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## 1 Identification and predictability of soil quality factors and indicators from

#### 2 conventional soil and vegetation classifications

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#### 26 ABSTRACT

Generally, the physical, chemical and biological attributes of a soil combined 27 with abiotic factors (e.g. climate and topography) drive pedogenesis. However, 28 biological indicators of soil quality play no direct role in traditional soil classification 29 and surveys. To support their inclusion in classification schemes, previous studies 30 have shown that soil type is a key factor determining microbial community 31 32 composition in arable soils. This suggests that soil type could be used as proxy for soil biological function and vice versa. In this study we assessed the relationship between 33 34 soil biological indicators with either vegetation cover or soil type. A wide range of soil attributes were measured on soils from across the UK to investigate whether; (1) 35 appropriate soil quality factors (SQFs) and indicators (SQIs) can be identified, (2) soil 36 classification can predict SQIs; (3) which soil quality indicators were more effectively 37 predicted by soil types, and (4) to what extent do soil types and/ or aggregate 38 vegetation classes (AVCs) act as major regulators of SQIs. Factor analysis was used 39 to group 20 soil attributes into six SOFs namely; Soil organic matter, Organic matter 40 humification, Soluble nitrogen, Microbial biomass, Reduced nitrogen and Soil 41 humification index. Of these, Soil organic matter was identified as the most important 42 SQF in the discrimination of both soil types and AVCs. Among the measured soil 43 attributes constituting the *Soil organic matter* factor were, microbial quotient and bulk 44 density were the most important attributes for the discrimination of both individual 45 soil types and AVCs. The *Soil organic matter* factor discriminated three soil type 46 groupings and four aggregate vegetation class groupings. Only the Peat soil and Heath 47 and bog AVC were distinctly discriminated from other groups. All other groups 48 overlapped with one another, making it practically impossible to define reference 49 values for each soil type or AVC. We conclude that conventionally classified soil 50

types cannot predict the SQIs (or SQFs), but can be used in conjunction with the conventional soil classifications to characterise the soil types. The two-way ANOVA showed that the AVCs were a better regulator of the SQIs than the soil types and that they (AVCs) presented a significant effect on the soil type differences in the measured soil attributes.

*Keywords:* Soil health; Soil quality factor; Multivariate classification; Discriminant
analysis; Cluster analysis

58

#### 59 **1. Introduction**

The multiple roles and functions of soil have resulted in several broad 60 definitions of soil quality. One of the most widely adopted definitions for soil quality 61 (SQ) was proposed by a committee for the Soil Science Society of America (chaired 62 by Karlen) as: "the capacity of soil to function, within natural or managed ecosystem 63 boundaries, to sustain plant and animal productivity, maintain or enhance water and 64 air quality, and support human health and habitation" (Karlen et al., 1997). The 65 quality of any soil has two parts: (1) the natural or inherent quality which is based on 66 the parent geological material and soil-state-factors and is rather static, and (2) the 67 dynamic soil quality which encompasses those soil properties that can change over 68 relatively short time periods in response to human use and management (Carter, 2002; 69 Fließbach et al., 2007; Bonfante et al., 2019). In contrast to the inherent SQ, the 70 dynamic SQ can be used to monitor temporal trends on the same soil. There is no 71 universally applicable set of inherent SQ criteria and optimum values (Carter, 2002) 72 because soils with differences in the soil forming factors have different absolute 73 capabilities (Seybold et al., 1998; Karlen et al., 2001). Therefore, soil quality and 74 indicators have been defined by very different criteria and approaches dependent on 75

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the various functions the soil performs (Rapport et al., 1997; Carter, 2002, Cherubin et
al., 2016). In spite of the lack of standard methodology and "critical limits", it is
possible to develop SQ ranges for specific soils evaluated with regard to specific land
use and management regimes.

Soil quality is evaluated in terms of measurable soil attributes that measure 80 specific physical, chemical, and biological properties; also known as soil quality 81 82 indicators (SQIs; Shukla et al., 2006; Cherubin et al., 2016). Many of these properties are interrelated and the best SQIs are those that integrate and have the combined 83 effect of several properties or processes that affect the capacity of a soil to perform 84 a specified function (Dagnachew et al., 2019). SQIs should generally be linked 85 and/or correlated with ecosystem processes and functions and should be responsive to 86 87 variations in management and climate on an appropriate time scale (Doran and Safley, 1997, Bonfante et al., 2019). The SQIs which respond over the medium term i.e. those 88 that are sensitive over few years and decades in land uses and management 89 practices, may be the most useful for indicating soil quality changes as opposed to 90 91 those which change either very rapidly (e.g. seasonally) or very slowly (e.g. over centuries) (Rapport et al., 1997; Dagnachew et al., 2019). Thus, measurement of key 92 SQIs over time can be used to establish whether the quality of a soil under a given 93 land use and management system is improving, declining or stable (Shukla et al., 94 2006; Ghaemi et al., 2014; Rayo et al., 2017). 95

Soil types are known to be inextricably determined by the physical, chemical and biological processes operating in soil, yet the biological indicators are rarely used in traditional soil classification and surveys (Cavigelli et al., 2005). Studies conducted by a number of researchers, such as Parkin (1993), Buyer et al. (2002), Girvan et al. (2003) and Ulrich and Becker (2006), have shown that soil type is a key factor

determining microbial community composition in arable soils. Furthermore, Rapport 101 et al. (1997) and Lagomarsino et al. (2009) reported that microorganisms and 102 microbial communities can provide an integrated measure of soil quality; an aspect 103 that cannot always be obtained with physical and chemical measures and/or analyses 104 of higher organisms. Currently, bioindicators are mostly based on so-called sum or 105 black-box parameters and generally include microbial indicators such as microbial 106 107 biomass, activity and biodiversity (Rapport et al., 1997; Nielsen and Winding, 2002; Schloter, et al., 2018). Recently, an alternative has been proposed, based on the use 108 109 of specific ratios that report on function such as the quotients of microbial respiration-C-to-microbial biomass-C  $(qCO_2)$  and the microbial biomass-C-to-110 organic matter-C ratio (qMic) (Schloter, et al., 2018). These indicators avoids the 111 problems of comparing trends in soils with different organic matter or microbial 112 biomass content and appears to provide a more sensitive indicator of soil changes than 113 either activity or population measurements alone (Lagomarsino et al., 2009). In this 114 study, we used multivariate statistical methods to explore these relationships using 20 115 physico-chemical and biological soil properties as Total Data Set (TDS). Using factor 116 analysis the 20 correlated variables were reduced to 6 uncorrelated factors (soil 117 quality factors; SQFs) also called Minimum Data Set (MDS) that were linear 118 functions of the original 20 variables. The main questions addressed in this study 119 were: (1) Can soil classification be used to predict SQFs and SQIs? (2) Which SQFs 120 and SQIs are more effectively predictable by soil type in UK soils? (3) To what extent 121 do soil types and/or AVCs act as major regulators of SQFs or SQIs? 122

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123

#### 124 **2. Materials and methods**

#### 125 2.1. Soil sampling and preparation

Soil samples were collected throughout the UK as part of the Centre for 126 Ecology and Hydrology Countryside Survey (CS) 2007 (Emmett et al., 2010) with 127 sites representing the main types of landscape and soil groups. To encompass all the 128 129 major soil and land use types, a total of 304 soil samples were collected throughout the UK, based on a stratified random sample of 1 km squares at gridpoints on a 15 km 130 131 grid using the Institute of Terrestrial Ecology (ITE) Land Classification as the basis of the stratification (Scott, 2008). Figure S1 shows the general location and distribution 132 of samples across the UK. At each grid intersection, a 1 km<sup>2</sup> sample area was 133 selected. Within the 1 km<sup>2</sup> sample area, 3 plots  $(5 \times 5 \text{ m}^2)$  were randomly located and 134 a single 15 cm long  $\times$  4 cm diameter soil sample was collected from each of the plots. 135 Topsoils were only selected for sampling to reflect standard practice in national 136 monitoring schemes (Bellamy et al., 2005) such as Soil Survey England and Wales 137 handbook (Hodgson, 1976), the National Soil Monitoring Network (Emmett, B.A., 138 2006) and the UK Soil Monitoring Network (Environmental Agency, 2008). 139

The soil leachate was collected according to Emmett et al. (2008). The soil 140 leachate replicate cores were first wetted to field capacity with artificial rainfall (125 141 μM NaCl, 15.7 μM CaCl2, 1.3 μM CaSO4, 15.3 μM MgSO4, 12.3 μM H2SO4) in the 142 143 dark at 10°C until the soils were fully wetted. The cores were then sprayed with artificial rainfall until a further 150 ml of leachate had been collected resulting in a 144 solution with a pH of approximately 4.6. After washing out the cores, a small amount 145 of suction was applied to drain larger pores. Cores were then incubated under 146 anaerobic conditions for 4 weeks, at 10 °C, approximately UK mean summer soil 147

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148	temperature Cores were then extracted with 1 M KCl, and ammonium and nitrate
149	concentrations were determined as a measurement of mineralisable N using a TOC-
150	VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan) as describe below.
151	Across all land uses (Supplementary Information S2) and aggregate vegetation
152	class (AVC) categories, the dominant soil types (% of total) were: Brown soils (32%),
153	Groundwater gleys (13%), Surface water gleys (19%), Lithomorphic soils (8 %),
154	Peats (15%), Pelosols (2%) and Podzolic soils (11%). See Table S1 for their
155	equivalents in the WRB classification. All the sites were characterised by a temperate
156	climate with a North-South mean annual temperature range of 7.5 to 10.6°C and East-
157	West mean annual rainfall range of 650 to 1700 mm.
158	
159	2.2. Aggregate vegetation classes
160	The vegetation data from the plots were analysed using the classification by
161	Aggregate Classes (ACs) or Aggregate Vegetation Classes (AVCs). The AVCs were
162	the vegetation types produced from a quantitative hierarchical classification of the
163	different species found in sample plots. The eight AVCs used for assessing vegetation
164	condition are listed in Table 1. Across all the soils sampled, the AVCs represented (%
165	of the total): 18% Crop and weeds, 17% Fertile grasslands, 22% Heath and bogs, 20%
166	Infertile grasslands, 2% Lowland woodland, 10% Moorland grass mosaics, 4% Tall
167	grass and herbs and 7% Upland woodland.
168	
169	Table 1. Summary of the Aggregate Vegetation Classes (AVCs) used for assessment of
170	vegetation condition. The brackets indicate the abbreviation of the vegetation class

171 *(adapted from Smart et al., 2003).* 

Aggregate vegetation class (AVC) +(abrev)	Description
1. Crops and weeds (Craw)	Weedy communities of cultivated and disturbed ground, including species-poor arable and horticultural crops.
2. Tall grass and herbs (Tgah)	Less intensively managed tall herbaceous vegetation typical of field edges, roadside verges, stream sides and hedge bottoms.
3. Fertile grassland (Frtg)	Agriculturally improved or semi improved grassland. Often intensively managed agricultural swards with moderate to high abundance of perennial rye grass.
4. Infertile grassland (Infg)	Less-productive, unimproved and often species rich grasslands in a wide range of wet to dry and acid to basic situations.
5. Lowland wooded (Lwlw)	Vegetation dominated by shrubs and trees in neutral or basic situations, generally in lowland Britain. Includes many hedgerows.
6. Upland wooded (Uplw)	Vegetation of broadleaved and conifer woodland often in more acidic situations, generally in upland Britain.
7. Moorland grass mosaics (Mrgm)	Extensive, often unenclosed and sheep grazed hill pastures throughout Britain.
8. Heath and bog (Htab)	Vegetation dominated by heathers. Includes drier heaths as well as bog. Mostly in the uplands.

### 175 2.3. Soil analysis

Soil pH was determined in soil-distilled water extracts (1:2.5 w/v soil to water 176 soil ratio) using a glass electrode (Gelplas general purpose electrode, BDH) and HI-177 209 pH meter (Orion research, Boston, MA, USA). Soil moisture was determined by 178 weight loss after oven drying at 105°C overnight (>16 h). Water content at field 179 capacity was estimated by saturating the soil followed by measuring the water 180 retained in the soil at -33 kPa. Bulk density was calculated (mass/volume) after 181 removal of stones from the cores (>2 mm in diameter). Loss on ignition (LOI) was 182 183 undertaken at 375°C for 16 h. Soil organic carbon (SOC) was calculated from the LOI values according to the method of Ball (1964) where 184

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186 Phosphorus was determined by the Olsen P method according to Emmett et al. (2008).

Total C and N were determined using UKAS accredited method SOP3102 on an
Elementar Vario-EL elemental analyser (Elementaranalysensysteme GmbH, Hanau,
Germany) according to Emmett et al. (2008, and 2010).

Soil respiration (SR) was determined on a 15 cm long, 2.5 cm diameter soil 190 cores with a 1250 cm<sup>3</sup> head space. The soils were incubated at 10°C for 1 h (at which 191 192 linearity was established). Subsequently, the head space gas was analysed for  $CO_2$ concentration using a Clarus 500 Gas Chromatograph (Perkin Elmer Corp., Beverley, 193 194 MA). The CO<sub>2</sub> flux was established by comparing the CO<sub>2</sub> concentration before and after incubation. Soil microbial biomass C and N were estimated on moist soil 195 samples (10 g) using the modified chloroform-fumigation-extraction (CFE) method of 196 Vance et al. (1987). For each soil 10g of the control and the fumigated samples were 197 extracted with 1 M KCl. The TOC and TON in the 1 M KCl extracts was determined 198 using a TOC-VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan). Extraction 199 efficiency correction factors of 0.45 and 0.54 were used for microbial C and N, 200 respectively (Joergensen and Mueller, 1996a; 1996b; Fließbach et al., 2006). Soil 201 microbial biomass was therefore calculated according to the formula:  $C_{mic} = EC/kEC$ , 202 where EC = (TOC in fumigated samples - TOC in control samples) and kEC = 0.45, 203 and  $N_{mic} = EN/kEN$ , where EN = (total N in fumigated samples – total N in control 204 samples) and kEN = 0.54. The microbial C:N ratios were subsequently calculated 205 from these values. 206

The metabolic and microbial quotients were calculated indices. The metabolic quotient or coefficient was calculated as the ratio between the CO<sub>2</sub>-C from basal respiration and the microbial biomass-C (CO<sub>2</sub>-C<sub>resp</sub>-to-C<sub>mic</sub>), expressed as  $\mu$ g CO<sub>2</sub>-C mg<sup>-1</sup> biomass-C h<sup>-1</sup>. It is also known as the specific respiration rate (*q*CO<sub>2</sub>) (Anderson and Domsch, 1993). The microbial quotient was calculated as the ratio between the microbial biomass-C-to-total organic C ( $C_{mic}$ -to- $C_{org}$ ).

213

#### 214 *2.4. Leachate analysis*

Leachate dissolved organic C (DOC) and total organic N (TON) were 215 measured using a TOC-VCSH/CSN analyzer (Shimadzu Corp., Kyoto, Japan) and the 216 217 DOC:TON ratio subsequently calculated. Nitrate and ammonium concentrations were measured with a Skalar SAN<sup>++</sup> segmented-flow autoanalyser (Skalar, Breda, 218 219 Netherlands), based on the cadmium (Cd) reduction method (Maynard and Kalra, 1993; Griffin, et al., 1995) and the modified Berthelot reaction (Searie, 1984) 220 respectively. Electrical conductivity (EC) was measured with a standard platinum 1 221 cm electrode on a 4520-EC meter (Jenway Ltd, Dunmow, Essex, UK). pH was 222 measured using a glass electrode (Gelplas general purpose electrode, BDH) on a HI-223 209 pH meter (Orion research, Boston, MA, USA). Total free amino acids were 224 determined using the fluorometric OPAME procedure of Jones et al. (2002) and a 225 Cary Eclipse Fluorescence Spectrophotometer (Varian Inc., Australia) using a leucine 226 standard. Humic substances were determined by measuring the absorbance of 350 µl 227 of leachate at 254 and 400 nm (UV and visible range respectively) on a PowerWave 228 XS scanning microplate spectrophotometer (BioTek® Instruments, Winooski, VT). 229 The absorbance of deionised water was used as a control. A humification index (HIX) 230 was calculated by dividing the absorbance at 254 nm by the absorbance at 400 nm 231 (Zsolnay et al., 1999; Embachar et al., 2007). Soluble phenolic concentrations were 232 assayed using a modification of the method of Box (1983) and Ohno and First (1998) 233 using Na<sub>2</sub>CO<sub>3</sub> (1.9 M) and the Folin-Ciocalteu reagent (Sigma-Aldrich, Poole, Dorset) 234

(DeForest et al., 2005). The blue-coloured phenolics were measured at 750 nm using a

236 PowerWave XS scanning microplate spectrophotometer (BioTek<sup>®</sup> Instruments).

237

#### 238 2.5. Statistical analyses

ANOVA, Factor, Discriminant and Cluster analyses were all determined using 239 SPSS version 15.0 (SPSS Inc., Chicago, IL) and GenStat version 8 (VSN 240 241 International Ltd, Hemel Hempstead, UK). They were used to analyse the measured attributes to investigate the effect of soil types and AVCs on the SOIs identified. To 242 243 identify significant differences between treatments, post hoc multiple comparison (pair-wise) tests were made using the Gabriel test where homogeneity of variance was 244 assumed and Games-Howell procedure where unequal variance occurred. Some 245 variables were clearly not normally distributed judging from the Q-Q plots (data not 246 presented); however, all the factors (SQFs) from factor analysis and discriminant 247 analysis were normally distributed. 248

For the cluster analysis, the average linkage method and a squared Euclidean distance measure were used with a rescaled distance cluster combined measure on the similarity axis. The variables were standardized to minimize the effect of scale differences since the variables possessed different units.

253

#### 254 **3. Results**

#### 255 3.1. Biological, physical and chemical properties of soils

The variability of individual soil quality indicators across the range of soil types is shown in Figure 1. The box plots shows the spread of each measured soil property for each soil type as well as the data's symmetry and skewness. (The boundary of the box closest to zero indicates the 25<sup>th</sup> percentile, the line within the

box marks the median (50<sup>th</sup> percentile), and the boundary of the box farthest from 260 zero indicates the 75<sup>th</sup> percentile while the whiskers below and above the box indicate 261 the 10<sup>th</sup> and 90<sup>th</sup> percentiles where outliers are present). From the box plots, most of 262 the soil quality indicators did not show differentiations among the soil types save for 263 the following: microbial quotient, SOC and Soil Respiration separated the peats from 264 the rest; pH and C:N leachate separated the peats and the podzols from the rest, while 265 266 the bulk density grouped the soils in three groups of Pelosols, the Browns, Groundwater gleys and the Surface-water gleys (average  $=1.1 \text{ g cm}^{-3}$ ) in one group; Podzols 267 and Lithomorphics ( $av = 0.5 \text{ g cm}^{-3}$ ) in the second group and peats (ave 0.2 g cm<sup>-3</sup>) in 268 the third group. All other properties were did not show effective differentiations 269 among the soil types. 270

Figure 1: Box plots showing the spread of each measured soil property for each of the major soil types from 304 individual soils sampled as part of a nationwide soil quality assessment in UK. The boundary of the box closest to zero indicates the 25<sup>th</sup> percentile, the line within the box marks the median (50<sup>th</sup> percentile), and the boundary of the box farthest from zero indicates the 75<sup>th</sup> percentile. Whiskers below and above the box indicate the 10<sup>th</sup> and 90<sup>th</sup> percentiles where outliers are present. GWG and SWG represent groundwater and surface water gley soils respectively.

278

#### 279 *3.2. Relationships among soil properties*

Correlation analysis of the 20 soil attributes representing soil biological, physical and chemical properties resulted in significant correlation (P < 0.05) in 112 of the 190 soil attribute pairs (Table 2). Of these, the highest significant (P < 0.01) positive correlations was between humic substances at 254 nm versus those at 400 nm (r = 0.97). Other highly significant (P < 0.01) positive correlations were between the

285	absorbance at 254 nm or 400 nm versus DOC ( $r = 0.78$ and $r = 0.71$ respectively);
286	leachate TON versus NO <sub>3</sub> <sup>-</sup> ( $r = 0.78$ ), and bulk density versus pH ( $r = 0.70$ ).
287	Additional notable significant ( $P < 0.01$ ) positive correlations ( $r > 0.50$ ) were between:
288	microbial-N versus microbial-C, SOC versus soil respiration, the leachate C:N ratio
289	versus SOC, electrical conductivity versus both nitrate and TON, phenolics versus
290	absorbance at 254 nm and DOC versus absorbance at 400 nm. The highest significant
291	(P < 0.01) negative correlation was between bulk density versus SOC $(r = -0.83)$
292	Other notable significant ( $P < 0.01$ ) negative correlations were between: bulk density
293	versus either microbial-C ( $r = -0.42$ ), soil respiration ( $r = -0.51$ ) or the leachate C:N
294	ratio ( $r = -0.47$ ); SOC versus $q$ Mic ( $r = -0.47$ ) and pH versus either SOC ( $r = -0.66$ ),
295	absorbance at 400 nm ( $r = -0.42$ ), leachate DOC ( $r = -0.40$ ) or leachate C:N ratio ( $r =$
296	-0.47)

297	Table 2.	Correlations	among phy	vsical, c	chemical	and b	oiologi	cal soil	attributes
			****** ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~ ~~	/~					

Varable	qMic	qCO2	Mic C	Mic N	Mic CN	SR	SOC	Nitrate	Amonia	рH
qMic	1	•								
qCO2	-0.07	1								
Mic C	0.23(**)	-0.07	1							
Mic N	0.17(**)	-0.05	0.63(**)	1						
Mic CN	0.18(**)	-0.02	0.03	-0.24(**)	1					
SR	-0.26(**)	-0.01	0.31(**)	0.09	-0.02	1				
SOC	-0.47(**)	-0.04	0.39(**)	0.09	0.04	0.61(**)	1			
Nitrate	0.21(**)	-0.01	-0.15(**)	-0.12(*)	0.20(**)	-0.22(**)	-0.33(**)	1		
Amonia	-0.05	-0.04	0.08	0.06	-0.03	0.02	0.04	0.06	1	
рН	0.35(**)	0.08	-0.31(**)	0	-0.11(*)	-0.39(**)	-0.66(**)	0.25(**)	-0.18(**)	1
Ec	0.03	0.06	-0.09	0.01	0.17(**)	-0.08	-0.03	0.59(**)	0.03	0.12(*)
Phenols	-0.23(**)	-0.01	0.19(**)	0.08	-0.01	0.27(**)	0.39(**)	-0.19(**)	0.38(**)	-0.36(**)
Absb @ 254	-0.24(**)	-0.01	0.10(*)	-0.03	0.05	0.22(**)	0.34(**)	-0.19(**)	0.23(**)	-0.42(**)
Absb @ 400	-0.23(**)	-0.01	0.10(*)	-0.06	0.05	0.21(**)	0.35(**)	-0.19(**)	0.23(**)	-0.42(**)
ніх	0.06	0.02	0	0.11(*)	0.13(**)	-0.07	-0.14(**)	0.24(**)	0.01	0.10(*)
amino acids	-0.04	-0.03	0.09	-0.04	0.28(**)	0.03	0.11(*)	-0.02	0.48(**)	-0.15(**)
TOC_L	-020(**)	0.01	0.12(*)	-0.02	0.06	0.29(**)	0.35(**)	-0.18(**)	0.32(**)	-0.40(**)
TON_L	0.18(**)	0.02	-0.08	-0.05	0.24(**)	-0.14(**)	-0.21(**)	0.78(**)	0.11 (*)	0.09
CN_L	-0.25 (**)	-0.04	0.16(**)	-0.02	0.03	0.33(**)	0.50(**)	-0.33(**)	-0.04	-0.47(**)
BD	0.46(**)	0.05	-0.42(**)	-0.22(**)	-0.07	-0.51(**)	-0.83(**)	0.35(**)	-0.14(**)	0.70(**)

**Table 2 continued** 

301

			Absob @	Absob @		amino				
Varable	Ec	Phenols	254	400	HIX	acids	TOC_L	TON_L	CN_L	BD
Ec	1									
Phenols	0.04	1								
Absb @ 254	-0.04	0.58(**)	1							
Absb @ 400	-0.08	0.60(**)	0.97(**)	1						
HIX	0.37(**)	-0.09	-0.04	-0.22(**)	1					
amino acids	-0.01	0.23(**)	0.09	0.09	0.11(*)	1				
TOC_L	0.01	0.56(**)	0.78(**)	0.71(**)	0.08	0.23(**)	1			
TON_L	0.66 (**)	-0.08	-0.13(**)	-0.14(**)	0.31 (**)	0.05	-0.05	1		
CN_L	-0.05	0.34(**)	0.38(**)	0.37(**)	-0.06	0.02	0.38(**)	-0.25 (**)	1	
BD	0.10(*)	-0.38(**)	-0.35 (**)	-0.33 (**)	0.04	-0.17 (**)	-0.37 (**)	0.21(**)	-0.4 (**)	1

302

Note: \*Correlation is significant at P < 0.05 level, and \*\* at the P < 0.01 level; qMic, microbial quotient; qCO<sub>2</sub>, metabolic quotient; Mic C,

microbial carbon (mg C kg<sup>-1</sup>); Mic N, microbial nitrogen (mg C kg<sup>-1</sup>); Mic C:N, microbial C:N ratio; SR, soil respiration (mg C kg<sup>-1</sup> h<sup>-1</sup>); SOC,

soil organic carbon (mg C kg<sup>-1</sup>); NO<sub>3</sub><sup>-</sup>, nitrate (mg N l<sup>-1</sup>); NH<sub>4</sub><sup>+</sup>, ammonium (mg N l<sup>-1</sup>); EC, (μS cm<sup>-1</sup>); Phenols, Soluble phenolics (mg l<sup>-1</sup>); Abs

306 @ 254 and 400, absorbance of soil solution at 254 and 400 nm; HIX, humification index; Am acids, Free amino acids (µM); TOC/N L, total

<sup>307</sup> organic carbon/nitrogen in leachate (mg l<sup>-1</sup>); BD, bulk density.

Due to differences in the units of individual variables, Factor Analysis (FA) was 308 performed using a correlation matrix on the standardised values of the measured 20 attributes. 309 The generalised least-squares method was used to extract factors because it is robust and requires 310 no assumptions of sample coming from a multivariate normal distribution (SPSS, 2006). The 311 first six factors with eigenvalues > 1 were retained for interpretation, whilst factors with 312 eigenvalues < 1 explained less total variation than individual soil attributes (Brejda et al., 2000). 313 The retained factors accounted for > 61% of the total variance in the measured attributes; see 314 Table 3. 315

316

Table 3. Total variance (Eigenvalue), proportion and cumulative variance (Prop Var and Cum
 Var) explained by factor analysis using correlation matrix (standardized data) on the measured
 attributes.

				Extra	ction sums o	f squared	Rotation sum of squared			
Factors	Initial eigenvalues				loadings		loadings			
	Total	Prop of	Cum	Total	Total Prop of Cum		Total	Prop of	Cum	
		Var (%)	Var (%)		Var (%)	Var (%)		Var (%)	Var (%)	
Factor 1	5.31	26.6	26.6	3.60	18.0	18.0	3.35	16.7	16.7	
Factor 2	2.64	13.2	39.8	3.22	16.1	34.1	2.96	14.8	31.5	
Factor 3	2.03	10.1	49.9	2.14	10.7	44.8	2.28	11.4	42.9	
Factor 4	1.73	8.7	58.6	1.56	7.8	52.6	1.65	8.3	51.2	
Factor 5	1.31	6.6	65.1	0.65	3.3	55.9	1.32	6.6	57.8	
Factor 6	1.18	5.9	71.1	1.15	5.7	61.6	0.76	3.8	61.6	

320

The retained factors were subjected to a varimax rotation. A varimax rotation redistributes the variance of significant factors and minimizes the number of variables that have high loadings on each factor, thereby simplifying the interpretation of the factors (SPSS, 2006). The relative importance of each soil attribute, in terms of its contribution to all of the factors,

was judged by its communality value (Field, 2005; Ayoubi and Khormali, 2008) and is shown in 325 Table 4. The six factors explained > 90% variance in absorbance (a) 254 and 400 (absb(a)254 and 326 400), microbial carbon (Mic C), and soil organic carbon (SOC); > 80% in total organic nitrogen 327 in leachate (TON L) and bulk density (BD); > 70% in microbial nitrogen (Mic N), Nitrate, 328 Ammonium, electrical conductivity (EC), and total organic carbon in leachate (TOC L); > 60 % 329 in microbial quotient (qMic), pH and humification index (HIX); > 50 % microbial C/N ratio 330 (Mic CN), soil respiration (SR), and phenolics; and < 50 % C/N ratio of the leachate (CN L) and 331 microbial metabolic quotient  $(qCO_2)$  (Table 4). Attributes with the low communality estimates 332 (e.g. qCO<sub>2</sub> and leachate C:N) were the least important for interpreting factors. The magnitudes of 333 the loadings were used as a criterion for interpreting the relationship between the soil attributes 334 and the factors. Soil attributes were assigned to the factor for which the loadings were highest. 335

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Communality
							extraction
qMic	-0.54	-0.13	0.12	0.45	-0.01	-0.07	0.67
qCO <sub>2</sub>	0.05	-0.05	-0.05	-0.18	0.01	0.00	0.10
Microbial-C	0.29	0.03	-0.04	0.89	0.09	-0.03	0.90
Microbial-N	0.05	-0.04	-0.08	0.75	-0.02	0.14	0.73
Microbial C:N	0.07	0.05	0.30	-0.03	0.11	0.01	0.51
Soil respiration	0.61	0.06	-0.07	0.09	0.03	-0.06	0.50
Soil organic C	0.92	0.16	-0.08	0.08	0.01	-0.06	0.91
Nitrate-N	-0.27	-0.09	0.81	-0.04	0.01	0.04	0.77
Ammonium-N	0.01	0.23	0.05	0.05	0.78	0.01	0.72
рН	-0.68	-0.28	0.02	-0.06	-0.18	0.05	0.68
Elec. conductivity	0.03	0.00	0.74	-0.03	-0.05	0.22	0.70
Soluble phenolics	0.29	0.52	-0.03	0.06	0.32	-0.10	0.55
Absorb @ 254 nm	0.17	0.98	-0.06	-0.01	0.04	0.03	1.00
Absorb @ 400 nm	0.17	0.96	-0.05	-0.02	0.04	-0.20	0.99
HIX	-0.06	-0.06	0.25	0.07	0.05	0.76	0.69
Amino acids	0.11	0.06	0.02	0.01	0.66	0.05	0.56
TOC (leachate)	0.24	0.71	-0.02	0.00	0.29	0.18	0.73
TON (leachate)	-0.12	-0.06	0.91	0.01	0.09	0.07	0.87
C:N (leachate)	0.47	0.26	-0.17	-0.02	-0.06	0.02	0.42
Bulk density	-0.86	-0.19	0.13	-0.18	-0.11	-0.05	0.87

337 **Table 4.** Proportion of variance (loadings) using varimax rotation and communality estimates

*for soil attributes of the retained factors.* 

339

The **first factor** explained 16.7 % (see Table 3) of the total variance. It was named *soil organic matter (SOM)* because it had high positive loading for SOC (0.92), soil respiration (0.61) and leachate C:N ratio (47), a high negative loadings for bulk density (-0.86), pH (-0.68) and moderately on qMic (-0.54). Grouping qMic with the *SOM* factor rather than factor 4 was as a result of its stronger correlation with attributes constituting the *SOM* factor namely, soil

respiration (r = -0.26), SOC (r = -0.47) and bulk density (r = 0.46) rather than with Microbial-C 345 (r = 0.23) and Microbial-N (r = 0.17) of factor 4 (Table 3). The second factor explained 15% of 346 the total variance with a high positive loading for soluble phenolics (0.52), leachate absorbance 347 at 254 nm (0.98), 400 nm (0.96) and leachate TOC (0.71) and consequently, was termed OM 348 humification. The third factor explained 11 % of the total variance with high positive loadings 349 350 for nitrate (0.81), leachate TON (0.91) and electrical conductivity (0.74) and was therefore termed soluble nitrogen factor. The fourth factor had positive loadings for Microbial-C (0.89), 351 Microbial-N (0.75) and a moderately high loading for qMic (0.45), and was termed microbial 352 biomass. The fifth factor had positive loading for ammonium (0.78) and amino acids (0.66) and 353 was termed *reduced N*. The sixth factor explained only 4 % of the total variance and had a high 354 positive loading for HIX (0.76) and was termed soil humification index. 355

356

#### 357 *3.3. Effect of soil types on attribute means and factor scores*

One way ANOVA revealed that most of the soil attributes and factor scores varied 358 significantly with soil types (Table 5). However, pairwise comparison showed that the effect of 359 soil types on most attribute was very small. In most cases, only the Peat soils were clearly 360 significantly (P < 0.01) different from all the other soil types. Only SOM and microbial biomass 361 factors (Factors 1 and 4 respectively) varied significantly (P < 0.05) with soil type. SOM factor 362 mean scores were negative for Brown, GWG, SWG and Pelosol soils and positive for 363 364 Lithomorphic, Peat and Podzolic soils. Peats had the highest score and were significantly different from all other soil types on the SOM factor. Furthermore, Peat soils had the highest 365 SOC content to which the analysis also confirmed. The mean scores for SOM factor did not vary 366 367 significantly (p > 0.05) within Browns, GWGs and Pelosols nor did it do so among the

- Lithomorphic, Podzolic and SWG soils. The *Microbial biomass* factor varied significantly (P <
- 0.05) between Brown versus GWG soil types and Lithomorphics only. Mean scores for OM
- humification, soluble N, reduced N and humification index did not vary significantly (P > 0.05)
- among all soil types.

**Table 5.** Soil attribute means and factor scores in the different soil types (The first 5 variables are the most important for discrimination between soil types)

Soil attributes	Soil types							SEM	ANOVA
	Brown	Groundwater	Lithomorphic	Peat	Pelosols	Podzolic	Surface	-	
		gley					water gley		
Microbial quotient	0.018 <sup>a</sup>	0.026 <sup>a</sup>	0.014 ac	0.003 <sup>b</sup>	0.014 abc	0.010 °	0.018 a	0.003	0.00
qCO <sub>2</sub>	0.073	0.002	0.001	0.011	0.01	0.002	0.003	0.012	NS
Microbial-C (g kg <sup>-1</sup> )	0.59 a	1.00 <sup>ab</sup>	1.03 ab	1.37 <sup>b</sup>	0.54 <sup>a</sup>	1.02 ab	0.89 <sup>ab</sup>	0.13	0.00
Microbial-N (mg kg <sup>-1</sup> )	85 <sup>a</sup>	119 <sup>ab</sup>	148 <sup>b</sup>	113 <sup>ab</sup>	71 <sup>ab</sup>	111 <sup>ab</sup>	99 <sup>ab</sup>	16	0.03
C:N (Microbial)	12.4	19.6	18.9	19.7	36.3	29.9	33.2	12	NS
Soil respiration (mg kg <sup>-1</sup> h <sup>-1</sup> )	0.63 <sup>a</sup>	1.10 a	0.93 <sup>a</sup>	3.35 <sup>b</sup>	1.63 ab	1.58 <sup>ab</sup>	1.18 a	0.45	0.00
Soil organic C (g kg <sup>-1</sup> )	42 a	45 a	132 ь	377 °	92 <sup>ab</sup>	151 <sup>b</sup>	98 <sup>b</sup>	23	0.00
Nitrate (mg N l <sup>-1</sup> )	3.00 <sup>a</sup>	2.04 ac	2.32 ac	$0.13^{b}$	1.13 °	$0.37  ^{bc}$	3.08 <sup>a</sup>	0.39	0.00
Ammonium (mg N l <sup>-1</sup> )	0.25	0.18	0.3	0.27	0.17	0.31	0.3	0.05	NS
рН	6.55 <sup>a</sup>	6.56 <sup>a</sup>	6.24 <sup>ac</sup>	4.71 <sup>b</sup>	6.18 ac	5.08 <sup>b</sup>	5.73 °	0.2	0.00
Elect. conductivity (μS cm <sup>-1</sup> )	129	107	124	99	74	81	116	16	NS
Soluble phenols (mg l <sup>-1</sup> )	0.33 ac	0.26 <sup>a</sup>	0.68 bc	1.10 <sup>b</sup>	0.56 abc	1.20 <sup>b</sup>	0.46 °	0.16	0.00
Absorbance @ 254 nm	0.25 <sup>a</sup>	0.28 <sup>a</sup>	0.29 <sup>ab</sup>	0.47 <sup>b</sup>	0.45 <sup>ab</sup>	0.48 <sup>b</sup>	0.32 <sup>ab</sup>	0.48	0.00
Absorbance @ 400 nm	0.028 <sup>a</sup>	0.033 a	0.032 <sup>ab</sup>	0.061 <sup>b</sup>	$0.047$ $^{ab}$	0.061 <sup>b</sup>	0.036 ab	0.009	0.00
Humification index (HIX)	9.0 <sup>ab</sup>	9.0 <sup>ab</sup>	8.7 <sup>ab</sup>	8.2 ª	8.3 <sup>ab</sup>	8.6 <sup>ab</sup>	9.3 <sup>b</sup>	0.3	0.03
Amino acids (µM)	1.52	1.83	1.67	1.95	1.15	3.1	2.08	0.4	NS
Leachate TOC (mg l <sup>-1</sup> )	7.5 <sup>a</sup>	6.9 <sup>a</sup>	8.2 <sup>ab</sup>	12.0 <sup>b</sup>	12.8 <sup>ab</sup>	12.3 <sup>b</sup>	9.8 <sup>ab</sup>	2.2	0.00
Leachate TON (mg l <sup>-1</sup> )	5.82 ª	3.47 ac	3.16 ac	0.78 <sup>b</sup>	1.62 °	1.81 bc	6.69 <sup>a</sup>	0.8	0.01
Leachate C:N	4.6 <sup>a</sup>	5.5 <sup>a</sup>	7.2 ª	19.0 <sup>b</sup>	9.1 ab	17.5 <sup>b</sup>	9.7 <sup>a</sup>	2.4	0.00
Bulk density	1.10 <sup>a</sup>	1.11 <sup>a</sup>	0.63 <sup>b</sup>	0.19 °	1.08 a	0.58 <sup>b</sup>	0.81 <sup>b</sup>	0.06	0.00
<u>Factors</u>	Factor scores								
Factor 1	-0.52ª	-0.63 a	0.15 <sup>b</sup>	1.58 °	-0.59 ª	0.2 <sup>b</sup>	-0.07 <sup>b</sup>	0.12	0
Factor 2	-0.17	-0.05	-0.13	0.22	-0.46	0.44	0	0.15	NS
Factor 3	0.09	-0.1	-0.06	-0.13	-0.4	-0.28	0.23	0.11	NS
Factor 4	-0.24 <sup>a</sup>	0.36 <sup>b</sup>	0.30 <sup>b</sup>	0.03 <sup>ab</sup>	-0.21 <sup>ab</sup>	0.01 ab	0.02 <sup>ab</sup>	0.19	0.04
Factor 5	-0.05	-0.22	-0.03	-0.11	-0.2	0.36	0.14	0.17	NS
Factor 6	0.06	-0.1	0.17	-0.3	-0.29	-0.2	0.23	0.18	NS

#### 375 *3.4. Soil quality indicators across soil types*

Discriminant analysis of the six statistical factors in relation to soil types, indicated that the 376 SOM was the most powerful in discriminating among the seven soil type groups based on the 377 magnitude of their discriminant coefficients (Eq. 2). The first canonical discriminant function 378 explained 90 % of the total variance based on Wilks's Lambda, (P < 0.001) (table not shown) 379 and therefore was the most important canonical discriminant function for discriminating soil 380 types using the soil quality factors identified. Although the second canonical discriminant 381 function was also significant (P = 0.03) based on Wilks's Lambda, it only accounted for 4 % of 382 the total variance and therefore was not used. 383

384 
$$Y_1 = 1.43 (SOM) + 0.29 (OM humification) - 0.14 (soluble N) + 0.08 (microbial)$$

385 
$$biomass$$
) + 0.03 (reduced N) - 0.22 (HIX) (Eq. 2)

Therefore the group differences across soil types shown by ANOVA can be explained in terms of *SOM*, judging from the discriminant coefficient which was five-fold larger than the coefficient for the *OM humification* factor and several fold greater than the rest of the factors. Discriminant analysis of the measured attributes constituting *SOM* (i.e. *q*Mic, soil respiration (SR), soil organic C (SOC), pH and bulk density (BD)) indicated that microbial quotient (*q*Mic) was the most powerful attribute discriminating the soil types (Eq. 3).

392 
$$Y_2 = 8.75 \times 10^{-6} (\text{SOC}) - 1.99 (q\text{Mic}) - 0.50 (\text{BD}) - 0.04 (\text{pH}) - 0.05 (\text{SR})$$
 (Eq. 3)

The discriminant coefficient for qMic was four-fold larger than the coefficient for bulk density and more than 40-fold for the rest. The qMic was significantly correlated with bulk density (0.46\*\*), soil organic C (-0.47\*\*), pH (0.35\*\*) and soil respiration (-0.26\*\*) while bulk density was significantly correlated with soil organic C (-0.83\*\*), pH (0.70\*\*) and soil respiration (-0.53\*\*) meaning that qMic and bulk density, though correlated, were the most important and dominant attributes for assessing soil quality across soil types. The mean comparisons using the Games-Howell approach indicated that the bulk density had similar discriminating power as the *SOM* factor among the soil types. *q*Mic mean values varied significantly with soil types separating Peat < Podzols < Browns, GWGs and SWGs soils in increasing order (Table 5).

- 403
- 404 *3.5. Effect of aggregate vegetation class on factor scores*

Aggregate vegetation class (AVC) showed more effects on factor scores than the soil types. 405 The significant effects were observed in SOM, OM humification, microbial biomass and 406 humification index. The soluble N and reduced N factors showed no significant variation among 407 the AVCs (Table 6). The SOM factor had the highest factor scores (P < 0.001) in Heath and Bog. 408 Mean scores between Moorland Grass Mosaics and Upland Woodland did not vary significantly 409 (P > 0.05); nor among Fertile Grasslands, Infertile Grassland, Lowland Woodland and Tall Grass 410 Mosaic. The mean scores were lowest in Crop and Weeds and were significantly different (P <411 0.001) from all other AVCs except in Tall Grass and Herbs. 412

	Average vegetation class mean factor scores										
Factors	Crops &	Fertile	Heath	Infertile	Lowland	Moorland grass	Tall grass	Upland	SEM	ANOVA	
	weeds	grasslands	& bog	grassland	woodland	mosaics	& herbs	woodland			
Factor 1	-0.80 <sup>a</sup>	-0.54 <sup>b</sup>	1.43 °	-0.50 <sup>b</sup>	-0.40 <sup>b</sup>	0.62 <sup>d</sup>	-0.64 <sup>ab</sup>	0.20 <sup>bd</sup>	0.10	0.00	
Factor 2	-0.40 <sup>a</sup>	-0.11 ab	0.30 <sup>b</sup>	0.02 <sup>b</sup>	0.41 <sup>ab</sup>	-0.11 <sup>ab</sup>	-0.06 <sup>ab</sup>	0.51 <sup>ab</sup>	0.19	0.00	
Factor 3	0.34	0.07	-0.09	-0.03	0.05	-0.34	0.12	-0.28	0.14	NS	
Factor 4	-0.49 a	0.16 <sup>b</sup>	$0.07  ^{\mathrm{b}}$	0.27 <sup>b</sup>	-0.19 <sup>ab</sup>	0.28 <sup>b</sup>	-0.61 <sup>a</sup>	-0.21 ab	0.16	0.00	
Factor 5	-0.39	0.09	0.13	0.02	-0.12	0.22	-0.20	0.18	0.14	NS	
Factor 6	-0.29 ª	$0.04^{ab}$	-0.35 <sup>ab</sup>	0.13 <sup>b</sup>	1.15 °	0.18 <sup>b</sup>	0.38 <sup>bc</sup>	0.63 bc	0.17	0.00	
<u>Soil</u>				S	oil attributa	maan valuas					
<u>attributes</u>				2		mean values					
Soil respiration	0.29ª	1.00 <sup>b</sup>	3.22 °	0.77 <sup>b</sup>	0.67 <sup>ab</sup>	1.44 <sup>b</sup>	0.43 ab	1.41 <sup>b</sup>	0.23	0.000	
Soil organic C	16.7ª	43.6 <sup>b</sup>	350.2 °	43.8 <sup>b</sup>	46.4 <sup>b</sup>	185.6 °	25.0 ab	119.8 °	11.2	0.000	
рН	7.3 ª	6.4 <sup>b</sup>	4.6 °	6.3 <sup>b</sup>	6.2 <sup>abd</sup>	5.2 <sup>d</sup>	6.6 <sup>ab</sup>	4.7 <sup>dc</sup>	0.2	0.000	
Bulk density	1.37 a	1.06 <sup>b</sup>	0.21 °	0.95 <sup>b</sup>	0.89 <sup>b</sup>	0.41 <sup>d</sup>	1.22 ab	0.48 <sup>d</sup>	0.05	0.000	
qMic	0.021ª	0.023 a	0.005 <sup>b</sup>	0.021 a	0.015 <sup>ab</sup>	0.009 <sup>b</sup>	0.015 <sup>ab</sup>	$0.010^{ab}$	0.003	0.000	

## **Table 6.** Effect of Aggregate Vegetation Class (AVC) on factor scores and soil attribute means.

Means scores for *OM humification* factor varied significantly (P < 0.001) between Crop 416 and Weeds verses Herb and Bog, and Infertile Grasslands; all other pairs did not vary 417 significantly. For microbial biomass factor, Crop and Weeds and Tall Grass and Herbs varied 418 significantly (P < 0.001) against the Fertile Grassland, Infertile Grasslands, Heath and Bog, and 419 420 Moorland Grass Mosaics, while all other pairs were not significantly different (P > 0.05). The *humification index* factor showed that the mean scores varied significantly (P < 0.001) among

Crop and Weeds versus Infertile Grassland and Moorland Grass Mosaics versus Lowland 422 Woodland only. 423

424

421

#### 3.6. Soil quality indicators across Aggregate Vegetation Classes (AVC) 425

The first canonical discriminant function of the discriminant analysis of the six factors 426 across the AVCs explained 94% of the total variance (Wilks's Lambda, P < 0.001) whose 427 428 coefficients were used in the equation below:

 $Y_3 = 2.12$  (SOM) + 0.49 (OM humification) - 0.35 (soluble N) + 0.30 (microbial biomass) 429 + 0.36 (reduced N) - 0.20 (soil HIX) 430 (Eq. 4)

From the discriminant coefficients in Eq. [4], SOM factor was the most powerful 431 discriminating among the eight different AVCs. The SOM factor was more than four-fold larger 432 433 than the coefficients of all others soil quality factors under consideration.

434 The discriminant analysis of the measured attributes constituting the SOM factor showed that BD and *q*Mic were the most powerful discriminating soil attributes among the seven habitats (AVCs) 435 (Eq. 5). 436

437 
$$Y_4 = 3.27$$
 (bulk density) - 2.45 (qMic) - 2.75 × 10<sup>-6</sup> (soil organic C) + 0.70 (pH) + 0.08  
438 (soil respiration) (Eq. 5)

Bulk density possessed similar discriminating power as the *SOM* factor among the AVCs. Bulk density values were significantly different (P < 0.001) among AVCs with the lowest mean values in Heath and Bog (0.21 g cm<sup>-3</sup>) < Upland Wooded (0.48 g cm<sup>-3</sup>) and Moorland Grass Mosaic (0.41 g cm<sup>-3</sup>) < Fertile Grass (1.06 g cm<sup>-3</sup>), Infertile Grass (0.95 g cm<sup>-3</sup>), Lowland Wooded (0.89 g cm<sup>-3</sup>) < Tall Grass and Herbs (1.21 g cm<sup>-3</sup>) and Crop and Weeds (1.37 g cm<sup>-3</sup>; Table 6).

445

#### 446 3.7. Main and interactions effect of soil types and AVCs

The results of the two-way ANOVA on the first canonical discriminate function on all 20 variables showed significant (P < 0.01) main and interaction effects. The main effect of soil types and the effect of soil types \* AVCs interaction on the attribute's scores was very small (Partial Eta Square = 0.09 and 0.16 respectively), while the main effect of the AVCs was large (Partial Eta Square = 0.42; Table. 7).

452 **Table 7.** Tests of between-subjects effects;

	Type IV sum of		Mean			Partial eta
Source	squares	Df	Square	F	Sig.	squared
Corrected model	553.14	38	14.56	36.36	0.001	0.844
Intercept	3.42	1	3.416	8.532	0.004	0.032
Soil Type * AVC_Desc	18.98	25	0.759	1.896	0.008	0.157
Soil Type	10.36	6	1.726	4.311	0.001	0.092
AVC_Desc	73.97	7	10.57	26.39	0.001	0.420
Error	102.09	255	0.400			
Total	655.24	294				
Corrected Total	655.24	293				

Notes: Dependent variable: 1st Canonical Discriminant function scores from of all the soil
attributes measured. AVC\_desc means AVC description; Soil Type\*AVC\_Desc means the
interaction between the soil type and AVC effects.

458

The cross tabulation of AVCs versus soil types (Table S3), showed that 27 out of 56 combinations or cells, the soil types were sampled less than the calculated expected counts in the AVCs. In 16 combinations, the soil types were not at all represented in the AVCs. The most affected were the Lowland Woodland and Tall Grass and Herbs where, only Brown and SWGs were samples in the former and only Browns, GWG and SWGs in the latter.

464

#### 465 **4. Discussion**

#### 466 *4.1. Effect of soil types and AVCs on the soil quality factors and/or indicators*

A set of 20 correlated soil attributes were grouped into six factors called soil quality factors, using factor analysis. The factors identified contribute to one or more key soil functions proposed by Larson and Pierce (1991) and therefore could be considered soil quality indicators (Brejda et al., 2000). Since the soil quality factors cannot be measured directly (Elliott, 1997; Brejda et al., 2000), the effect of soil types and the AVCs on these factors were inferred by monitoring soil attributes that comprised them.

Not all the soil quality factors varied significantly with soil types or with AVCs. Only *SOM* and *microbial biomass* factors varied significantly (P < 0.001) by soil types. *SOM* was able to discriminate the highest number of soil groups, separating the Peats (1) with the highest scores, from Lithomorphics, Podzols, and SWGs (2) with intermediate scores, and from Browns, GWGs and Pelosols (3) with the lowest scores (Fig. 5), thus rendering three distinct soil type

groupings. The *microbial biomass* factor had a minor effect, discriminating the Browns from 478 GWGs and Lithomorphics only. The soil attributes constituting these soil quality factors (Soil 479 respiration, SOC, pH, bulk density, qMic, Microbial-C and -N) showed significant (P < 0.01) 480 variations discriminating at most three groups of soil types. In all the attributes considered, 481 Browns, GWGs and Pelosols were grouped together. SOM factor, SOC and bulk density 482 483 attributes separated the Peats as a unique soil group from all other soil types, which is not entirely a surprising result, since the peats are highly organic in nature with low BD as opposed 484 to mineral soils with low OM content and higher bulk densities. The most important soil quality 485 indicator associated with specific soil types or groups was the SOM factor with qMic > bulk486 density as the most important attributes. 487

Similarly, the most important SQF differentiating the AVCs across the GB was SOM 488 factor with bulk density > qMic attributes being the most important attributes. Four distinct AVC 489 groups were separated based on SOM factor and BD. Heath and bog was exclusively separated as 490 one group (1). Other groups were: (2) Crop and weeds with Tall grass and herb; (3) Fertile 491 grassland, Infertile grassland, Lowland woodland, Tall grass and herbs, and Upland woodland; 492 (4) Moorland grassland mosaic with Upland woodland. The Upland woodland and Tall grass and 493 herbs were intermediate habitats classifying in more than one of these groups. The rest of the 494 factors and attributes discriminated three or less groups. The soil attributes were generally better 495 in discriminating the AVCs than the SQF (Table 6) 496

Since *q*Mic and bulk density were moderately correlated ( $r=0.46^{**}$ ), they may be redundant as indicators to be used together. If only one attribute were to be used to monitor soil quality in soil types and AVCs, *q*Mic and BD respectively seems to offers the greatest potential judging from their high weights on the respective prediction models. However the *q*Mic may be

a 'MUST be included' soil attribute in the minimum data set, due to its important role in several 501 soil functions, being a fraction of soil carbon. Soil C influences a wide range of soil functions 502 including bulk density, infiltration, pesticide buffering, aeration, aggregate formation, pH, buffer 503 capacity, cation-exchange properties, mineralization, and the activity of soil organisms (Larson 504 and Pierce, 1991; Seybold et al., 1997). However, since the measurement of bulk density is 505 506 reasonably easy to obtain, it is therefore reasonable to consider it together with SOC, microbial and biomass C as minimum data set for assessing soil quality across average vegetation classes 507 in the study area. 508

Pedogenesis has taken place over thousands of years in the UK. During this period there 509 has been a range of climate change related vegetation colonization phases starting from tundra 510 heath and cycling through a range of forest types (Fitzpatrick, 1980). During this period parent 511 material/topography, climate and vegetation would have been stable for long periods of time 512 leading to the differentiation of soils. This was followed by progressive forest clearance which 513 started approximately 1000-3000 years ago with vegetation cover becoming more grassland and 514 heathland dominated. The last 200 years, however, has seen intense management of these soils 515 with the addition of fertilisers, lime and organic wastes combined with mechanical mixing of the 516 soils which has reversed centuries of acidification and soil horizon development. This 517 homogenisation of the soil has led to shifts between soil types even over short timescales (e.g. 518 humic-podzolic to brown soils on improved upland grasslands) and the loss of peat soils in 519 520 intensive agricultural areas (e.g. East Anglia; Taft et al., 2018). One key question is therefore whether it is historical soil type or current vegetation that is more important in driving soil 521 processes in the short term (e.g. over a 10-25 year timescale)? Here we found that more soil 522 523 quality factors showed an AVC effect rather than a soil type effect. All soil quality factors varied

significantly (P < 0.01) by AVCs except *soluble* N and *reduced* N factors though none discriminated more than four groups. It is possible that some of the soil quality factors that were insensitive to vegetation may represent inherent soil qualities that are controlled by other key factors of soil formation (e.g. parent material/topography), while those which significantly varied by AVCs may represent dynamic soil qualities, possessing great potential for assessing management practices on soil quality (Jenny, 1994; Soybold et al., 1997; Brejda et al., 2000; Bünemann, et al., 2018).

Most indicators available in literature have not been validated nor their sensitivity tested in a wide range of situations (Velasquez et al., 2007). Some of the attributes measured and the soil quality indicators identified in this work are not usually used in the monitoring of soil quality, but are candidates for potential alternatives (Schloter, et al., 2018).

535

#### 536 *4.2. Prediction of SQF and SQI by soil type or AVC*

The clusters from multivariate classification are "natural" groups, which uses the 537 "minimum-variance" solution; where a population is partitioned into cluster subsets by 538 minimizing the total within group variation while maximising between groups variance (Wishart, 539 1968). The groups/cluster formed from the multivariate analysis need to have no significant 540 overall spread. The clusters therefore, should correspond to data modes (distinct modes). 541 However, most of our cluster modes defined by soil types were not always distinct. Most of them 542 were separated from each other by significant "noise" data, making it impossible to resolve all 543 the clusters. Thus, the definition of the reference values for each soil type or AVC was 544 ambiguous, since most soils types or AVC groups could not be differentiated (Fig. 2). Forming, 545 546 describing and defining the groups could involve the use all measured attributes even though

only a few could be differentiating (Soil Survey Staff, 1999). Even when the soil quality factors/indicators and attributes were used in combination, some groups/clusters could still not be resolved. Therefore, the soil quality indicators and attributes identified in this study can only be used to characterise soil types and AVCs groups rather than for prediction or classification. From the discriminant plots and the dendrogram in Fig. 3 three groups can be defined in soil types and four groups in the AVC.

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Figure 2. Discrimination plots showing 95% confidence circles around the means for soil types
(Panel A) and AVCs (Panel C). Panels B and D are the respective cluster analyses dendrograms
using a complete linkage method.

557

Defining differentiating criteria for these groups in the soil types could involve the use of 558 bulk density attribute to define unique property ranges for the first groups, a combination of soil 559 respiration and SOC attributes for the second group, and a combination of qMic, soil respiration, 560 SOC, pH and bulk density attributes for the third group. The Pelosols were the most dispersed 561 and unreliable group for the purpose of attribute membership prediction, probably due to the fact 562 563 that they were under sampled, considering that only six samples were included in the analysis. The classification of the AVCs using discriminate and cluster analyses on key attributes yielded 564 four clusters. Defining differentiating criteria for these groups could involve the use of a 565 566 combination of soil respiration, SOC, pH attributes to define property ranges for the first, second and fourth groups and bulk density attribute for the third group. Tall Grass and Herbs and 567 Lowland Wooded were under sampled (with 11 and 6 samples respectively; Table S3) which 568

greatly compromised their predictive accuracy as can be observed from the large 95%
confidence circles which overlapped with other AVC groups.

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#### 4.3. To what extent do soil types and/ or AVC act as major regulators of SQI?

The two-way ANOVA and the tests of between-subjects effects on the first canonical 573 discriminant (CD) function from the canonical discriminant analysis (CDA) of the 20 physical, 574 chemical and biological properties showed significant differences between groups (soil types and 575 AVCs) as well as significant differences in the effect of soil type on the soil attributes between 576 the AVC (significant interaction of soil type  $\times$  AVC; Table 7). The 'practical' significance of 577 each term from Partial Eta Square values indicates that AVCs (with a large Partial Eta Square = 578 0.42), were a better regulator of the SQIs than soil types (with a weak Partial Eta Square = 0.09). 579 The effect size for the interaction was equally relatively weak (Partial Eta Square = 0.16). The 580 conclusion of the significant (P < 0.01) interaction effect of soil type × AVC is that the soil type 581 differences in the first CD function (or attributes) partly depended on the AVCs where the soil 582 was sampled. A multiple comparison of all soil type groups with AVC groups would be required 583 to draw specific conclusions regarding the interaction effects, which is quite complex and is 584 beyond the scope of this thesis. Suffice to say that there was a partial and varied soil type  $\times$  AVC 585 interaction across all levels. These interactions confirm Jenny's (1994) theory that the biotic 586 587 factor (of which vegetation plays a major role) is amongst others an important soil forming 588 factor. However, the results from the cross tabulation indicated that not all soil types were well represented in the AVCs in going by the calculated expected counts. In some cases soil types 589 were not at all represented (See Table S3). This problem can contribute to the complexity and 590 591 accuracy in the interpretation of the interaction effect observed above.

#### 593 **5. Conclusions**

The dominant SQFs/Is and attributes varied by both soil type and AVC. The SOM factor 594 was the most discriminating factor for both soil types and AVCs with microbial quotient and 595 bulk density as the most discriminating measured attributes. The discriminant analysis on the 596 597 important measured attributes comprising the SOM factor produced three fairly homogenous groups for soil types and four groups for AVCs. It was however, impossible to define reference 598 values in the SQF/I or attributes for separate individual soil types or AVCs, as property ranges 599 greatly overlapped due to large between group variability (probably due to integrating large 600 spatial areas). Some of the differences observed in soil types with regard to soil attributes were in 601 part dependent on the AVCs differences. 602

Therefore, whether SQIs can be predicted by soil types remains an open question. This study has shown that soil types or AVCs are poor predictors for SQF and indicators derived from factor analysis. However, different sets of SQIs and attributes for different regions have been used in the past in different studies (e.g. Brejda et al., 2000a; Brejda et al., 2000b; Shukla et al., 2006; Valesquez et al., 2007; Ayoubi and Khormali, 2008) suggesting that there may not be a universal optimum set of indicators for use across different regions of differing climatic conditions. Therefore, the search for SQIs which can be predicted by soil types continues.

For future work it might be worthwhile to make special consideration for the climatic, spatial and parent material variability in the sampling designs in addition to the inclusion of other promising soil attributes. Management factors should also be included (e.g. fertilizer regime). In terms of other key soil quality indicators, it would be interesting to include measures of key soil enzymes (e.g. cellulase, protease, phosphatase, sulfatase), their potential to release N<sub>2</sub>O and CH<sub>4</sub>, their hydrophobicity and clay effects. A further consideration should be in the sampling design,
to ensure equal and adequate representation of soil types in the aggregate vegetation classes in
order to accurately capture the interaction effect.

618

#### 619 **Supporting information**

Figure S1 Map of the UK showing the individual soil sampling locations used in the study. Thetotal land area is 209,331 km2.

**Table S2:** Shows conceptually comparable classification of the soils in the World reference base

623 (WRB) Classification. Number in brackets indicates the number of samples for that soil type

**Table S3:** Land class classification with the corresponding land uses

Table S4: The cross tabulation table of Aggregate Vegetation classes (AVCs) versus soil types.

627

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Figure 1 continued





Figure 2





# FigureS1