 34' E	Wheat	3.2	Bsh	27	182

Bsh= Warm Semi-Arid Climate, Bwh= Warm Desert Climate, Bwk= Cold Desert Climate, Cwb= Temperate Continental/ Humid Continental Climate, Dsa,= Warm continental climate, MAT= Mean Annual Temperature (1981-2010) , MAP= Mean Annual Precipitation (1981-2010)

Microbial biomass carbon, soil organic carbon content and its fractions: Microbial biomass carbon (MBC) was measured by using fumigation extraction techniques (Vance *et al.*, 1987; Fontaine *et al.*, 2004). Briefly, fresh soil (10g) was fumigated in ethanol-free chloroform and kept in the dark for 48 h then extracted with 40ml of 0.5 M K_2SO_4 solution. Similarly, 10 g of fresh soil was also extracted with 0.5 M K_2SO_4 directly, without submitting them to chloroform fumigation. The difference of dissolved organic carbon between fumigated and non-fumigated extracts were considered as microbial biomass C.

Soil organic carbon (SOC) was determined by using Walkley-Black method (Walkley and Black, 1934). For this, air dried soil (1.0 g) was digested in 1N $K_2Cr_2O_7$ solution. The mixture was continuously stirred for two minutes after adding 20 ml conc. H_2SO_4. The mixture was left on a bench to cool off for 30 mins. Two hundred ml of distilled water was then added followed by the addition of 10 ml of concentrated orthophosphoric acid. The mixture was titrated against 0.5M ferrous ammonium sulphate using 10-15 drops of ferroin indicator.

SOC fractions were determined by modified Walkley and Black method (Chan *et al.*, 2001). The method involves oxidation of SOC under increasing oxidizing conditions. In the classic Walkley Black method, 20 ml of conc. H_2SO_4 (corresponding to 20 N) is used to oxidize SOC. However, in the modified method, 5 and 10 ml of conc. H_2SO_4 i.e., 12 N and 18 N respectively, are used to oxidize the easily oxidizable and relatively protected SOC. This can be further explained as follows:

Oxidizable soil organic carbon (SOCO): (12 N H_2SO_4) - organic carbon that is easily oxidizable by using 5ml H_2SO_4.

Intermediate soil organic carbon (SOCI): (18 N-12 N H_2SO_4) - soil organic carbon that is relatively protected and requires little harsher oxidizing conditions for digestion. It is calculated by taking the difference between organic matter oxidized with 18N and 12N H_2SO_4.

Recalcitrant soil organic carbon (SOCR): (24 N-18 N H_2SO_4) - soil organic carbon that is bound with the strongest organo-mineral interactions and difficult to oxidize. It is calculated by taking the difference between organic matter oxidized with 24N and 18N H_2SO_4.

Measurement of specific potential respiration: Eighty grams of soil from each sampling site was packed in an appropriate size beaker. It was sealed in a 1L jar for pre-incubation in triplicate after adjusting the moisture to 60% of water holding capacity (WHC). After 7-days of pre-incubation, CO_2 evolution was periodically measured for 29-days keeping moisture at 60% WHC and temperature at 25°C, conditions typically used in incubation based measurements of soil respiration (Rehman *et al.*, 2018; Shahzad *et al.*, 2019). Soda traps, 10 ml 1N NaOH, were placed inside mason jars to capture CO_2 being emitted from soils. The traps were replaced on days 1, 2, 3, 7, 13, 16, 23, and 29 of incubation keeping CO_2 absorption in NaOH significantly lower than what a 10 ml 1N NaOH can hold. At each measurement, fresh NaOH was placed inside the mason jars. Absorbed CO_2 was measured by titrating NaOH from microcosm with 1N HCl using phenolphthalein indicator (Isermeyer, 1952; Kaneez-e-Batool *et al.*, 2016). Jars were ventilated with fresh air after each respiration measurement to avoid CO_2 saturation.

Statistical analysis: Multiple variable analysis (MVA) was employed to find the mutual correlation of all parameters (climate, soil properties, SOC variables) by developing correlation matrix. Furthermore, backward step regression (BSR) was performed to select a set of predictors (from climate and soil properties) that can best explain SOC parameters. Principal component analysis (PCA) was performed to confirm the results and draw relationships, unexplained by MVA and BSR tests.

RESULTS

Description of sampled sites and physicochemical characteristics of soils: The sampling sites are in cold desert climate to warm desert climate spanning the warm semi-arid climate to temperate continental to humid continental climate within an MAT range of 2.7 °C to 26.7 °C and MAP within 10.7- to 104 mm (Table 1). Annual crop yield from sampled sites has been estimated between 0.3 to 846 ‘000’ tons.

The sampled soils showed a large variation in terms of physicochemical characteristics (Table 2). Briefly, all the soils were basic or alkaline in nature showing pH ranging from 7.2 to 8.6. Electrical conductivity (EC) ranged between 341.7 to 874 mS cm^{-1}. Particle distribution was also wide, with clay particles ranging from 21% to 46%. Sandy clay, sandy clay loam, sandy loam, clay loam, and clay textures were represented. Concentration of different cations also varied widely across sampling sites with K^+ ranging from 1424 to 9045 mg kg^{-1} soil, Na^+ from 161 to 926 mg kg^{-1} soil, Ca^{2+} from 500 to 13779 mg kg^{-1} soil and Mg^{2+} from 5676 to 17542 mg kg^{-1} soil. The sampled soil represented a wide range of weathering status with TRB ranging between 61 to 171 c mol (+) kg^{-1} soil.

Carbon pools: All the soils studied were poor in soil organic carbon (SOC) concentration (Table 3). The SOC ranged between 0.9 to 1.63% whereas the SOC stocks ranged between 2.16 and 4.54 kg C m^{-2}. However, higher SOC content did not necessarily translate into higher C stocks owing to variation in bulk density of soils.

The distribution of various fractions, based on their lability, showed interesting results. Their distribution was not linearly linked with total SOC content and sometimes was counter intuitive when seen with reference to total SOC content. The oxidizable soil organic carbon (SOCO), the most easily degradable pool of the SOC, ranged between 0.085 and 0.65%. Interestingly, the lowest SOCO content was found in a soil that had 1.38% SOC, which was among top quartiles of the studied soils in terms of total SOC. Whereas, 0.65% SOCO, the highest amount of labile C, was found in soils having 0.9% and 1.19% SOC that were in lower quartile in terms of SOC. The pool that has the lability of the intermediate level (SOCI) ranged from 0.043 to 0.78 %. The soil with the lowest SOCO had the highest SOCI i.e. 0.78%. The most complex SOC pool, i.e. SOCR, ranged between 0.199% and 0.63%. The lowest SOCR was found in the soil having the SOCO (0.64%) in the upper quartile among the studied soils in terms of total SOC.

Microbial biomass-C was found to be between 171 to 651 (mg C kg^{-1}). Microbial quotient (MBC/SOC) was found between 107 and 583 (Table 3). This variable showed a negative correlation with pH.

Specific potential respiration: Specific potential respiration (SPR, µg CO_2-C g^{-1} SOC h^{-1}) was determined to assess the pools of the SOC that are readily available for microbial decomposition at room temperature. This variable was determined in addition to SOCO, which is also a pool readily available for microbial decomposition, for two reasons. Firstly, the effect of the MAT of sites was removed because the incubation was conducted at room. Secondly the initial SOC content of the soil was normalized by dividing the measured respiration with it thereby taking its effect out of equation.

Evidently, the soil showed a large variation in specific potential respiration (Table 3). The soil having the SOC content in the upper quartile i.e., 1.38% showed the lowest specific potential respiration, that was 271 µg CO_2-C g^{-1} SOC h^{-1}. It must be mentioned that this very soil showed the lowest SOCO. The highest SPR of 558 µg CO_2-C g^{-1} SOC h^{-1} was shown by the soil that had the highest SOCO i.e., 0.64%. In addition to providing direct information on the oxidizable SOC in the absence of effect of temperature of origin and total SOC content, these results also validated the fractionation method of

SOC pools. Metabolic quotient (qCO_2) was found between 4.2 and 7.9. It was negatively correlated to SOC content and displayed a small positive relation with pH.

Table 2: Physicochemical properties of the studied soils.

ID	Clay (%)	Silt (%)	Textural class	Ca^{2+} (mg kg^{-1} soil)	Mg^{2+} (mg kg^{-1} soil)	K^{+} (mg kg^{-1} soil)	Na^{+} (mg kg^{-1} soil)	TRB (cmol kg^{-1} soil)	pH	EC (mS $cm^{-1)}$
P	38 ± 0	25 ± 0	Clay loam	5820 ± 84	12754 ± 184	7391±107	281±4	155±2.2	7.8±0.01	484±1.7
C	31 ± 0	30 ± 0.	Clay loam	2243 ± 32	10127 ± 146	5869±85	272±4	111±1.6	7.8±0.01	372.5±0.86
VF	27 ± 0	15 ±0.01	Sandy clay loam	2531 ± 36	7995 ± 115	4421±64	504±7	92.±1.3	7.8±0.03	485±.57
V	27 ± 0.01	21± 0.01	Sandy clay loam	2728 ± 39	9808 ± 142	6643±96	924±13	116±1.7	7.72±0.01	630±10.3
LW	24 ± 0.01	30 ± 0.01	Sandy clay loam	2513 ± 36	10679 ± 154	4420±64	245±3	113±1.6	7.9±0.02	381±.57
M	27 ± 0.06	18 ±0.01	Sandy clay loam	2748 ± 39	11968 ± 173	5345±77	926±13	131±1.9	8.6±0.04	476.5±2
GP	22 ± 0	12 ±0.01	Sandy loam	2072 ± 30	9829 ± 142	5721±82	303±4	108±1.6	7.7±0.01	440±4.6
G	21 ±0.05	23 ±0.01	Sandy clay loam	1574 ± 22	9022 ± 130	5395±78	260±3	98±1.4	7.46±0.01	392.5±2.02
ZW	27 ± 0	16 ±0.01	Sandy clay loam	2814 ± 40	9690 ± 140	9045±130	609±8	120±1.7	8.1±0.08	859.5±1.44
D	22 ± 0	6 ± 0.01	Sandy clay loam	1168 ± 16	6046 ± 87	1424±20	909±13	63±.92	7.7±0.01	386±1.15
KW	37 ± 0	8 ±0.01	Sandy clay	8296 ± 120	12856 ± 186	2923±42	302±4	157±2.3	8.0±0.02	341.5±4.3
CHW	41 ± 0	9 ± 0.01	Sandy clay	1918 ± 27	6565 ± 95	2809±40	496±7	73±1	7.7±0.03	487±0.57
RW	45 ± 0.08	13 ± 0.01	Clay	3861 ± 55	8502 ± 123	2182±31	234±3	96±1.4	8.0±0.02	477.5±0.86
B	46 ± 0.01	13 ± 0.01	Clay	4355 ± 63	17542 ± 154	5112±74	241±3	182±2.6	7.8±0.03	378.5±3.7
A	40 ± 0	20 ± 0.01	Clay	2394 ± 34	6846 ± 99	6078±88	161±2	85±1.2	7.5±0.02	395±2.3
S	42 ± 0	28 ±0.01	Clay	2439 ± 35	7122 ± 103	5480±79	203±2	86±1.2	7.6±0.01	480.5±7.8
MW	43 ± 0.01	11± 0.01	Sandy clay	3760 ± 54	13463 ± 194	5644±81	383±5	147±2	7.9±0.01	874±5.78
SW	35 ± 0	22 ± 0.019	Sandy clay	1120 ± 16	8658 ± 125	2317±33	708±10	86±1.2	7.2±0.02	381±0.57
F	30 ± 0	21 ± 0.01	Sandy clay loam	500 ± 7	5676 ± 82	4377±63	165±2	61±89	7.6±0.01	485.5±1.44
LL	46 ± 0	23 ± 0	Clay	13779 ± 199	10902 ± 157	3834±55	359±5	171±2.4	7.8±0.01	426.5±9.5
MK	35 ± 0	11 ± 0.01	Sandy clay	1600 ± 23	11042 ± 159	7301±105	510±7	120±1.7	7.8±0.01	484±0.57

Values are means of three replicates whereas values followed by ± represent standard errors of means.

Table 3: SOC pools, stocks, and fractions as well as metabolic and microbial quotients of the soils.

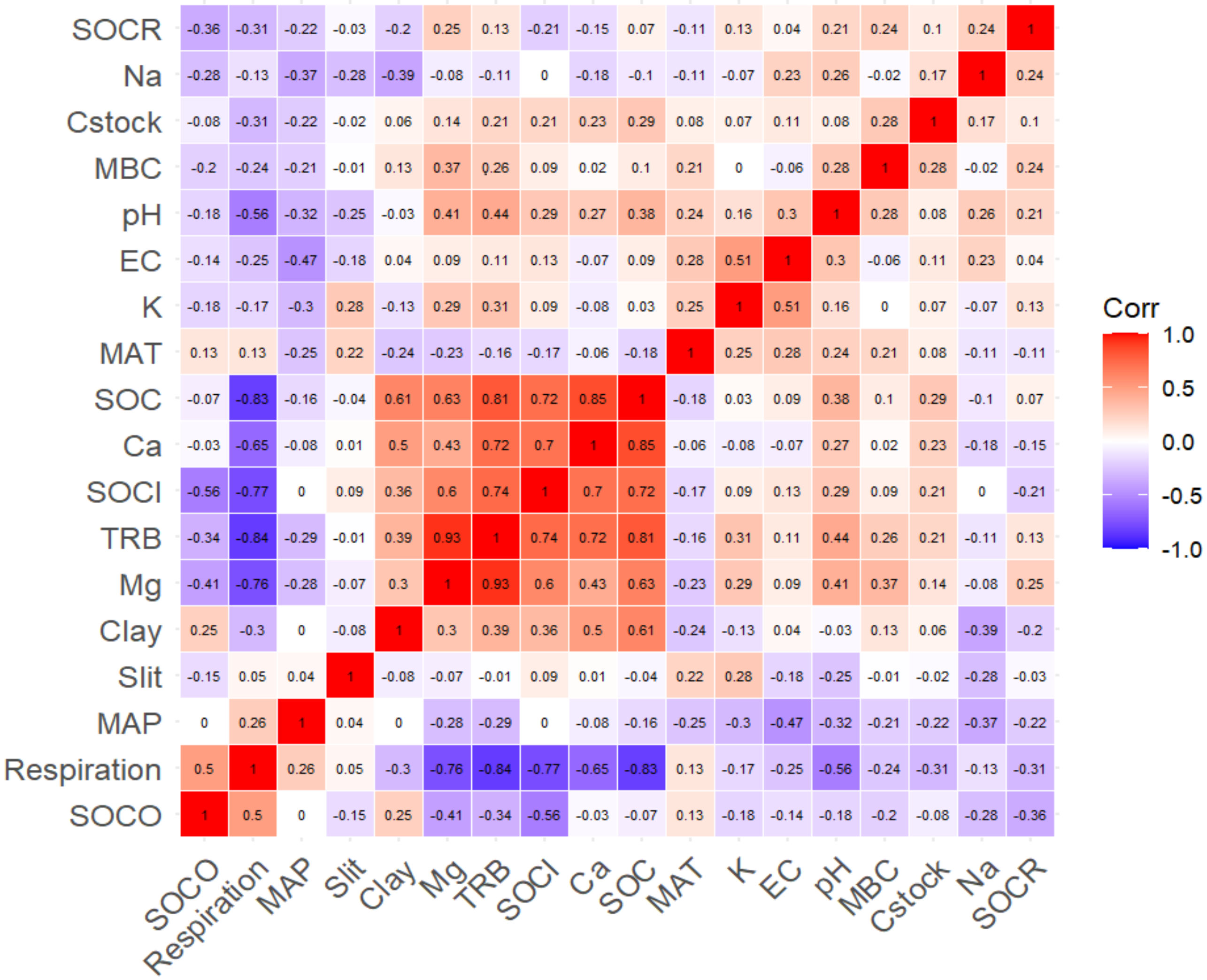
ID	MBC mg.kg^{-1}	SOC %	Soil Organic Carbon Fractions			Carbon Stock kg.m^{-2}	SPR ug/g SOC h^{-1}	qCO_2	qMic
			SOC0 %	SOCI %	SOCR %				
P	544±110	1.38±0	0.085±0.01	0.788±0.04	0.50±0.03	3.06±0.01	271.21±0.4	5.5 ± 1.4	394.7 ± 80
C	543±73	1.02±0	0.29±0.01	0.0450±01	0.68±0.01	2.38±0.01	429.99±0.33	8.2 ± 1.3	531.2 ± 72
VF	497±71	1.09±0	0.38±0.04	0.180±05	0.53±0.01	4.40±1.8	411.79±1.12	8.6 ± 1.22	453.7 ± 65
V	480±201	1.13±0	0.17±0	0.44±0	0.51±0	4.54±1.9	331.38±7.5	16.7 ± 11.8	421.9 ± 176
LW	512±55	1.09±0	0.17±0.01	0.44±0.04	0.48±0.04	2.52±0	336.55±.68	6.7 ± 0.65	468.7 ± 50
M	641±9	1.15±0.01	0.20±0.04	0.45±0.02	0.49±0.04	2.650±0.01	337.54±4.1	5.2 ± 0.09	554.7 ± 9
GP	492±67	0.99±0.01	0.53±0.01	0.043±0.01	0.43±0.03	2.34±0.07	497.72±16.8	10.4 ± 12	499.3 ± 84
G	159±81	0.95±0	0.12±0	0.27±0	0.56±0.03	2.24±0.07	444.48±9.3	79.2 ± 57	170.8 ± 85
ZW	157±60	1.13±0.	0.13±0	0.48±0.01	0.52±0.01	2.60±0.01	315.40±2.14	33.2 ± 18	138.9 ± 53
D	267±79	0.96±0	0.27±0.01	0.043±0.01	0.65±0	2.27±0	427.68±0.81	18.7 ± 4.8	276.9 ± 83
KW	515±45	1.29±004	0.22±0.05	0.75±0.07	0.31±0.03	2.91±0.08	276.19±7.9	5.4 ±0.47	397.5 ± 37
CHW	580±65	0.99±0	0.43±0.01	0.095±0.01	0.47±0	2.33±0.01	479.05±1.7	8.4 ± 1.06	583.6 ± 64
RW	337±113	1.33±0	0.48±0	0.32±0.01	0.53±0	2.98±0	348.67±0.17	14.1 ± 5.9	253.2 ± 85
B	651±29	1.35±0	0.20±0.03	0.51±0.03	0.63±0	3.01±0	277.62±1.3	4.27 ± 0.2	482.4 ± 22
A	272±59	0.98±0.01	0.34±0	0.35±0.01	0.29±0.01	2.31±0.01	509.42±3.6	21.3 ± 6.06	275.9 ± 59
S	535±52	1.087±0.05	0.55±0	0.0930.01	0.44±0.03	2.51±0.09	450.09±18.05	8.6 ± 1.06	491.1 ± 28
MW	511±35	1.24±0.04	0.24±0	0.54±0	0.46±0.04	2.81±0.07	308.48±10.2	6.0 ± 0.5	411.3 ± 28
SW	194±93	1.05±0.03	0.24±0.01	0.49±0.03	0.31±0.01	2.44±0.06	457.37±4.1	45.3 ± 25	181.9 ± 82
F	193±4	0.90±0.03	0.64±0.01	0.061±0.02	0.199±0.03	2.16±0.05	558.29±18.8	28.9 ± 0.8	213.1 ± 10
LL	174±20	1.63±0	0.50±0.01	0.750	0.37±0.02	3.51±0.01	275.16±1.5	16.1 ± 1.7	107.2 ± 13
MK	171±15	1.19±0.01	0.64±0.01	0.052±0.01	0.50±0.01	2.71±0.03	398.59±7.5	23.5 ± 1.8	144.2 ± 15

SOC=Soil Organic Carbon, SOCO=Soil Organic Carbon Oxidizable, SOCI=Soil Organic Carbon Intermediate, SOCR=Soil Organic Carbon Recalcitrant, SOCstock, Soil Organic Carbon Stock, SPR=Specific Potential Respiration, MBC=Microbial Biomass Carbon, qCO_2= Respiration quotient (Respiration/MBC), qMic= MBC/SOC

Values are means of three replicates followed by standard errors of means after the symbol ±.

Climatic and edaphic controls on SOC and SOC pools: To determine the relationships of various physicochemical properties of the soils among themselves as well as with the SOC pools, three different statistical analyses were used. They were multiple variable analysis (MVA), backward stepwise regression analysis, and principal component analysis.

Multiple variable analysis: The results of multiple variable analysis are presented in Figure 2 in the form of a correlation matrix. Given that all the studied soils were basic or alkaline in nature, the pH was determined mainly by the concentration of Ca^{2+} and TRB cations. The electrical conductivity was significantly positively linked with MAP, while it was significantly negative related to potassium ions. Interestingly, according to MVA, microbial biomass was not found in a significant relationship with any of the climatic or edaphic factors as well as any of the SOC pools and fractions. The SOC was in a strongly positive relation with the clay content as well as calcium and magnesium ions. Consequently, TRB was also strongly positively influencing the SOC content. Moreover, as the pH increased the SOC content decreased. Three fractions of the SOC were found in interesting relationships with soil minerals. For instance, the Mg^{2+} was found in a significant negative relationship with the oxidizable SOC (SOCO), whereas the intermediate SOC fraction (SOCI) was in a strong positive relationship with both Mg^{2+} and Ca^{2+} and by consequence with the TRB. Moreover, unlike SOCO, the SOCI was in significant positive correlation with the SOC and in an evident negative correlation with the SOCO. Surprisingly, none of the variables tested was observed in a significant relationship with the recalcitrant SOC. Overall, neither the SOC nor any of its fractions was found in any significant correlation with the two climatic variables tested in this study.



	SOCO	Respiration	MAP	Slit	Clay	Mg	TRB	SOCI	Ca	SOC	MAT	K	EC	pH	MBC	Cstock	Na	SOCR
SOCR	-0.36	-0.31	-0.22	-0.03	-0.2	0.25	0.13	-0.21	-0.15	0.07	-0.11	0.13	0.04	0.21	0.24	0.1	0.24	1
Na	-0.28	-0.13	-0.37	-0.28	-0.39	-0.08	-0.11	0	-0.18	-0.1	-0.11	-0.07	0.23	0.26	-0.02	0.17	1	0.24
Cstock	-0.08	-0.31	-0.22	-0.02	0.06	0.14	0.21	0.21	0.23	0.29	0.08	0.07	0.11	0.08	0.28	1	0.17	0.1
MBC	-0.2	-0.24	-0.21	-0.01	0.13	0.37	0.26	0.09	0.02	0.1	0.21	0	-0.06	0.28	1	0.28	-0.02	0.24
pH	-0.18	-0.56	-0.32	-0.25	-0.03	0.41	0.44	0.29	0.27	0.38	0.24	0.16	0.3	1	0.28	0.08	0.26	0.21
EC	-0.14	-0.25	-0.47	-0.18	0.04	0.09	0.11	0.13	-0.07	0.09	0.28	0.51	1	0.3	-0.06	0.11	0.23	0.04
K	-0.18	-0.17	-0.3	0.28	-0.13	0.29	0.31	0.09	-0.08	0.03	0.25	1	0.51	0.16	0	0.07	-0.07	0.13
MAT	0.13	0.13	-0.25	0.22	-0.24	-0.23	-0.16	-0.17	-0.06	-0.18	1	0.25	0.28	0.24	0.21	0.08	-0.11	-0.11
SOC	-0.07	-0.83	-0.16	-0.04	0.61	0.63	0.81	0.72	0.85	1	-0.18	0.03	0.09	0.38	0.1	0.29	-0.1	0.07
Ca	-0.03	-0.65	-0.08	0.01	0.5	0.43	0.72	0.7	1	0.85	-0.06	-0.08	-0.07	0.27	0.02	0.23	-0.18	-0.15
SOCI	-0.56	-0.77	0	0.09	0.36	0.6	0.74	1	0.7	0.72	-0.17	0.09	0.13	0.29	0.09	0.21	0	-0.21
TRB	-0.34	-0.84	-0.29	-0.01	0.39	0.93	1	0.74	0.72	0.81	-0.16	0.31	0.11	0.44	0.26	0.21	-0.11	0.13
Mg	-0.41	-0.76	-0.28	-0.07	0.3	1	0.93	0.6	0.43	0.63	-0.23	0.29	0.09	0.41	0.37	0.14	-0.08	0.25
Clay	0.25	-0.3	0	-0.08	1	0.3	0.39	0.36	0.5	0.61	-0.24	-0.13	0.04	-0.03	0.13	0.06	-0.39	-0.2
Slit	-0.15	0.05	0.04	1	-0.08	-0.07	-0.01	0.09	0.01	-0.04	0.22	0.28	-0.18	-0.25	-0.01	-0.02	-0.28	-0.03
MAP	0	0.26	1	0.04	0	-0.28	-0.29	0	-0.08	-0.16	-0.25	-0.3	-0.47	-0.32	-0.21	-0.22	-0.37	-0.22
Respiration	0.5	1	0.26	0.05	-0.3	-0.76	-0.84	-0.77	-0.65	-0.83	0.13	-0.17	-0.25	-0.56	-0.24	-0.31	-0.13	-0.31
SOCO	1	0.5	0	-0.15	0.25	-0.41	-0.34	-0.56	-0.03	-0.07	0.13	-0.18	-0.14	-0.18	-0.2	-0.08	-0.28	-0.36

Figure 2. Correlation matrix showing relationship between different climatic, and physicochemical variables.

Backward stepwise regression: The results of the backward stepwise regression are presented in Table 4. The microbial biomass C, specific potential respiration (or simply respiration), SOC stock, SOC (%), SOCO, SOCI, and SOCR were tested as dependent variables against the mix of physicochemical and climatic parameters. MBC was best predicted by MAP, Ca^{2+}, Mg^{2+}, and TRB, where Ca^{2+} and Mg^{2+} show a positive relationship with the MBC and the other two variables showed a negative relationship. Interestingly, the respiration was best predicted by Ca^{2+}, Mg^{2+} and Na^{+} concentrations of the soils. The higher the concentration of these elements in the soils, lower is the specific potential respiration i.e., the carbon respired per unit of the SOC of the soil. Although the SOC stocks were predicted by the Ca^{2+} concentration in the soil, the value of the adjusted coefficient of variation was very low (R^2 = 20.37) indicating that none of the variables in the models were strong predictors of the SOC stocks. The SOC content (%) was strongly predicted by clay concentration and TRB, both having a positive relationship with the SOC content. Although, the Mg^{2+} was also a significant predictor of the SOC content, its relationship was negative. Similarly, the oxidizable SOC (SOCO) was predicted by the clay content and Mg^{2+}. The intermediate fraction of the SOC (i.e., SOCI) was strongly predicted by TRB cations, whereas the recalcitrant fraction of the SOC could not be predicted by any of the variables.

Table 4: Backward step regression analysis between soil properties and soil organic matter variables.

Dependent variables	MBC	Respiration	SOC stock	SOC	SOCO	SOCI	SOCR
MAT (℃)	NA	NA	NA	NA	NA	NA	NA
MAP (mm)	-2.57 (0.0197)	NA	NA	NA	NA	NA	NA
Clay (%)	NA	NA	NA	2.83057 (0.0111)	2.1527 (0.0444)	NA	NA
Silt (%)	NA	NA	NA	NA	NA	NA	NA
Ca^{2+} (mg kg^{-1} soil)	4.07 (0.0008)	-4.80754 (0.0001)	2.52438 (0.0202)	NA	NA	NA	NA
Mg^{2+} (mg kg^{-1} soil)	4.59 (0.0003)	-3.64378 (0.0019)	NA	-2.46712 (0.0239)	-2.78508 (0.0118)	NA	NA
Na^{+} (mg kg^{-1} soil)	NA	-2.69134 (0.0149)	NA	NA	NA	NA	NA
K^{+} (mg kg^{-1} soil)	NA	NA	NA	NA	NA	NA	NA
TRB (cmol kg^{-1} soil)	-4.37943 (0.0004)	NA	NA	5.09541 (0.0001)	NA	5.02736 (0.0001)	NA
EC (m S cm^{-1})	NA	NA	NA	NA	NA	NA	NA
Ph	NA	NA	NA	NA	NA	NA	NA
Adj R^2	53.8871	81.6132	20.3716	82.483	27.3705	53.6161	0
St.EE	119.027	36.6404	0.1588	0.02625	0.14565	0.16743	0.12212
MAE	90.9608	25.4085	0.09247	0.01889	0.10122	0.12185	0.09381

Principal component analysis: The oxidizable soil organic carbon showed close relationship with the soil pH. Like in backward stepwise regression (BSR), the MAP and MBC were linked negatively (Figure 3). Moreover, the SOC stocks were also in a negative relationship with the MAP. Moreover, like in the correlation matrix and backward stepwise regression, the total SOC content as well as the intermediate fraction of the SOC, i.e. SOCI, were very strongly and positively linked with Ca_2^{+}, Mg^{2+} and TRB. To some extent they were also determined by the clay content in soil, whereas silt content had no link with any fraction or the total SOC content of the soil. However, the recalcitrant fraction (SOCR) was negatively linked with the MAP.

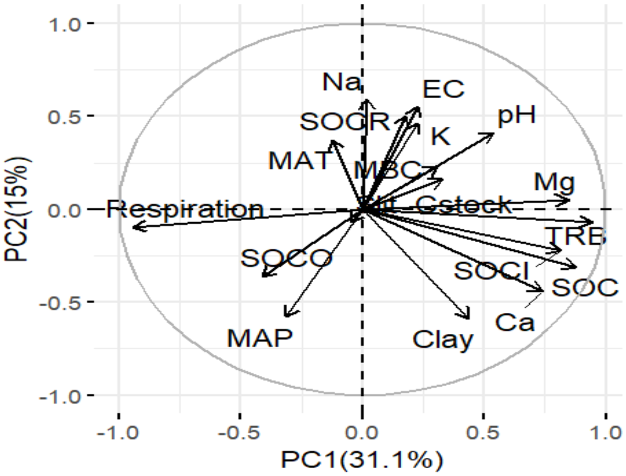


Figure 3. Principal component analysis explaining the variance in the overall data. Vectors show strength and direction of each variable to the overall distribution.

DISCUSSION

The soils studied here represent a long transect of ~1600 km from north to south of Pakistan. In addition, they represent a large variation in MAT, MAP climate classes, as well as the physicochemical properties of the soil (Tables 1 & 2). They were all sampled from cultivated fields to represent an almost similar crop type in general, though there were remarkable differences of productivity among these sites. However, tillage and extractive management practices, where the economic as well as the biological yield is taken out of the field at harvest, were also common on these sites. Consequently, all the soil was more or less poor in terms of SOC (Table 3). However, there was still a wide range of SOC represented in this study i.e., from 0.9 to 1.63% SOC (Table 3). On top of that, the variation among soils in terms of various fractions, ranging from labile to recalcitrant, was even larger. Therefore, we can say that the selected soils provided a sufficient range in terms of climatic conditions, physicochemical variables, and SOC pools to meaningfully determine different controls on SOC pools by relating various predictor variables with the response variables.

Overall, climatic factors were found to show little control on soil C pools. Mean annual temperature or mean annual precipitation did not determine any pool of the soil C according to the three tests applied on the data. Except, in backward linear regression analysis, MAP was found negatively influencing microbial biomass (Table 4). This sounds counterintuitive given that a higher MAP is associated with higher plant productivity in general thereby leading to higher inputs of organic carbon into soils and the consequent substrate for microorganisms to grow (Xu *et al.*, 2020; Zhang and Xi, 2021). The sites studied here benefit from the irrigation water for crop production and moisture availability is overwhelmingly thanks to irrigation. This may have confounded the effects of precipitation on microbial biomass as well as other soil C pools. For example, the sites at Vehari, Chishtian, Multan and Mirpur Mathelo receive the MAP that falls in the lowest quartile among the studied sites and experience the highest mean annual temperature. However, the same sites showed the SOC content falling in the upper quartile except one Chishtian site under crops.

The interesting result vis-à-vis climatic factors was the inexistence of any relationship between MAT and any of the soil C pools. There is an overwhelming amount of literature reporting an inverse correlation between SOC and temperature (Bradford *et al.*, 2016; Crowther *et al.*, 2016; García-Palacios *et al.*, 2021). Most of these studies include soil warming experiments and assess the connection between mineralization of soil carbon and temperatures. However, the on-site association between temperature and SOC pools that develop over hundreds of years show different dynamics. Our results are more closely related to (Doetterl *et al.*, 2015b) who found no relationship between temperature or precipitation and soil C variables over a 4000 km north south transect in Chile spanning over a wide range of climatic and physicochemical gradients. It should be recalled that these soil C pools, particularly SOC content, and intermediate and recalcitrant fractions, have developed over many decades on our sites. Consequently, the SOC pools on such sites are a product of physicochemical and climatic factors over the long term. On the other hand, the SOC pools are well explained by climatic factors like temperature and precipitation only over a short term (Bradford *et al.*, 2008; Alvarez *et al.*, 2018; Georgiou *et al.*, 2021).

Interestingly, almost all the soil C pools studied were in strong relationships with one or more physicochemical characteristics of the soils. Overall, soil clay particles have often been associated positively with SOC content and stocks (Stewart *et al.*, 2008; Wiesmeier *et al.*, 2019). Clay particles protect organic matter in two ways. Firstly, being the finest particles of mineral soils, they provide protection to organic C by making aggregates thereby making it difficult for microorganisms to access and mineralize it (Dungait *et al.*, 2012). Secondly, being charged particles thanks to mineral ions, they make bonds with the charged particles of organic matter thereby resulting in mineral-associated organic matter, which is not easily accessible to microorganisms for decomposition. These charged minerals may vary depending on the weathering and/or soil formation processes as well as the parent material from which the soil is formed. For instance, in our case, it was mainly the Ca^{2+} particles that were associated with organic carbon in the studied soils, whereby the higher the number of these particles higher is the SOC content of the soil. Overall, TRB was observed in a strong positive correlation with SOC (Tables 4 & 5). This further underlines the importance of charged particles protecting the SOC. The two indicators of labile components of SOC, i.e., respiration per unit of SOC as well as oxidizable carbon, were in a negative relationship with the three cations. The higher the concentration of the cations higher is the protection of the SOC from microbial decomposition thereby resulting into lower specific respiration (Doetterl *et al.*, 2015b).

While the backward stepwise regression model disentangled the role of climatic and soil factors in determining control on SOC and its pools thereby emphasizing that the soil factors were overwhelmingly stronger in determining these pools, there were also interesting relationships among different pools of organic carbon. For instance, the generally lower SOCO and specific respiration were found in those soils that had the SOC content in the upper quartile (Table 3). Moreover, the higher SOC containing soils had generally higher proportion of the intermediate SOC (SOCI).

Recently, the paradigm is emerging that the labile and easily decomposable fractions are the key contributor to the buildup of the SOC stocks in the soils (Cotrufo *et al.*, 2019). It has been postulated and indicated that the microbes process the very labile carbon like root exudates and turn them into mineral-associated organic carbon (Cotrufo *et al.*, 2015; Lavallee *et al.*, 2019). Interestingly, our results corroborate this paradigm. The soils rich in clay particles and TRB showed the SOC content in upper quartile among the studied sites. Moreover, the higher the SOC content in a soil, the lower the respiration per unit of the SOC or the overall oxidizable SOC. This indicates that the clay particles and TOC afforded the protection to SOC via aggregation as well as mineral associate linkages thereby raising the overall SOC content of these soils. Moreover, the SOC in these soils was most likely stabilized via the microbes, which is now known to process the labile carbon of rhizodeposition thereby turning them into mineral-associated organic carbon (Cotrufo *et al.*, 2019; Sokol *et al.*, 2019). This was confirmed by the higher microbial biomass found in soils rich in SOCR and SOC stocks.

While predicting the SOC pools using various variables in different multivariate analyses, it was evident that different tests had different predicting powers. However, there were many commonalities across the three predictor tests used here, which indicate that predictors were confirmed by multiple tests. For instance, across the three tests used, the SOC content was strongly related to the amount of clay fraction in soils (Table 4, Figures 2 and 3). It is well known that the clay particles are known for their organo-mineral linkages with the SOC (Stewart *et al.*, 2008; Colman and Schimel, 2013; Wiesmeier *et al.*, 2019). However, it should be considered that in the soil carbon saturation concept, the silt particles are also accounted for while calculating the total C storage potential of a soil, assuming that the silt particles protect organic carbon via their role in aggregation and hence physical protection of the organic matter (Stewart *et al.*, 2008). In our study, none of the tests showed control of silt particles over SOC pools. There may be an explanation for this anomaly. The SOC stocks in the studies soils mainly consisted of SOCI that was mostly likely composed of microbial necromass. The same is mainly retained on soil by interactions of metal cations and organic ligands of organic matter rather than occlusion of microbial debris through aggregate formation. Additionally, statistically significant relationships of metal cations, TRB and SOM fractions further support this proposed route of carbon storage in these soils. Hence, the silt particles did not show any link to SOC.

Organo-mineral associations are strongly affected by weathering processes. Highly weathered soils produce clays with high surface area and likely to offer more carbon sportive sites (Mayer, 1994). Considering clay content as the only determinant of SOC dynamics can disguise the role of soil mineralogy and may not efficiently explain specific SOM stabilization routes. Other soil properties and their mutual interactions may better clarify SOM stabilization than clay content alone. Studies suggest that weathering-related predictors of SOM stabilization are much complicated and related to surface chemistry and mineralogy of secondary clays (Masiello *et al.*, 2004; Doetterl *et al.*, 2015a; b; Zhao *et al.*, 2019; Reichenbach *et al.*, 2021). It is well established that negative charge on mineral surfaces repel negatively charged organic ions, and polyvalent cations present on the mineral surface exchange complex supports attachment among them. In alkaline/neutral soils similar to our study, Ca^{2+} and Mg^{2+} are the main cations, while hydroxypolycations of Fe^{3+} and Al^{3+} are major cations in acidic soils (Singh *et al.*, 2018). In accordance with this well-established phenomenon, in our soils, it is observed that the base cations Mg^{2+} and Ca^{2+} and the overall TRB cations were significantly positively linked with the SOCI and SOCR fractions of the SOC. However, the Mg^{2+} content was particularly negatively related to the SOCO, the most labile component of the SOC. These cations adsorbed on negatively charges mineral surfaces behave as cation bridges and help in adoption of negatively charged organic polymers that are partially decomposed microbial, necromass attributed as SOCI in this study (Torn *et al.*, 1997; Lützow *et al.*, 2006; Mikutta *et al.*, 2007; Yang *et al.*, 2017; Singh *et al.*, 2018; Lavallee *et al.*, 2019; Zhao *et al.*, 2022).

Conclusion: Our results indicate that SOC amounts were greater in fine-textured soils, predominantly those with a higher clay percentage and increased levels of basic cations. These soils have a greater amount of SOC bound through cation bridging indicating that physicochemical properties, such as organo-mineral interactions thanks to clay particles, play key role in protection of SOC pools. We can conclude that in these alkaline soils, the percentage of clay particles as well as total reserve in base cations are the key determinants of the SOC. Furthermore, the positive correlation between SOC stocks and recalcitrant fraction of the SOC with microbial biomass confirms the recent paradigm about microbial origin of protected carbon. Interestingly, no significant link among SOC content and climate parameters was observed in any of the three statistical models. This indicates that climate factors do not have a strong direct control on SOC pools in these sites. They may indirectly control SOC dynamics through factors like plant productivity, rock weathering, and microbial community composition.

Acknowledgements: This research work was funded by the ILSI Research Foundation through BRPGP010-13 – ILSI Research Foundation Grant to the corresponding author, Tanvir Shahzad. Furthermore, the corresponding author is thankful to Pakistan Academy of Sciences on whose funding this work was presented in the 9th International Symposium on Soil Organic Matter held in Morocco on May 26-31, 2024.

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