Using paper chromatography for assessing soil health in southwestern Australia

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Cover Photograph Chromatogram of soil collected from Little Grove, Albany, WA.

DISCLAIMER

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1 Introduction

Local natural resource management groups have a strong interest in improving soil health in catchments, and have adopted various land management practices aimed at enhancing soil condition. In order to track whether their endeavours have led to an improvement in soil health, land managers need to monitor soil condition. Given that many laboratory soil tests can be costly, community members are particularly interested in the development and use of cost-effective methods to assess soil for humus, minerals and microbiology. Pfeiffer's Circular Chromatography (PCC), a simple qualitative test first utilised by Ehrenfried E. Pfeiffer in 1953 represents such an inexpensive approach (Pfeiffer, 1984). The method was first developed to provide farmers and composters a snapshot of the biological activity and health of soil, compost, plants and food, and has been used in the UK, North and South America, and parts of Australia. Paper chromatography has been described as a cheap, easy to learn and highly effective way of assessing the health of soils, and landowners have been encouraged to use the method for the assessment of the biological quality of soils.

The aim of this report is to evaluate whether PCC can be used to assess soil health in southwestern Australia. To achieve this, we quantified patterns in chromatograms created from soils over gradients of characteristics (i.e. electrical conductivity (EC), pH, total organic carbon (TOC), and microbial activity (CO_2 burst tests)) and from several land use types (pasture, vegetable gardens, orchards, and remnant vegetation).

2 How to make chromatograms

Briefly, soils are placed in a solution of sodium hydroxide which is applied to circular filter paper that has been treated with silver nitrate. The soil mixture is poured into a petri dish, and is drawn up through a wick inserted through the middle of the filter paper. The different elements in this soil mixture move through the paper at different rates through capillary action, resulting in distinctive patterns.

2.1 Soil collection

Soil cores should be collected with a clean stainless-steel corer, with at least five 10cm deep cores being taken from each plot. Cores are placed in a container and mixed thoroughly, removing as much grass and rocks as possible (Figure 2.1). At least 250g of soil is weighed from the mixture and placed in a bag which has been labelled with information such as site number and date, with additional soil required if other tests (i.e. soil chemistry) are to be performed. If applicable, environmental parameters such as soil moisture should be recorded at this time.

2.2 Infusing filter paper with Silver Nitrate

Using Whatman #1 (150mm diameter) filter papers, and holding only the edges of the paper, first puncture a small hole (≈ 2mm) in the centre of the paper. Using a pencil create marks at 4cm and 6cm from the centre of the paper. Make a 2cm long wick by rolling a ≈2cm x 3cm piece of filter paper and place the wick in the punctured hole. Fill a small petri dish approximately 2-3mm deep with 0.5% silver nitrate solution (0.5g of $AgNO_3$ in 100ml distilled water) and put this in a larger petri dish. Place the filter paper with wick inserted over the large petri dish, ensuring the wick is in the

silver nitrate solution (Figure 2.2 & Figure 2.3). Allow the solution to soak through the filter paper until it is 1-2mm short of the 4cm mark. Remove the paper from the petri dish, extract and discard the wick, place the filter paper on a clean sheet of paper in a dark room and allow to dry overnight.

Figure 2.1. Collection of soil, taking soil cores (left), and mixing soil cores from a plot (right).

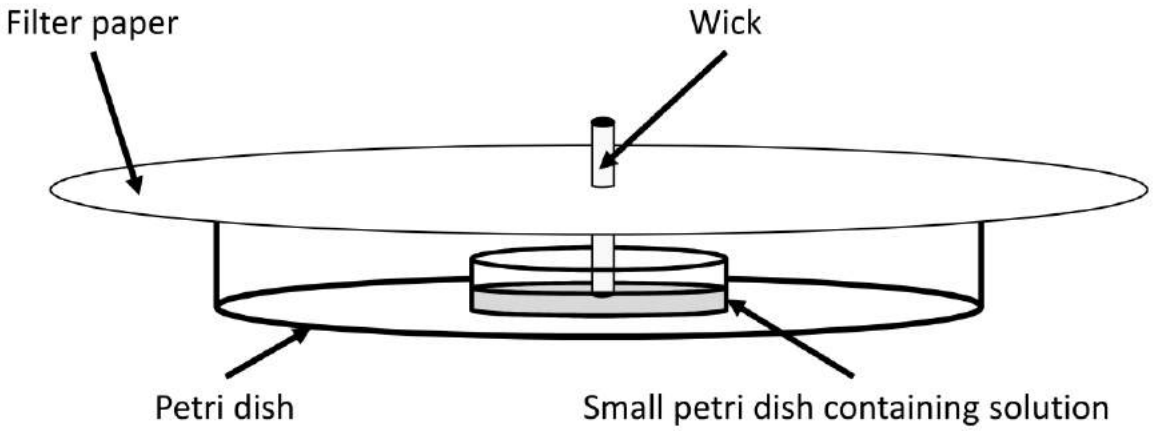

Figure 2.2. Diagram depicting the preparation of a chromatogram

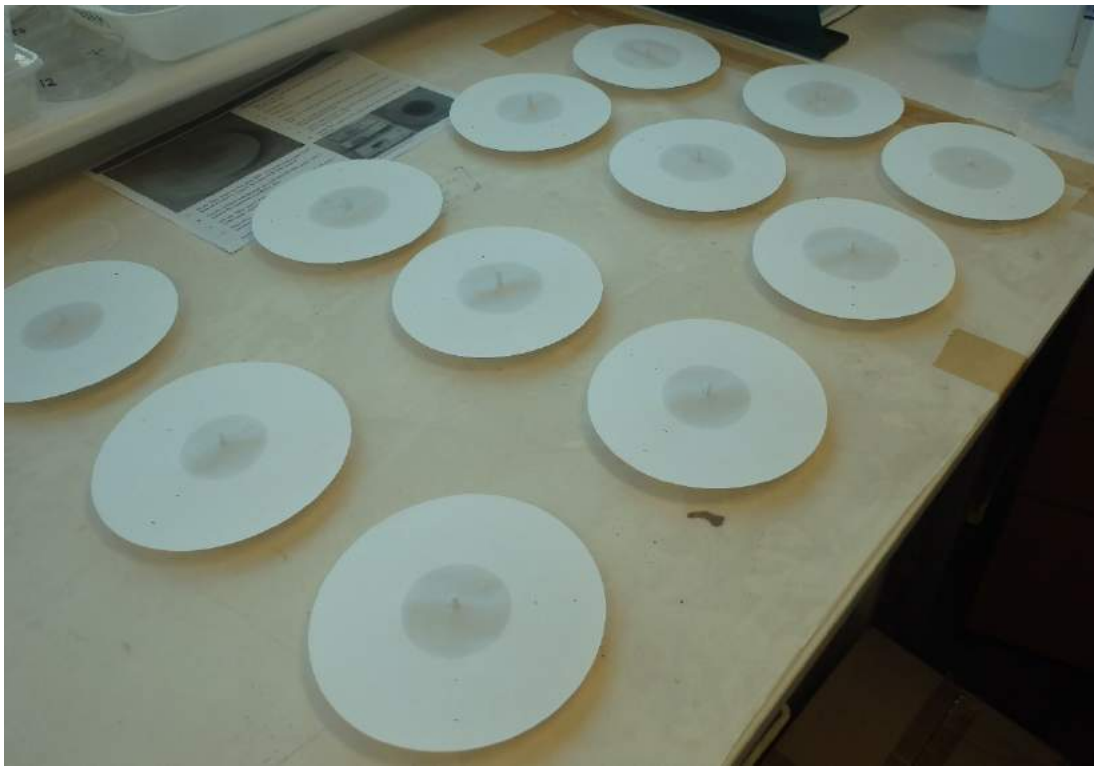

Figure 2.3. Filter papers being prepared with silver nitrate solution.

2.3 Soil preparation

The collected soil should be oven dried at 55-60°C for 6-24 hours. When fully dried, soil samples are first sieved to remove particles greater than 2mm, then finely ground using a mortar and pestle or equivalent. Once ground, 5g of soil is to be placed in a container with 50mL of 1% sodium hydroxide solution (1g NaOH in 100ml distilled water) to digest. After 15 minutes, swirl the solution and leave to rest for one hour, then swirl again. Leave digest to rest for a further five hours.

2.4 Running soil solution through $AgNO_3$ infused filter papers

Create a humid environment in a wooden box or cupboard, using a container of boiling water or humidifier. It is essential to be able to see into the box or cupboard when it is closed. Carefully decant 10-15mL of the sodium hydroxide digest solution into a small petri dish, ensuring no soil is transferred, and put the petri dish in a larger one (as with Section **Error! Reference source not found.** above) and this is placed in the humid environment. Insert a wick into a dried filter paper which has been infused with silver nitrate, and place this over the large petri dish, ensuring the wick is in the sodium hydroxide solution (Figure 2.2 & Figure 2.4). Allow the digest solution to spread until it is 1-2mm short of the 6cm mark, the remove the filter paper from the petri dish, discard the wick, and place the filter paper on clean paper to dry overnight. When dry, attach the chromatogram to a south facing window (southern hemisphere) to develop for seven to 10 days.

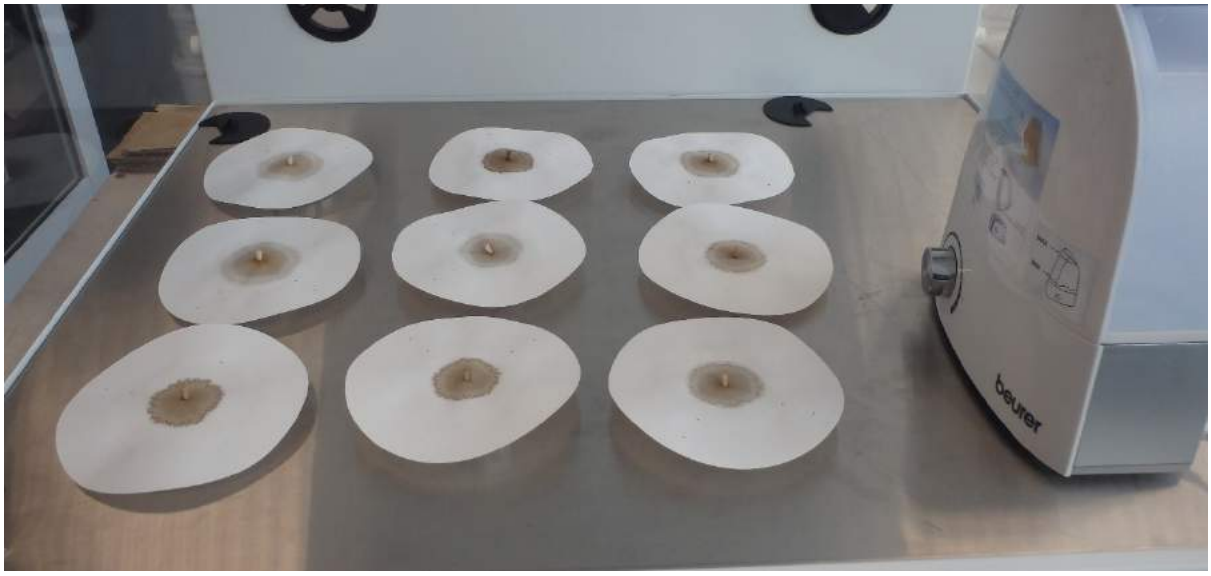

Figure 2.4. Prepared filter papers absorbing the sodium hydroxide digest solution.

3 Description of patterns produced

3.1 Regions of chromatograms

This 'pictograph' of what's happening in the soil, termed a 'chromatogram', is thought to vary in form, colour, and pattern, depending on the quality of the soil. The circular image generally has three distinct zones (inner, median and outer regions) (Figure 3.1). The inner region is thought to reflect mineral content, the median region, the presence of organic carbon and organic matter, and the outer region, humus content (Pfeiffer, 1984; Khemani *et al.*, 2008). The presence of separate zones with little irregularities or 'interactions' is thought to reveal poorer quality soils, while more complex patterns are thought to be indicative of better quality soils (Follador, 2015; Kokornaczyk *et al.*, 2017).

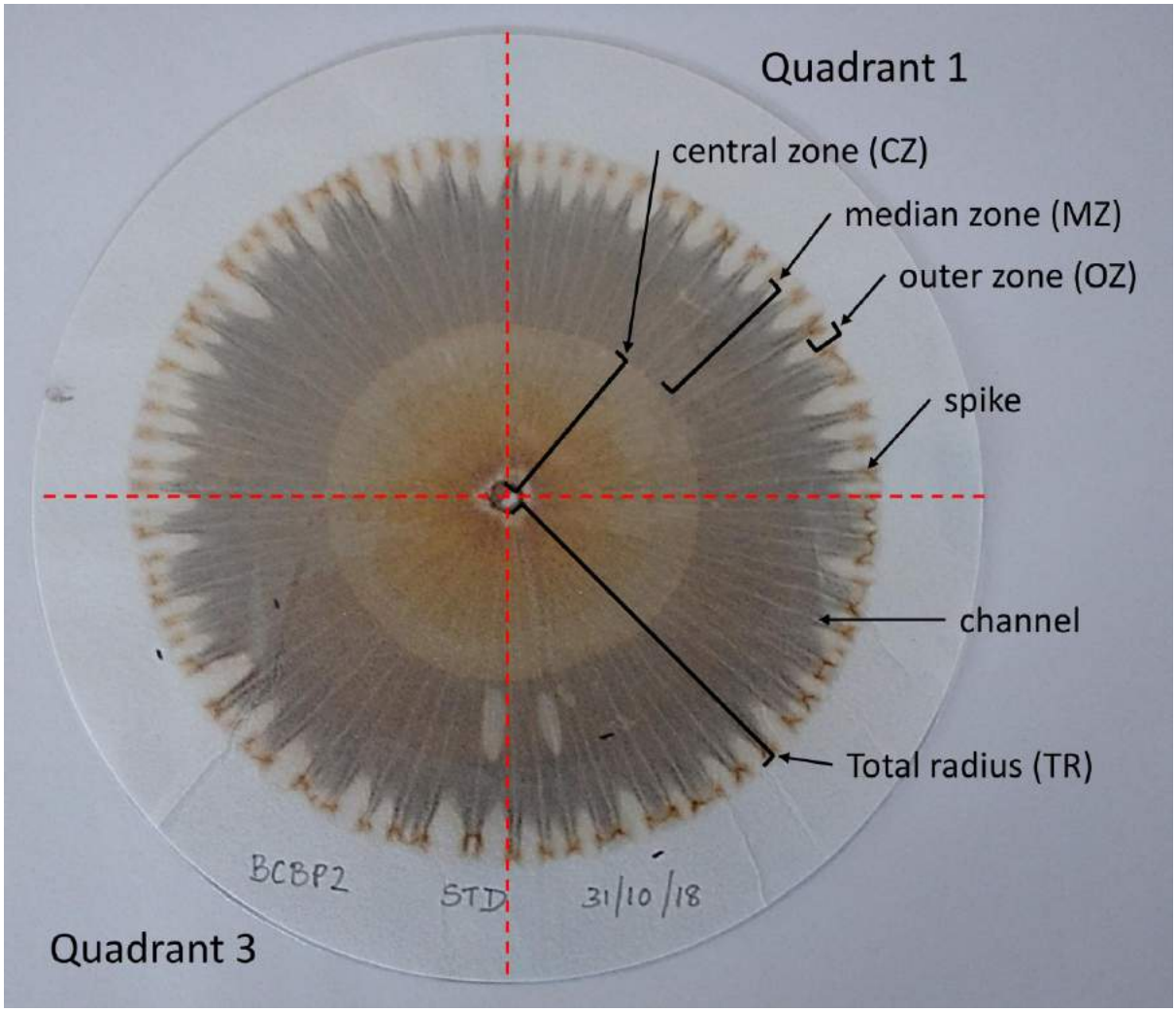

Figure 3.1. Chromatogram displaying quadrants and quantifiable attributes.

3.2 Description of quantifiable parameters

One way of assessing the meaning of the array of forms, colours and patterns would be to develop a set of quantifiable measurements as was done by Kokornaczyk *et al.* (2017) who measured the zones, counted the number of concentric rings and radial features such as channels and spikes, and scored colour intensity. In this way, chromatograms can be quantitatively assessed through a suite of continuous and ordinal variables. Each chromatogram can be divided into 4 quarters and two opposing quarters selected for measurements to be performed upon. Within each of these quarters, four measurements can be made following the methodology proposed by Kokornaczyk *et al.* (2017): total radius (TR) (mm), central zone (CZ) radius (mm), median zone (MZ) breadth (mm), and outer zone (OZ) breadth (mm). In addition, for each quarter, counts can be made of the number of channels, spikes, and concentric rings (within MZ and/or CZ). Channel structure (1=absent, 5=fully developed), development of spikes (1=absent, 5=fully developed), and colour intensity (1=blurred, 5=intense) can be scored for each quarter by the same evaluator to maintain consistency in scoring (Figure 3.1, Table 4.1). Averages can then be taken over measured variables to characterise each chromatogram. Additional variables, such as the summed radii of the median zone and central

zones (MZ+OZ), and comparing this value with the radius of the central zone (CZ:MZ+OZ) are also features which may be considered (Table 4.1).

4 How to interpret chromatograms

4.1 Summary of existing literature

Although the procedure for obtaining a chromatogram is well described, a robust, standardized procedure for interpretation is missing (Khemani *et al.*, 2008), with rigorous evaluation of the effectiveness of soil chromatography for reflecting soil condition limited. Pfeiffer (1984) presented a detailed, descriptive account of chromatograms produced from a variety of soils, but did not evaluate the consistency of the procedure for assessing soil health. This author distinguished the three main zones, and suggested that the width of the outer and middle zones should reflect the amounts of organic matter in the samples. In a 'case based reasoning' approach, Khemani *et al.* (2008) scanned chromatograms and undertook image analyses, and recorded corresponding soil properties in an attempt to evaluate whether quantitative information could be extracted from chromatogram images, but did not report on their results. In a more recent study, Kokornaczyk *et al.* (2017) examined 16 soil samples using paper chromatography and standard chemical analysis. Although based on a limited data set, these authors reported a strong correlation between chromatogram patterns and organic matter content, total nitrogen, assimilable phosphorus and bromine levels. Overall, they concluded that a strong development of radial features such as channels and spikes was indicative of better quality soils, while concentric features such as the number of concentric rings were indicative of soils of poorer quality (Kokornaczyk *et al.*, 2017). This led these authors to conclude that differences in patterns on chromatograms produced using Pfeiffer's circular chromatography (PCC) may be a reliable indicator of soil health. A recent study by Saavedra *et al.* (2018), compared chromatograms produced by soils from various agricultural land use practices. These authors found chromatogram colours to be sensitive to changes in nutrient (nitrogen) loading and general soil health. Changes in chromatogram structural features were also found to represent changes in the relations between microbiology, organic matter, and minerals (Saavedra *et al.*, 2018).

Table 4.1. Description of chromatogram features and parameters measured to quantify variability among chromatograms.

Feature	Parameters measured	What it represents	Abbreviation
Central	Central Zone radius (mm)	Patterns in the central zone inform about the presence of minerals. These are the heaviest contents of the digest to move into the filter paper and are thus move the least distance from the centre of the filter paper.	CZ
Median	Median Zone radius (mm)	Structure indicates the presence of proteins, organic carbon and organic matter (minerals and humus).	MZ
Outer	Outer Zone radius (mm)	"Clouds" at the ends of spikes indicate available nutrients. Bacterial enzyme activity displayed in this zone.	
Total	Total radius (mm)		TR
Combinations	Median + Outer Zone radius (mm)		MZ+OZ
	Central Zone radius: Median + Outer Zone radius		CZ:MZ+OZ
Channels	Channels (1=absent, 5=fully developed)	Greater number of channels suggests increased organic matter and nutrients. Channels extending across zones indicate integration of soil components.	Channels
# channels	Number of channels in quadrant		# channels
Spikes	Spikes (1=absent, 5=fully developed)	Greater number of spikes suggests increased organic matter and nutrients. Well-developed spikes are thought to represent healthy soil.	Spikes
# spikes	Number of spikes in quadrant		# spikes
Colour	Colour intensity (1=blurred, 5=intense)	Warm colours (gold, red, yellow, orange, cream) and/or high colour intensity indicate healthy soil. Colder colours (grey, dark brown, or blueish) suggest soils with less microbial activity.	Colour
Rings	Number of concentric rings	Strong rings indicate possible excess of soluble minerals	Rings

4.2 Interpreting chromatograms for soils from southwestern Australia

In order to realize the full potential of this approach for assessing soil health, chromatograms for soils across known gradients of organic content, microbial activity, pH and salinity were examined to establish a relationship between the form, colour and pattern of these chromatographs and general health of the soils. A total of 361 chromatograms were created from soils sampled from four land uses – pasture (233 soil samples), orchards (52 samples), vegetable gardens (41 samples), and soil from remnant vegetation (35 samples).

4.2.1 Measures of soil health

Sub samples of field moist soil were oven dried (70° C, 24 hrs) and sieved (<2mm). Portions of the soil fine fraction (ie <2mm) were analysed for electrical conductivity (EC) and pH in both water and 0.01M CaCl2 solution (only water results are presented). A further portion of the soil fine fraction was finely ground in a mortar and pestle and analysed for total organic carbon (expressed as percent C per 100g of soil). These analyses were performed according to the Australian standards (Rayment & Lyons, 2011). Soil microbial respiration was measured using the Solvita® soil CO_2 burst test (Haney *et al.*, 2008). This cost effective, rapid method has proved effective in discriminating soils, with microbial activity shown to be significantly correlated with soil organic carbon and microbial abundance (Munoz-Rojas *et al.*, 2016). For this method, 40g of dried soil (< 3% soil moisture) was placed in a 50cc beaker and deionised water added based on the settled volume of the soil until soil was moistened to 50% water-filled pore space. The beaker and a CO_2 probe were placed into a sealed glass jar, and after 24 hours, the probe was removed and inserted into a digital colour reader. Solvita® fertility test colour and level of CO_2-C expressed in mg/kg (ppm) were recorded.

4.2.1.1 Electrical conductivity

Electrical conductivity (EC - µS/cm) of soil samples was measured to represent salinity. As all but one values of EC were in the "Non-saline" category of Hazelton and Murphy (2016), quartiles of the values of EC for the full collection of soil samples were used to create categories of EC relevant for this study. Values below the first quartile were classified as "Very low", and values within the second quartile were classed as "Low". Values falling in the third and fourth quartiles were classed as "High" and "Very high" respectively (Table 4.2).

Table 4.2. Categories and corresponding value ranges of EC (µS/cm).

Category	Range (µS/cm)
Very low	3 – 131
Low	132 - 177
High	178 - 245
Very high	246 +

4.2.1.2 pH

Values of pH were categorised based on the classification of pH presented by Hazelton and Murphy (2016), and classed as either "Very strongly acid", "Strongly acid", "Moderately acid", "Slightly acid", "Slightly alkaline", "Moderately alkaline", "Strongly alkaline", or "Very strongly alkaline" (Table 4.3).

Table 4.3. Categories and corresponding value ranges of pH.

Category	Range
Very strongly acid	<3 – 3.99
Strongly acid	4 – 5.49
Moderately acid	5.5 – 5.99
Slightly acid	6 – 6.99
Slightly alkaline	7 – 7.99
Moderately alkaline	8 – 8.99
Strongly alkaline	9 – 9.99
Very strongly alkaline	10 - 11

4.2.1.3 Total organic carbon

Organic content was characterised through Total organic carbon (TOC %) and classified based on a modified version of classifications of soil carbon content by Griffin *et al.* (2013) and Hazelton and Murphy (2016). This classification was modified as nearly all TOC values were found to be in the "Very high" and "Extremely high" categories of Griffin *et al.* (2013) and Hazelton and Murphy (2016). For the purposes of this study, TOC was classified into seven categories, being "Very low", "Low", "Moderate", "High", "Very high 1", "Very high 2", or "Extremely high" (Table 4.4).

Table 4.4. Categories and corresponding values of TOC (%).

Category	Range (%)
Very low	<0.5%
Low	0.5 – 0.99%
Moderate	1 – 1.99%
High	2 – 3.99%
Very high 1	4 – 5.99%
Very high 2	6 – 8%
Extremely high	> 8%

4.2.1.4 CO_2 burst tests

The values of the Solvita® CO_2 burst tests provide information pertaining to biological fertility and soil condition. CO2 burst test results were classed according to the Soil CO_2-Burst official Solvita® instructions (Woods End® Laboratories Inc., Mt. Vernon, ME), see Table 4.5 for details on interpreting values.

Table 4.5. Categories and corresponding attributes of soil for CO2 burst tests. Table modified from Solvita® instruction guide.

Category/Range (ppm)	Biological fertility & soil condition
< 5	Very low in microbes, No N-min potential
< 12	Low biology soil, Very low N-min potential
< 30	Medium – low biology soil, Low N-min potential
< 70	Medium biology soil, Some N-min potential
< 165	High biology soil, Strong N-min potential
< 400	Very high biology soil, High N-min potential

4.2.2 Testing chromatogram characteristics against soil health

To test for variability in chromatogram variables explained by soil health variables, Kruskal Wallis tests were performed with the continuous chromatogram response variables (CZ, MZ, OZ, MZ+OZ, CZ:MZ+OZ, TR, # channels, and # spikes). Significant Kruskal Wallis results were followed by pairwise Dunn tests with false discovery rate (fdr) adjustment for multiple comparisons. Differences in the ordinal chromatogram variables (Channels, Spikes, Colour, and # rings (included due to low range in number of rings)) among soil variable categories was tested for using Fisher's exact test, with significant results followed by pairwise tests of independence for data with an ordered variable, with fdr adjustment for multiple comparisons. Boxplots were used to visualise the distribution of continuous chromatogram variables across soil variable categories. Ordinal chromatogram variables were visualised through bubble plots, where the size of the bubble represents the proportion of observations across soil variable categories for each level of the ordinal chromatogram variable.

4.2.3 Results

4.2.3.1 Soil health variables

Overall, soil health parameters varied considerably over all study sites, with most variables ranging over several categories (Table 4.6). The vast majority of values however, were found within the mid ranges of most categories, the exception being CO_2 burst test results, where a considerable proportion of samples were in the higher categories for microbial activity (Figure 4.1).

Table 4.6. Summary of soil quality variables from all soil samples, and the categories the values reflect.

	mean	SD	min	max	categories
EC (µS/cm)	220.17	214.65	7	2400	Non saline (only one sample >2000)
pH	5.81	0.70	4.2	7.81	Strongly acid – Slightly alkaline
TOC (%)	5.85	2.80	0.78	17.60	Low – Extremely high
CO_2 (ppm)	159.96	94.49	2.30	416	Soil very low in microbes – Unusual High-Biology Soil

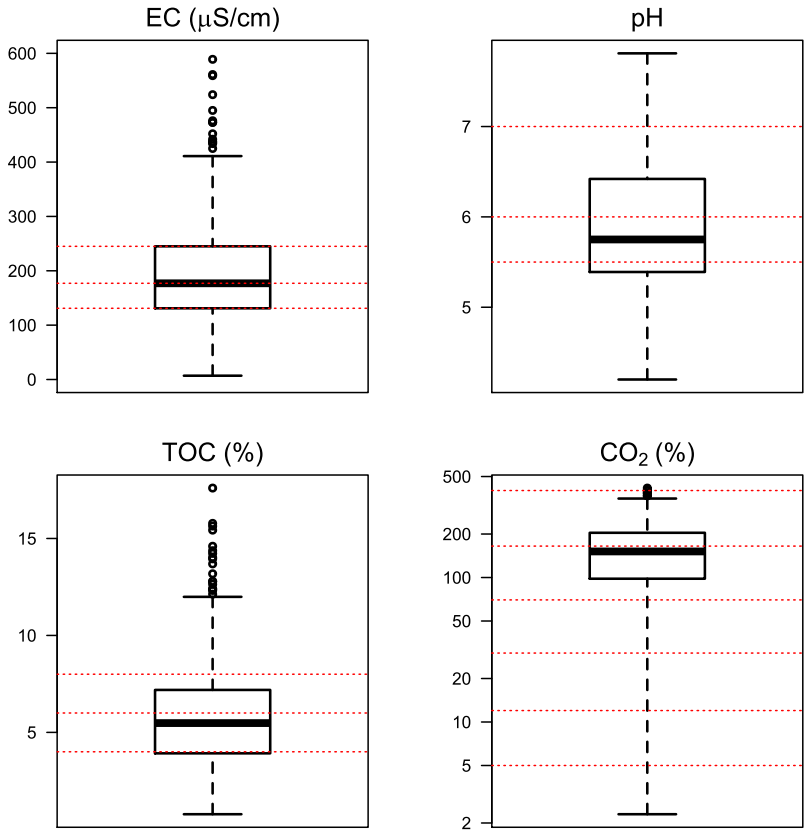

Figure 4.1. Summary of soil health variables measured over all soil types used in this study. Red lines indicate categories used in this study. Note: outliers of EC extend to 2400, with five values above 1000. Y axis for CO_2 burst is log_{10} scale.

4.2.3.2 Chromatogram characteristics

Across all soil types, chromatogram variables typically exhibited less variability than the soil variables (Figure 4.2, Table 4.7). This result, however, may be an artefact arising from what patterns can be produced within a 150mm filter paper, i.e. only a certain number of channels or total radius can be achieved. See Figure 4.3 for examples of ranges in chromatogram variables.

Table 4.7. Summary of chromatogram variables from all soil samples. Mean summarises continuous variables, median summarises count or ordinal variables.

	mean/median	SD	min	max
TR	55.19	3.28	42.90	63.50
CZ	24.82	3.91	15.75	46.85
MZ	22.37	2.73	8.10	30.70
OZ	7.41	1.36	3.45	16.35
MZ+OZ	29.78	2.47	12.30	38.45
CZ:MZ+OZ	0.85	0.24	0.46	3.66
#channels	12.5	2.43	7	20.5
#spikes	12	2.46	4.5	19.5
Channels	2.5	0.70	0.5	4.5
Spikes	2.5	0.70	1	4.5
Colour	2.5	0.62	1	4
#rings	5	0.77	3	8

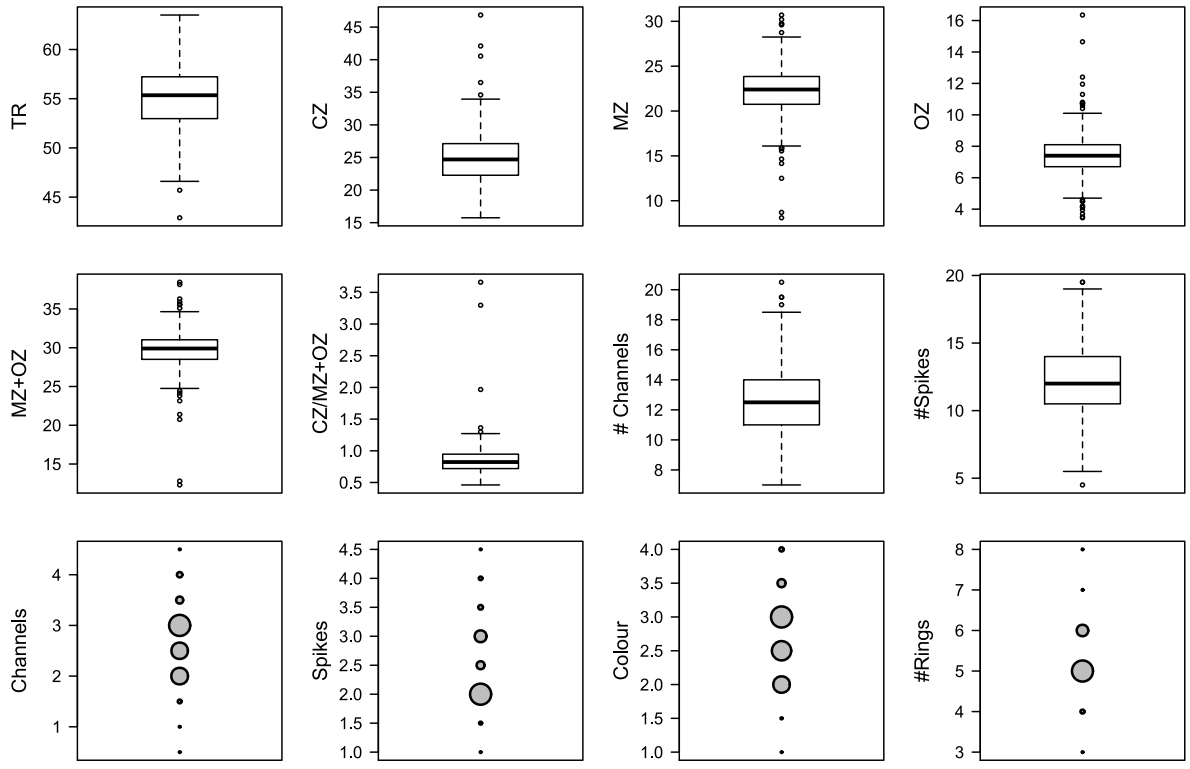

Figure 4.2. Summary of chromatogram variables measured over all soil types used in this study. Chromatogram variable abbreviations are listed in Table 4.1.

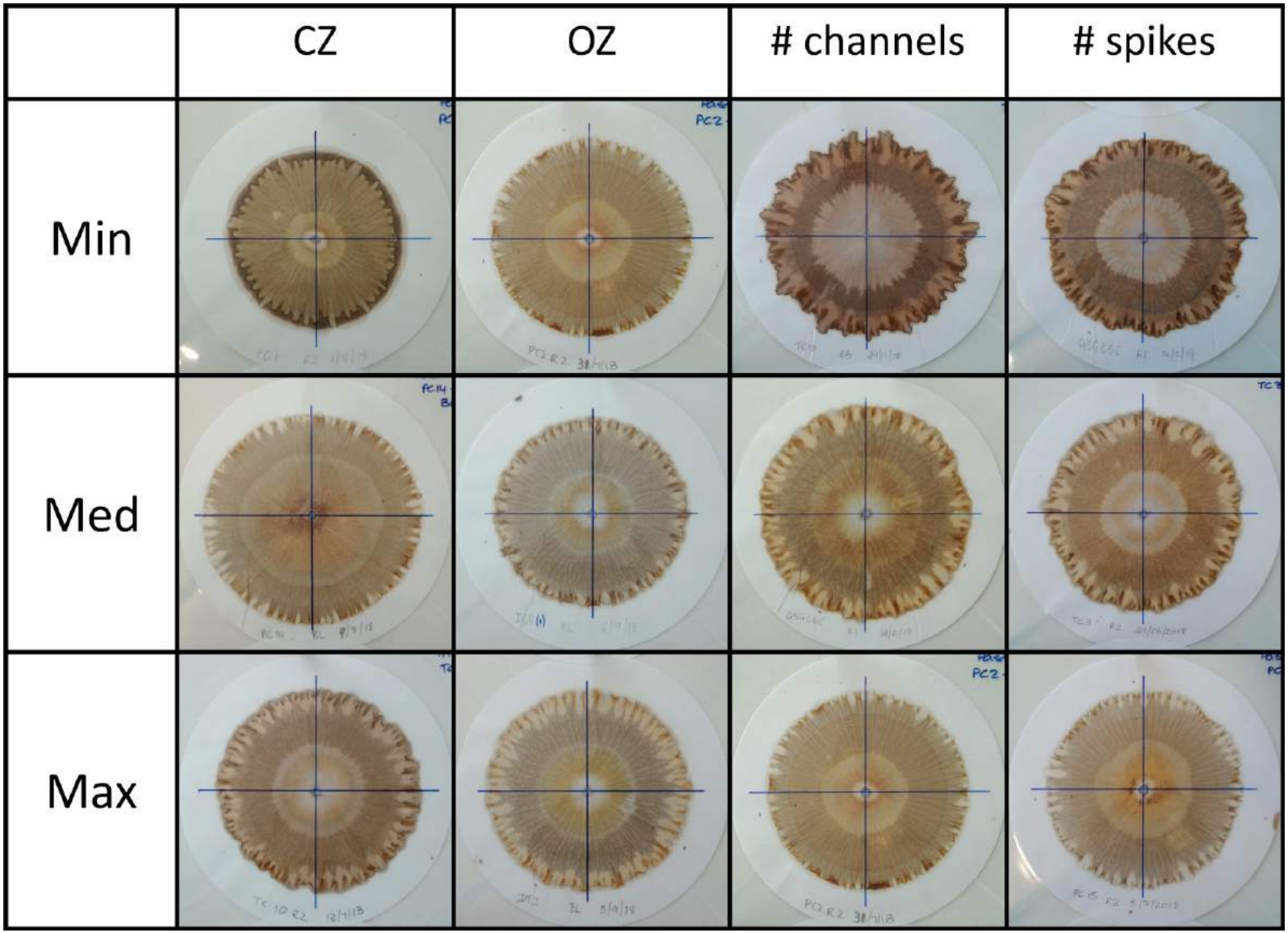

Figure 4.3. Selection of chromatograms displaying minimum, median, and maximum values in CZ, OZ, # channels, and # spikes.

4.2.3.3 Pasture soils

Soils collected from pastures demonstrated considerable ranges in values for each of the soil variables measured, particularly EC and CO_2 (Table 4.8). Features of chromatograms from pasture soils displayed ranges which indicated a sufficient sample size to capture variability for each of the features measured (Table 4.9).

Of the chromatogram variables measured, CZ, CZ:MZ+OZ, # spikes, spikes, and # rings were found to significantly differ among EC categories for pasture soils (Figure 4.4). The chromatogram variables which may be of use in interpreting soil EC are (definition of) spikes, and number of rings. Undefined spikes appear to be associated with "High" and "Very high" EC values, while there is a trend of a high number of rings being associated with "Very low" and "Low" levels of EC and low numbers of rings being associated with "High" and "Very high" levels of EC (Figure 4.4).

With the exception of MZ+OZ, Channels, Colour, and # rings, chromatogram features differed significant differences among pH categories (Figure 4.5). The chromatogram characteristics which appear to have the potential in identifying changes in pH levels in pasture soils include TR, which decreases with decreasing pH, OZ, which increases with decreasing pH, and spike definition, which decreases with decreasing pH. While not significant, colour intensity also displayed a decreasing trend with decreasing pH (Figure 4.5).

Most measured chromatogram characteristics displayed differences among TOC categories for pasture soils, the exceptions being MZ+OZ, CZ:MZ+OZ, and number of rings (Figure 4.6). The chromatogram features most useful in identifying levels of TOC appear to be channel definition (decreases with increasing TOC), spike definition (decreases with increasing TOC), and colour intensity (decreases with increasing TOC). On a coarser level, number of channels and number of spikes also appear to differentiate among "High", "Very high 1", and greater values (Figure 4.6).

Only CZ, OZ, and CZ:MZ+OZ chromatogram features differed significantly among levels of CO_2 burst tests (Figure 4.7). Both CZ and CZ:MZ+OZ displayed weak unimodal relationships with increasing CO_2 burst test results (Figure 4.7), and thus are not useful as a diagnostic of microbial activity in pasture soils in southwestern Australia. Outer Zone radius (OZ) has the potential to distinguish between low (<70) and greater CO_2 burst test results, and channel definition may be able to identify high (<400) levels of CO_2 burst test results, with most chromatograms displaying low channel resolution also having high CO_2 burst test results (Figure 4.7).

Chromatogram features with the potential for use in southwest Australian pasture soil health assessment are summarised in Table 4.10.

Table 4.8. Summary of soil quality variables from pasture samples, and the categories the values reflect.

	mean	SD	min	max	category
EC	209.52	150.31	64	161	Very low – Very high
pH	5.68	0.64	4.2	7.81	Strongly acid – Slightly alkaline
TOC	6.30	2.48	0.78	14.02	Low – Extremely high
CO_2	173.16	94.87	2.3	416	Very low soil microbes – Unusually high soil biology

Table 4.9. Summary of chromatogram characteristics from pasture samples. Mean summarises continuous variables, median summarises count or ordinal variables.

	mean/median	SD	min	max
TR	55.42	3.49	46.6	63.5
CZ	25.54	3.66	15.9	36.5
MZ	21.557	2.27	12.5	30.2
OZ	7.64	1.15	3.95	12.4
MZ+OZ	29.20	2.03	20.75	38.15
CZ: MZ+OZ	0.88	0.16	0.51	1.36
#channels	12	2.18	7	19.5
#spikes	11	2.04	4.5	17.5
Channels	2.5	0.64	0.5	4.5
Spikes	2	0.64	1	4
Colour	2.5	0.62	1	4
#rings	5	0.75	3	7

Table 4.10. Summary of chromatogram features able to detect changes in pasture soil health variables. Black ticks indicate significant and/or strong patterns, grey ticks indicate non-significant and/or weak relationships.

	EC	pH	TOC	CO_2
TR		✓		
CZ		✓		
MZ		✓	✓	
OZ		✓	✓	✓
MZ+OZ				
CZ: MZ+OZ	✓	✓		
#channels		✓	✓	
#spikes	✓	✓	✓	
Channels			✓	
Spikes	✓	✓	✓	
Colour	✓	✓	✓	
#rings	✓			

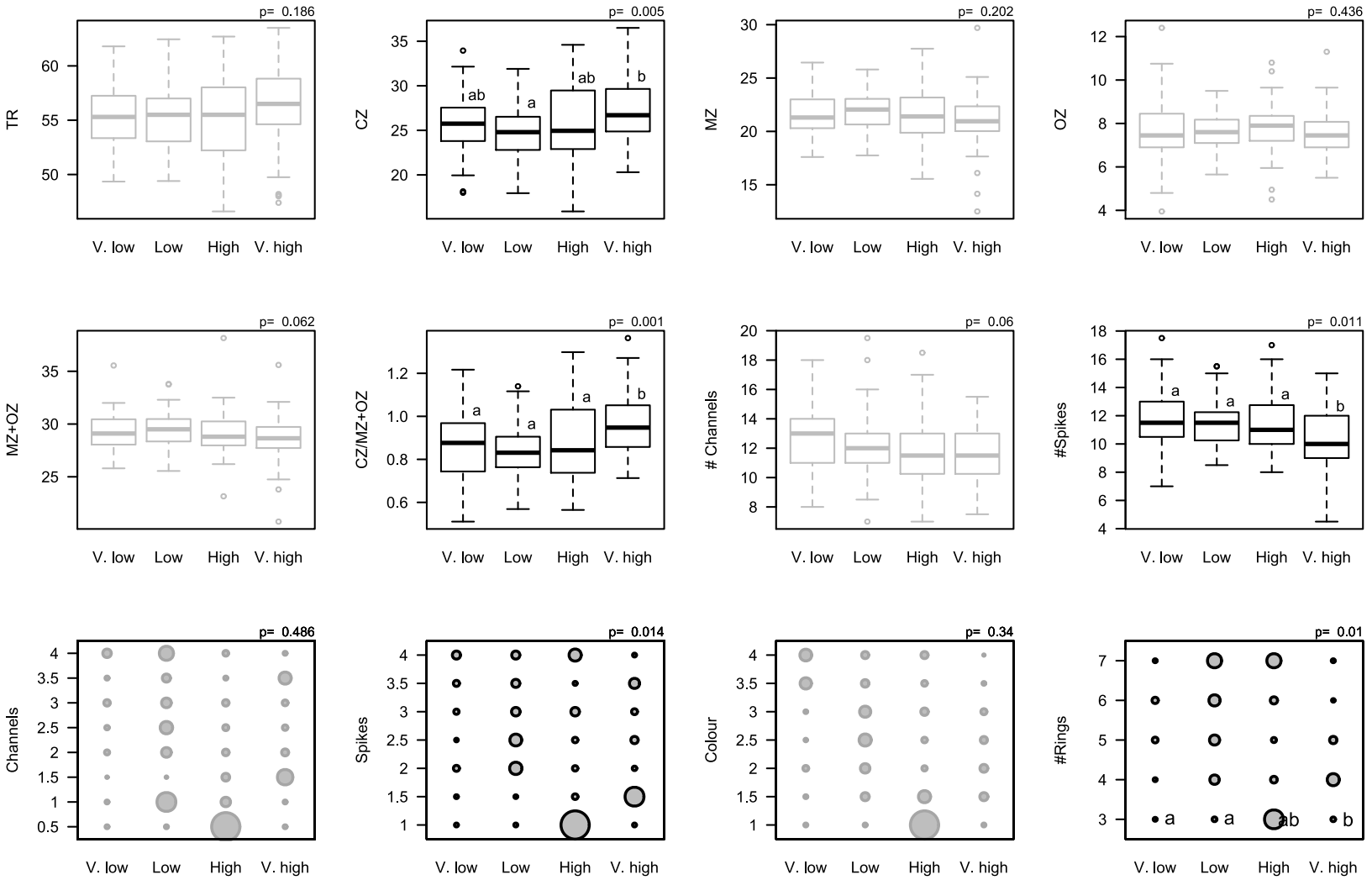

Figure 4.4. Distribution of chromatogram characteristics over soil quality parameters for electrical conductivity (EC) from pasture samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

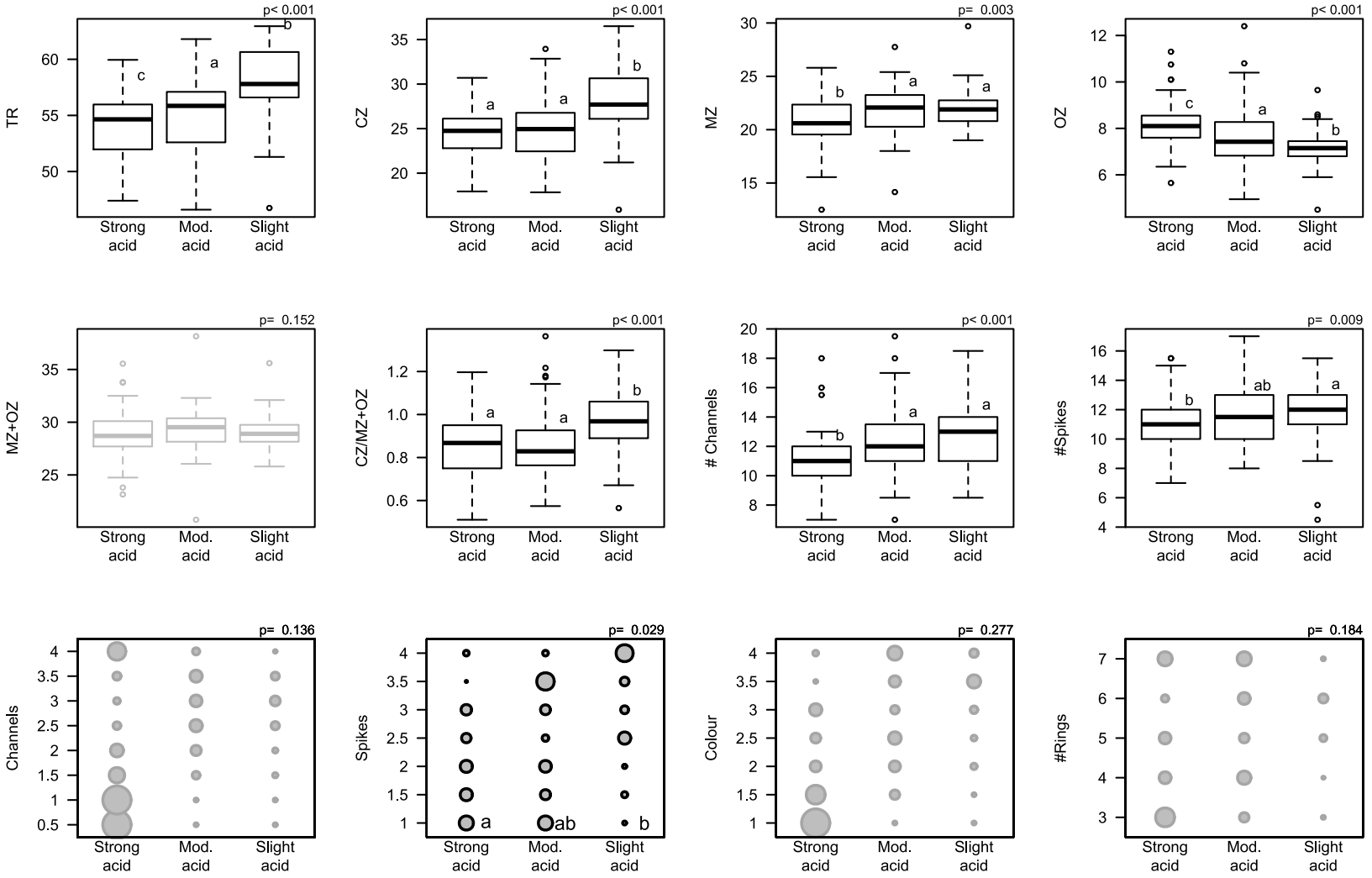

Figure 4.5. Distribution of chromatogram characteristics over soil quality parameters for pH from pasture samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

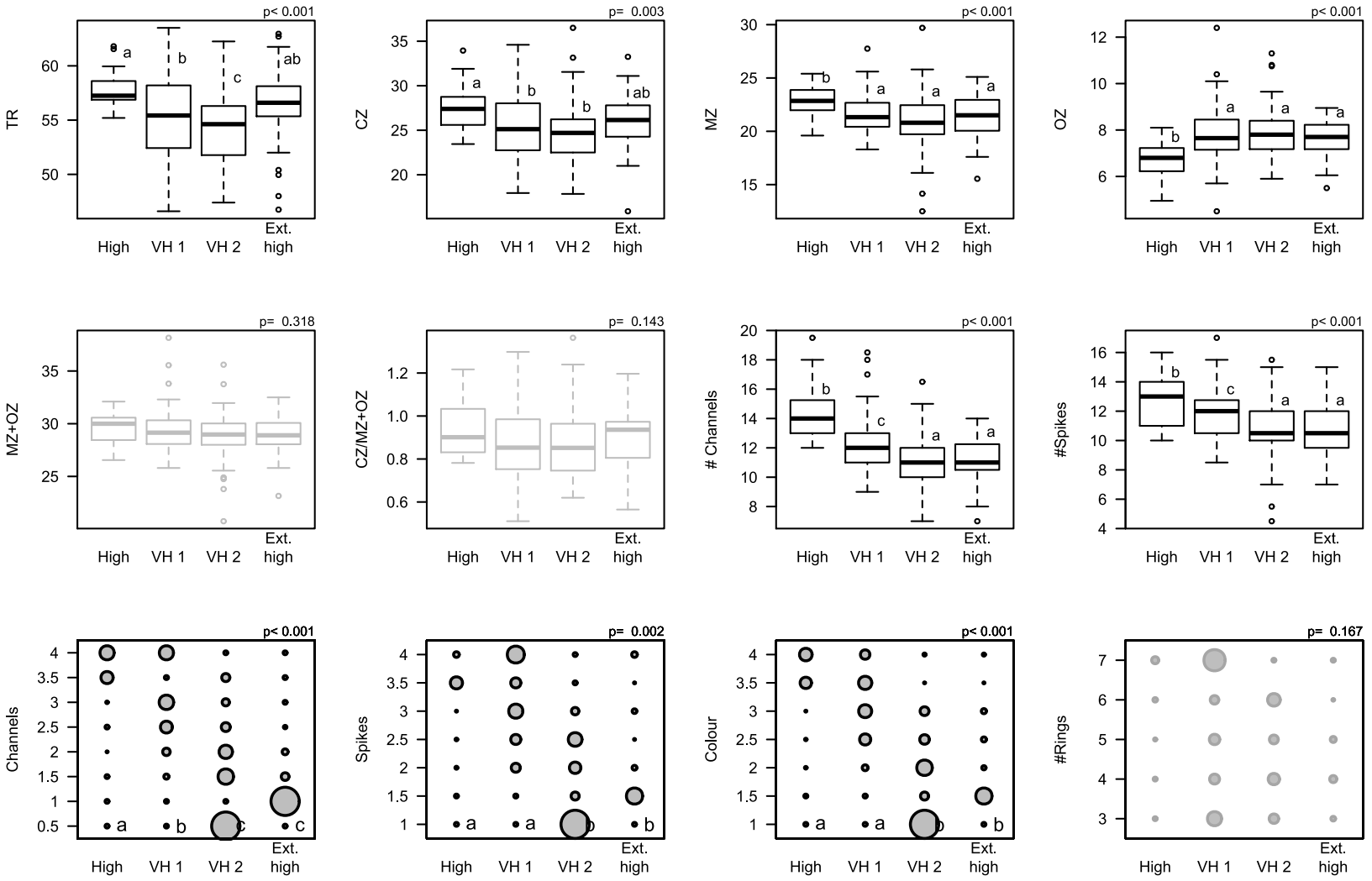

Figure 4.6. Distribution of chromatogram characteristics over soil quality parameters for total organic carbon (TOC) from pasture samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

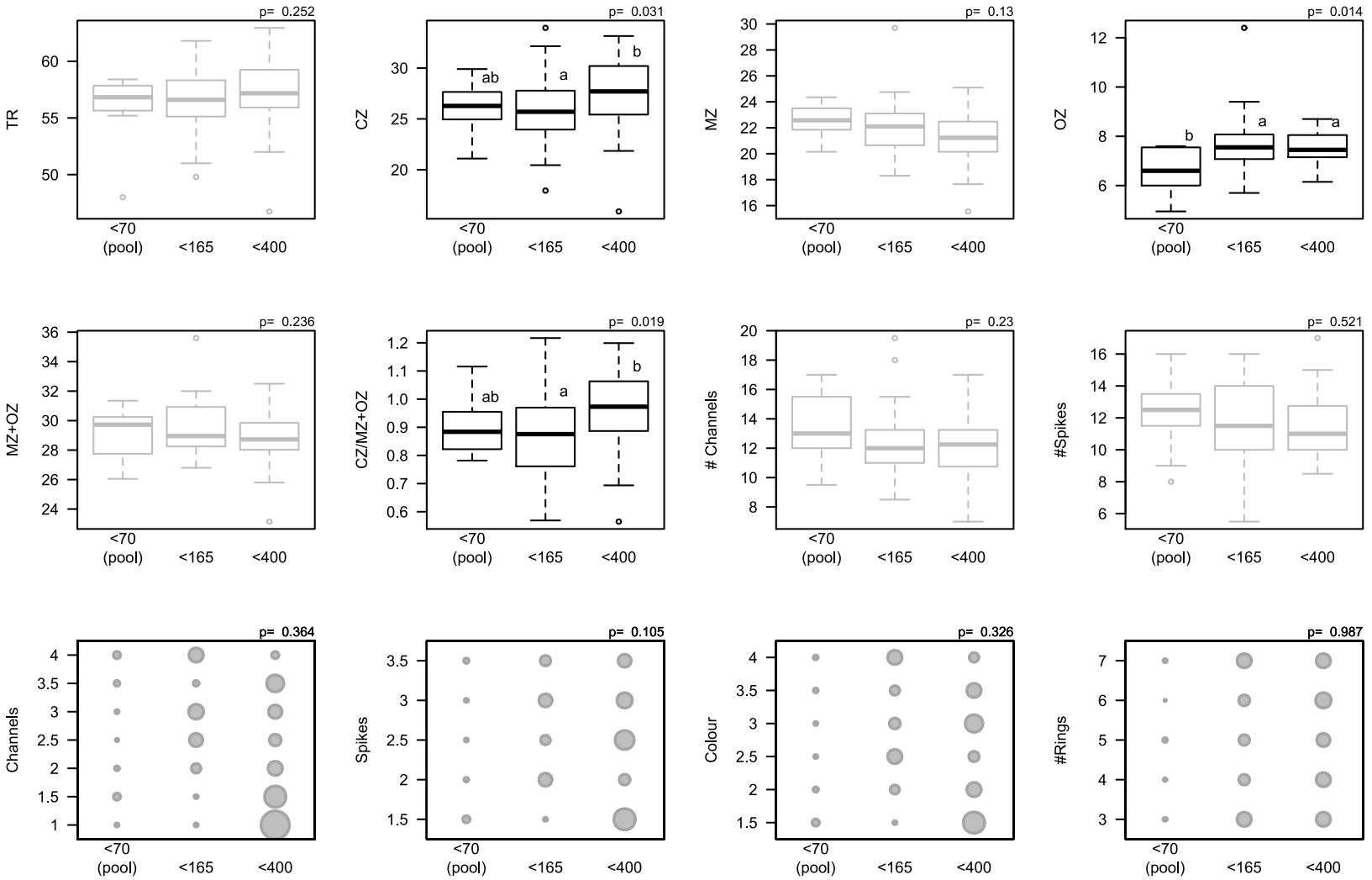

Figure 4.7. Distribution of chromatogram characteristics over soil quality parameters for CO_2 burst tests from pasture samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

4.2.3.4 Orchard soils

Most soil health variables from orchard samples exhibited ranges of values encompassing most categories. The CO_2 burst test results were based on few (five) samples and are not likely to be indicative of actual values or patterns (Table 4.11). Ranges in values of chromatogram features also suggested that the potential range of values had been reasonably observed (Table 4.12).

Most measured features of chromatograms from orchards displayed significant differences among EC categories, the exceptions being MZ, MZ+OZ, spikes, and rings (Figure 4.8). In terms of characteristics useful as an indicator of EC levels in orchard soils, number of channels, number of spikes, channel definition, spike resolution, and colour intensity all are high at "Low" levels of EC, but are not able to distinguish among the higher levels of EC (Figure 4.8).

The features of chromatograms from orchard soils which differed among pH categories were TR, CZ, number of channels, and number of rings (Figure 4.9). In terms of usefulness in assessing soil pH, TR, CZ, and number of channels all increased with decreasing pH, while no strong patterns were evident in the number of rings (Figure 4.9).

Most orchard chromatogram features differed among TOC categories, the exceptions being MZ+OZ, CZ:MZ+OZ, and number of rings (Figure 4.10). To identify levels of TOC in orchard soils, OZ, number of channels, number of spikes, channel definition, spike definition, and colour intensity all appear to be able to discriminate at least between the High and Very high categories (Figure 4.10).

Chromatogram features with the potential for use in southwest Australian orchard soil health assessment are summarised in Table 4.13.

Table 4.11. Summary of soil quality variables from orchard samples, and the categories the values reflect.

	mean	SD	min	max	category
EC	122.00	78.17	45	326	Very low – Very high
pH	5.96	0.83	4.51	7.2	Slightly alkaline - Strongly acid
TOC	3.59	1.93	0.92	8.08	Very low – Extremely high
CO_2	26.20	13.77	10.2	47.8	Very low biology soil – Medium biology soil

Table 4.12. Summary of chromatogram characteristics from orchard samples. Mean summarises continuous variables, median summarises count or ordinal variables.

	mean/median	SD	min	max
TR	54.15	2.27	46.75	59.5
CZ	23.22	3.99	16.3	42.1
MZ	23.87	2.35	14.65	28.75
OZ	6.96	1.17	4.6	9.55
MZ+OZ	30.83	1.91	21.4	33.55
CZ: MZ+OZ	0.76	0.21	0.55	1.967
#channels	14.5	3.09	7.5	19.5
#spikes	14	3.07	7	19.5
Channels	3	0.60	1.5	4
Spikes	2	0.52	1.5	4
Colour	2.5	0.54	1.5	4
#rings	5	0.67	4	6

Table 4.13. Summary of chromatogram features able to detect changes in orchard soil health variables. Black ticks indicate significant and/or strong patterns, grey ticks indicate non-significant and/or weak relationships.

	EC	pH	TOC
TR		✓	
CZ		✓	
MZ			✓
OZ			✓
MZ+OZ			
CZ: MZ+OZ			
#channels	✓	✓	✓
#spikes	✓		✓
Channels	✓		✓
Spikes			✓
Colour			✓
#rings			

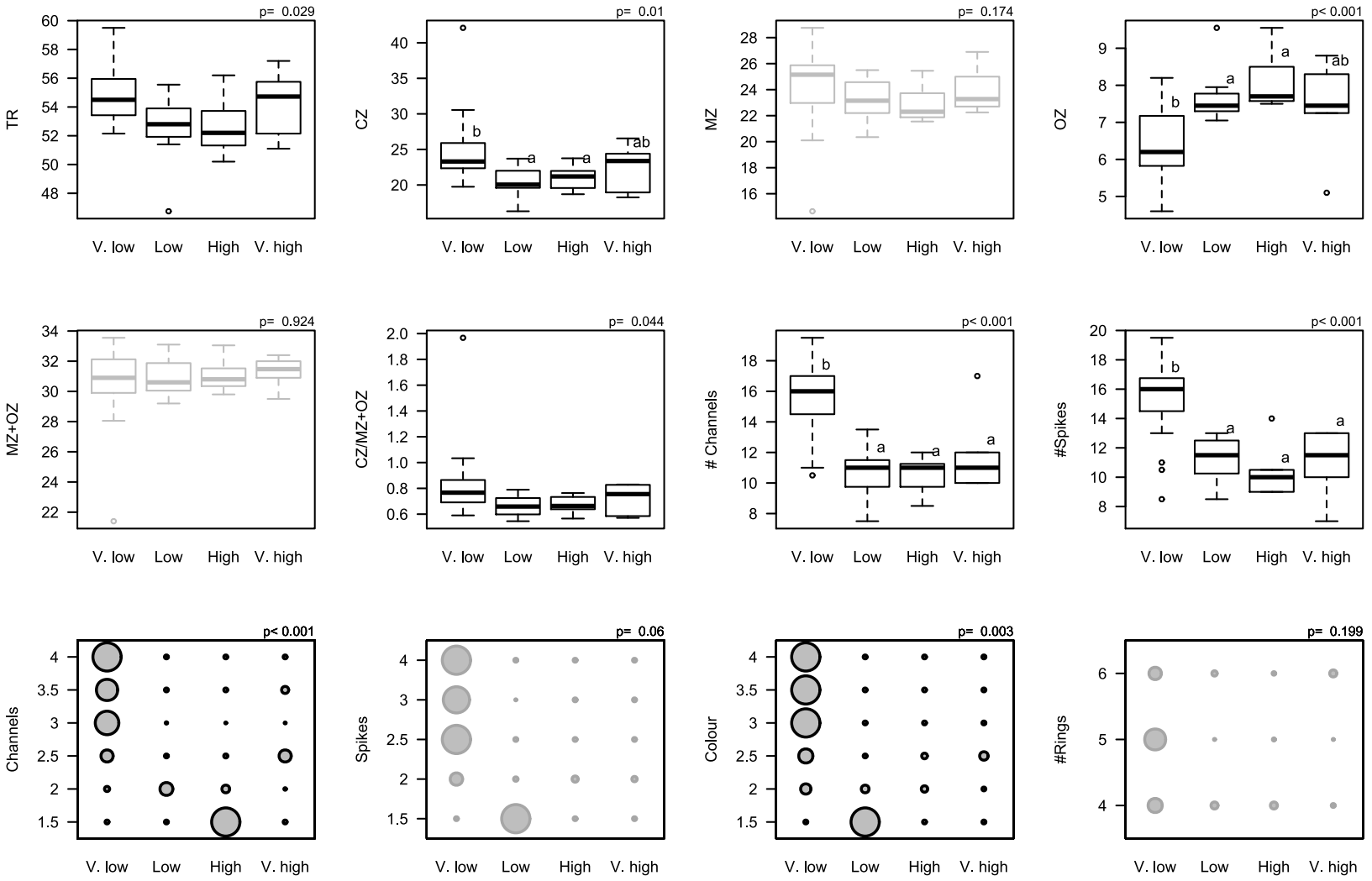

Figure 4.8. Distribution of chromatogram characteristics over soil quality parameters for electrical conductivity (EC) from orchard samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

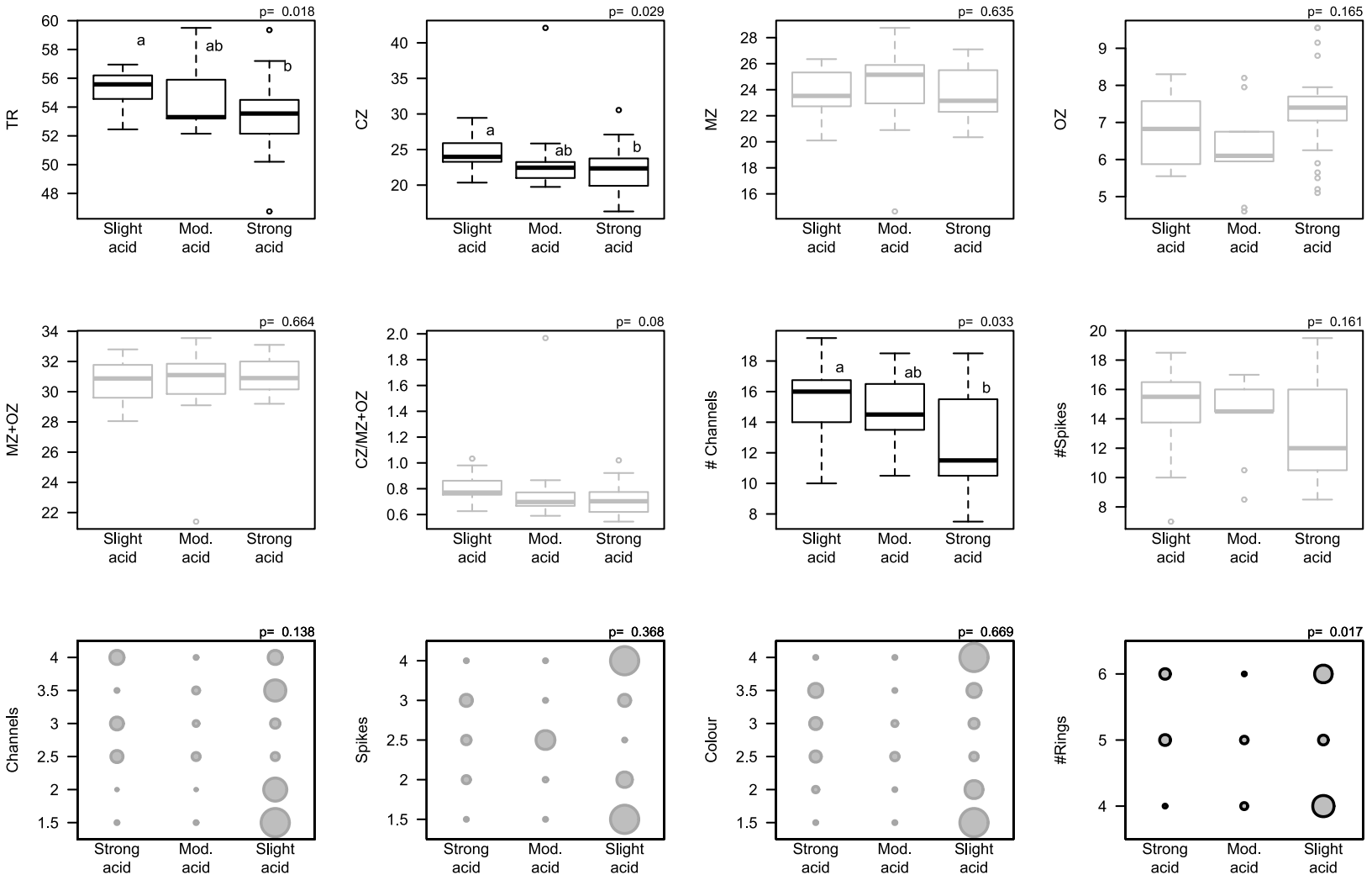

Figure 4.9. Distribution of chromatogram characteristics over soil quality parameters for pH from orchard samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

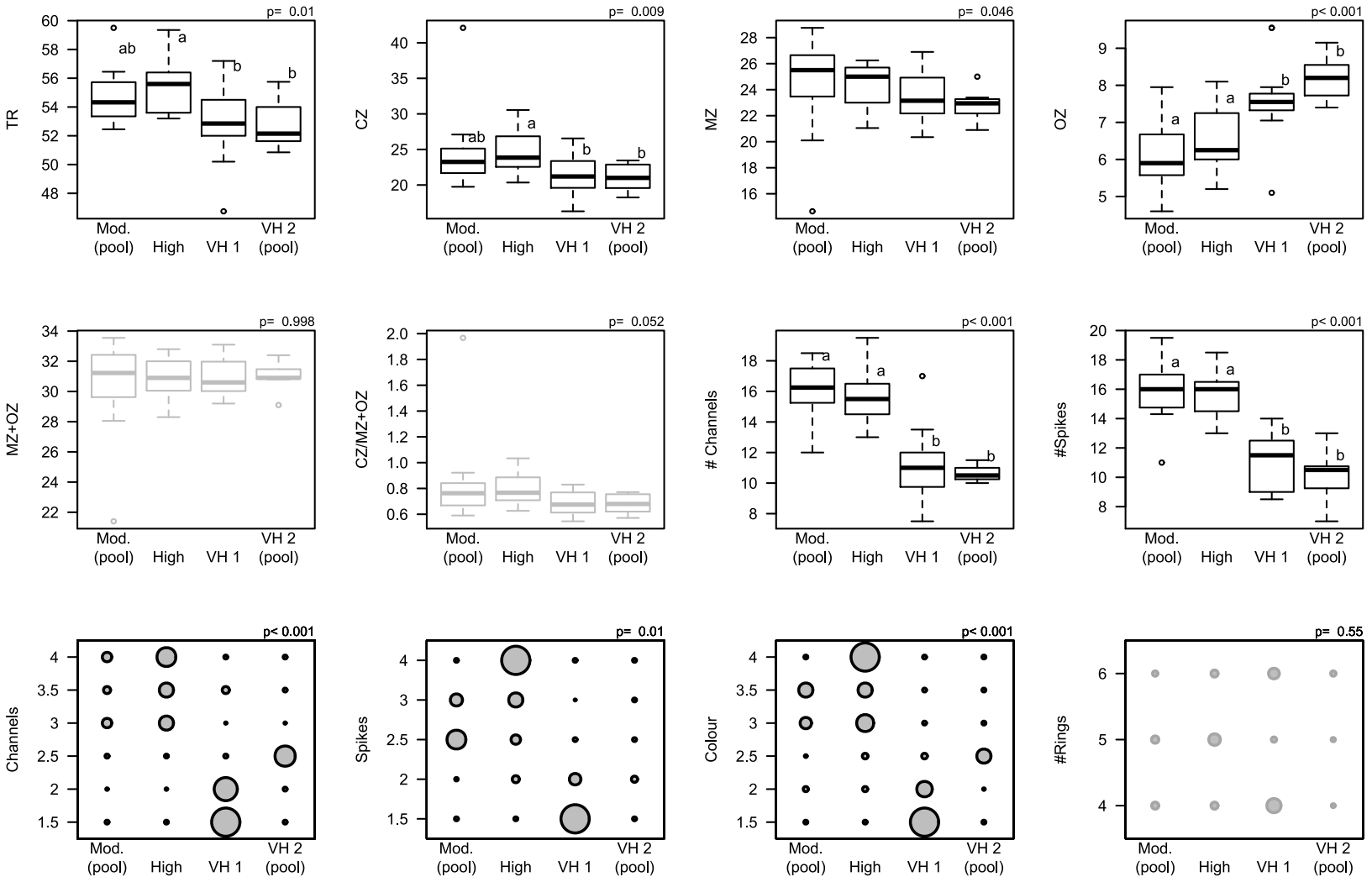

Figure 4.10. Distribution of chromatogram characteristics over soil quality parameters for total organic carbon (TOC) from orchard samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

4.2.3.5 Garden soils

Soils from gardens displayed a relatively reduced range in pH values, and the maximum value of EC (1696 µS/cm) was one of three anomalous values for this soil variable. Both TOC and CO_2 burst test results displayed ranges over several categories, although the CO_2 burst test results did not have values in the lower categories, which could be expected of soils in vegetable gardens (Table 4.14). Chromatogram features from garden soils displayed a range of values indicating sufficient variability among chromatograms to detect any changes in features due to gradients in soil health variables (Table 4.15).

Only MZ+OZ and channel definition from chromatograms from garden soils were found to significantly differ across EC categories (Figure 4.11). While MZ+OZ demonstrated a loose unimodal pattern with increasing EC, channel definition tended to increase with increasing EC. Other chromatogram features which may have potential in assessing EC levels in garden soils are CZ, CZ:MZ+OZ, spike definition, and colour intensity, although these variables were not found to differ over EC categories (Figure 4.11).

Only MZ, channel definition, and colour intensity were found to differ across pH categories for garden soils, although none of these variables demonstrated a pattern conducive to assessing soil health (Figure 4.12). This result may be an artefact of the low number of samples in the "Slight alkaline" (three samples) and "Mod. acid" categories (five samples). Although not significant, TR, MZ+OZ, and number of channels show potential as pH indicators for garden soils, possibly requiring more samples to be useful (Figure 4.12).

Low sample numbers required the pooling of data to assess chromatogram applicability for TOC assessment in garden soils. This resulted in a low number of categories, and only spike definition differed between TOC categories (Figure 4.13). The pattern observed for spike definition across TOC categories was not useful for assessment of TOC in garden soils.

Chromatogram features with the potential for use in southwest Australian garden soil health assessment are summarised in Table 4.16.

Table 4.14. Summary of soil quality variables from garden samples, and the categories the values reflect.

	mean	SD	min	max	category
EC	311.66	325.99	46	1696	Very low – Very high
pH	6.62	0.31	5.95	7.09	Moderately acid - Slightly alkaline
TOC	3.97	1.54	0.92	9.13	Very low – Extremely high
CO_2	73.93	39.04	12.2	128	Medium/low biology soil – High biology soil

Table 4.15. Summary of chromatogram characteristics from garden samples. Mean summarises continuous variables, median summarises count or ordinal variables.

	mean/median	SD	min	max
TR	54.04	2.85	45.7	63.4
CZ	22.78	3.03	15.75	29.55
MZ	23.52	2.19	17.6	27.9
OZ	7.33	1.61	3.45	14.65
MZ+OZ	30.85	1.94	25.95	35.9
CZ: MZ+OZ	0.74	0.13	0.46	1.14
#channels	13	1.86	10.5	20.5
#spikes	13	1.32	11	16.5
Channels	3	0.63	2	4
Spikes	2.5	0.80	2	4
Colour	2.5	0.60	2	4
#rings	5	0.90	4	8

Table 4.16. Summary of chromatogram features able to detect changes in garden soil health variables. Black ticks indicate significant and/or strong patterns, grey ticks indicate non-significant and/or weak relationships.

	EC	pH	TOC
TR		✓	
CZ	✓		
MZ		✓	
OZ			
MZ+OZ		✓	
CZ: MZ+OZ	✓		
#channels		✓	
#spikes			
Channels	✓		
Spikes	✓		
Colour	✓		
#rings			

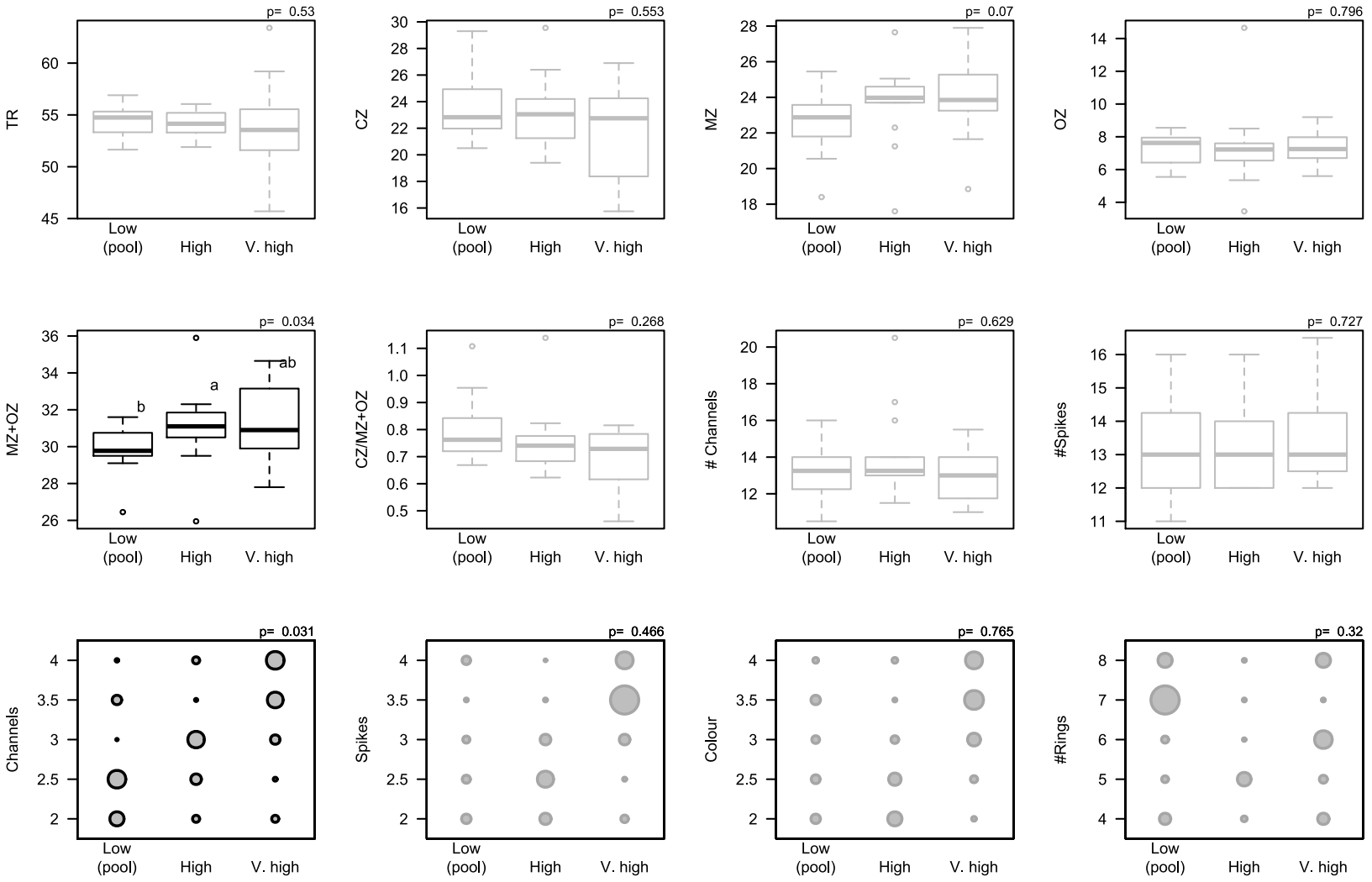

Figure 4.11. Distribution of chromatogram characteristics over soil quality parameters for electrical conductivity (EC) from garden samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

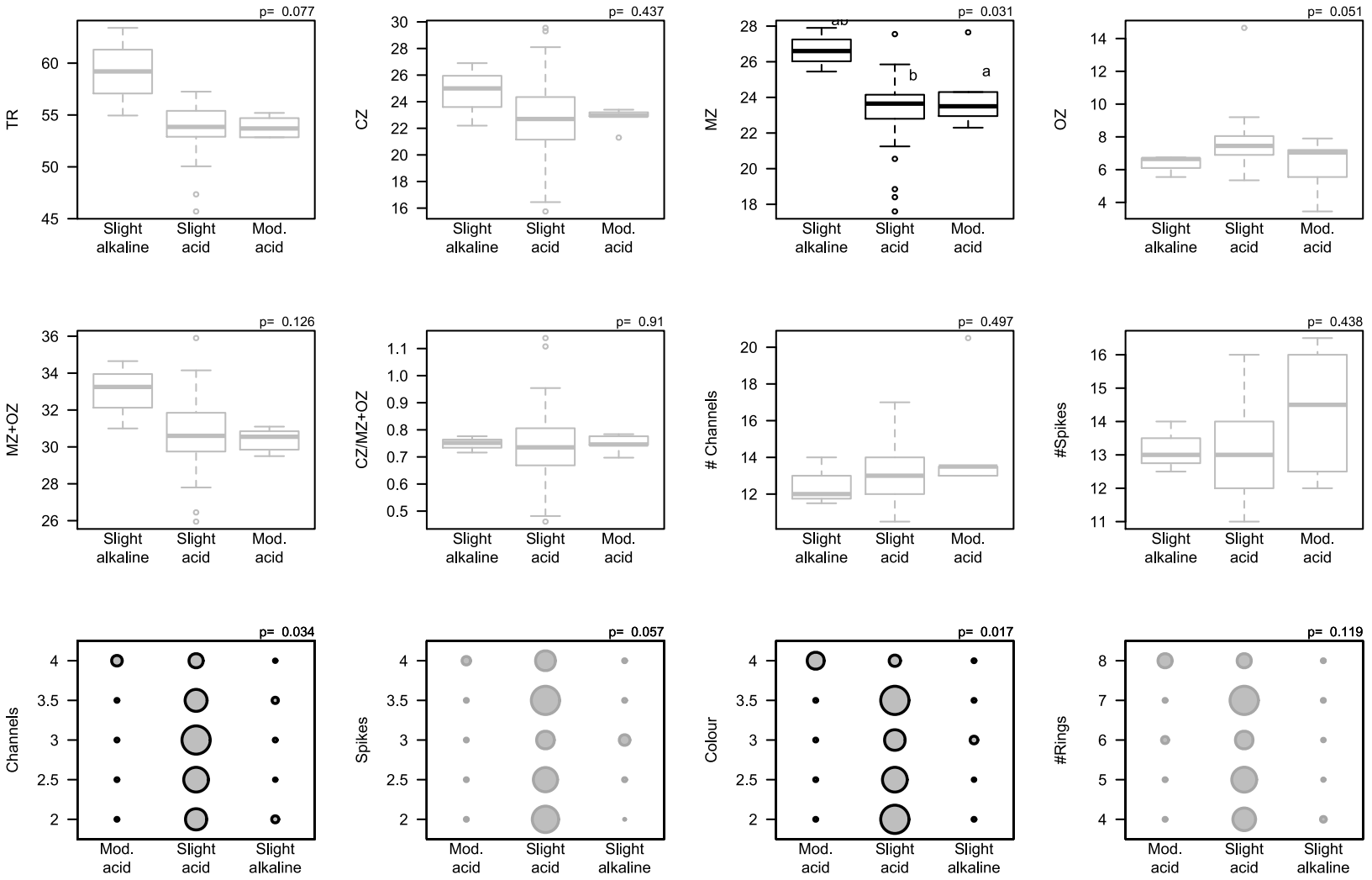

Figure 4.12. Distribution of chromatogram characteristics over soil quality parameters for pH from garden samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups. NOTE: "Slight alkaline" & "Mod. Acid" comprised few samples.

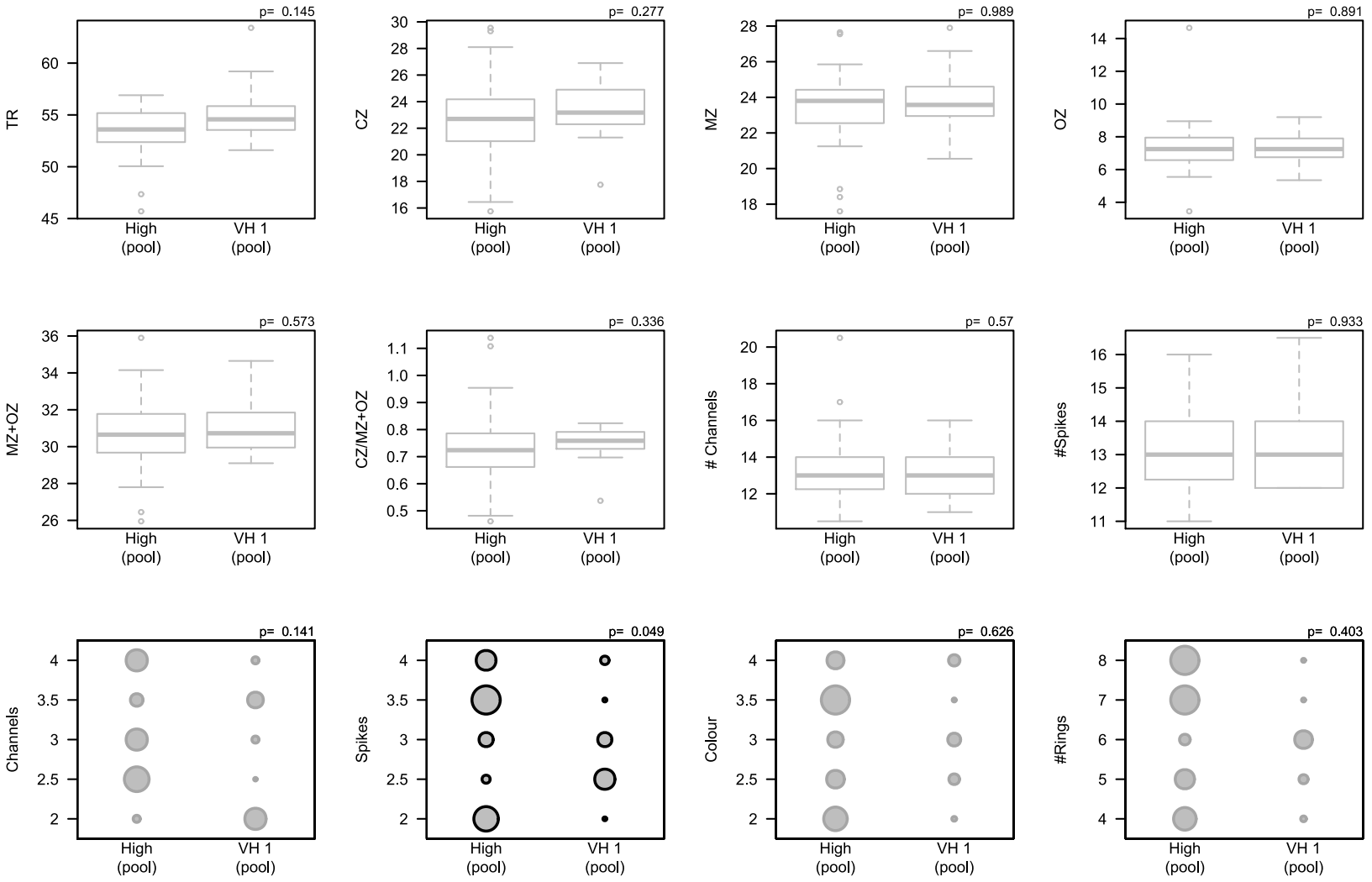

Figure 4.13. Distribution of chromatogram characteristics over soil quality parameters for total organic carbon (TOC) from garden samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

4.2.3.6 Remnant soils

Soil variables from remnant vegetation soils were typically similar to those from the other land use samples. The CO_2 burst test results, however, were higher than other land uses, in particular orchards and gardens (Table 4.17). Except for "CZ" and "MZ", features of chromatograms from remnant vegetation soils also demonstrated ranges in their values. Both "CZ" and "MZ" had restricted ranges, with their maximum and minimum values respectively being one of two anomalous values (Table 4.18).

Chromatogram features OZ, number of channels, number of spikes, and spike definition were found to differ across EC categories for chromatograms created from remnant vegetation soils (Figure 4.14). Both number of channels and number of spikes are able to discriminate very low EC levels from higher levels, and spike definition shows a relatively strong decreasing pattern with increasing EC. Although OZ did not differ among EC groups after adjustment for multiple comparisons, it displays promise as a potential indicator of EC levels in remnant vegetation soils. Channel definition and colour intensity also show promise as potential indicators of EC in remnant vegetation soils (Figure 4.14).

No features of chromatograms from remnant vegetation soils were found to differ over pH categories, and no trends are apparent (Figure 4.15).

Significant changes in MZ, OZ, MZ+OZ, number of channels, number of spikes, channel definition, and spike definition were identified over TOC categories for chromatograms from remnant vegetation soils (Figure 4.16). Of these, number of channels and number of spikes are able to differentiate the "High" category of TOC from the higher levels. While pairwise comparisons did not detect significant differences among categories of TOC, both channel and spike definition show trends of decreasing definition with increasing TOC levels (Figure 4.16).

No features of chromatograms from remnant vegetation soils were found to differ over CO_2 burst test result categories, possibly due to low sample numbers (Figure 4.17).

Chromatogram features with the potential for use in southwest Australian remnant vegetation soil health assessment are summarised in Table 4.19.

Table 4.17. Summary of soil quality variables from remnant vegetation samples, and the categories the values reflect.

	mean	SD	min	max	category
EC	185.91	211.09	7	949	Very low – Very high
pH	5.51	0.82	4.46	6.76	Strongly acid – Slightly acid
TOC	5.80	2.85	1.3	11.34	Moderate – Extremely high
CO_2	195.14	61.23	86.3	268	High biology soil – Very high biology soil

Table 4.18. Summary of chromatogram characteristics from remnant vegetation samples. Mean summarises continuous variables, median summarises count or ordinal variables.

	mean/median	SD	min	max
TR	57.80	2.33	50.05	61.4
CZ	27.06	4.88	21.55	46.85
MZ	23.92	4.52	8.1	30.7
OZ	6.12	1.21	3.5	8.25
MZ+OZ	30.03	4.81	12.3	36.3
CZ: MZ+OZ	0.99	0.63	0.60	3.66
#channels	14.5	2.52	8	18.5
#spikes	13.5	2.40	7.5	18
Channels	3.5	0.78	1.5	4.5
Spikes	3.5	0.75	1.5	4.5
Colour	3.5	0.55	2	4
#rings	5	0.85	3	7

Table 4.19. Summary of chromatogram features able to detect changes in remnant vegetation soil health variables. Black ticks indicate significant and/or strong patterns, grey ticks indicate non-significant and/or weak relationships.

	EC	pH	TOC	CO_2
TR				
CZ				
MZ				
OZ	✓			
MZ+OZ				
CZ: MZ+OZ				
#channels	✓		✓	
#spikes	✓		✓	
Channels	✓		✓	
Spikes	✓		✓	
Colour	✓			
#rings				

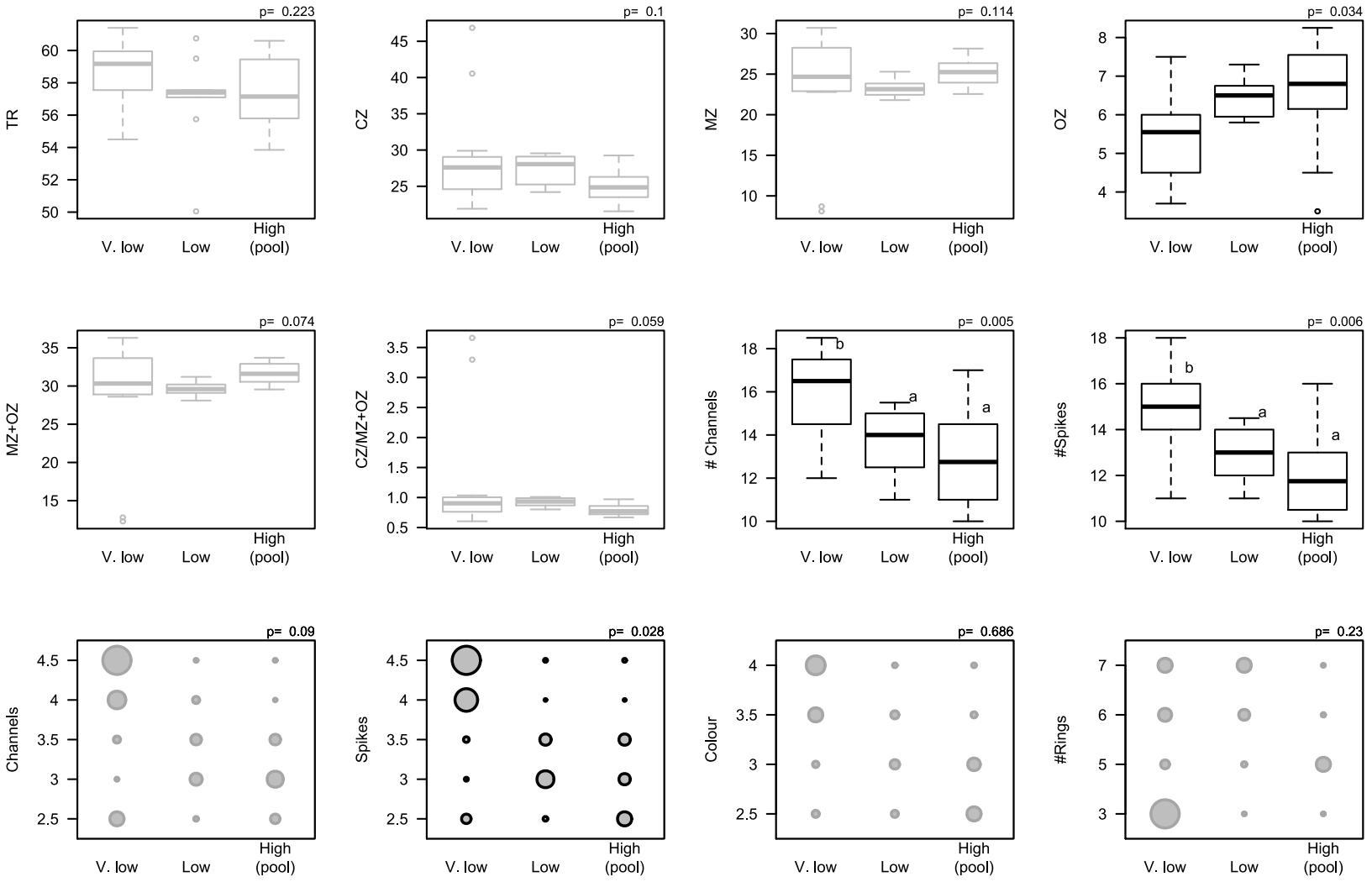

Figure 4.14. Distribution of chromatogram characteristics over soil quality parameters for electrical conductivity (EC) from remnant vegetation samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

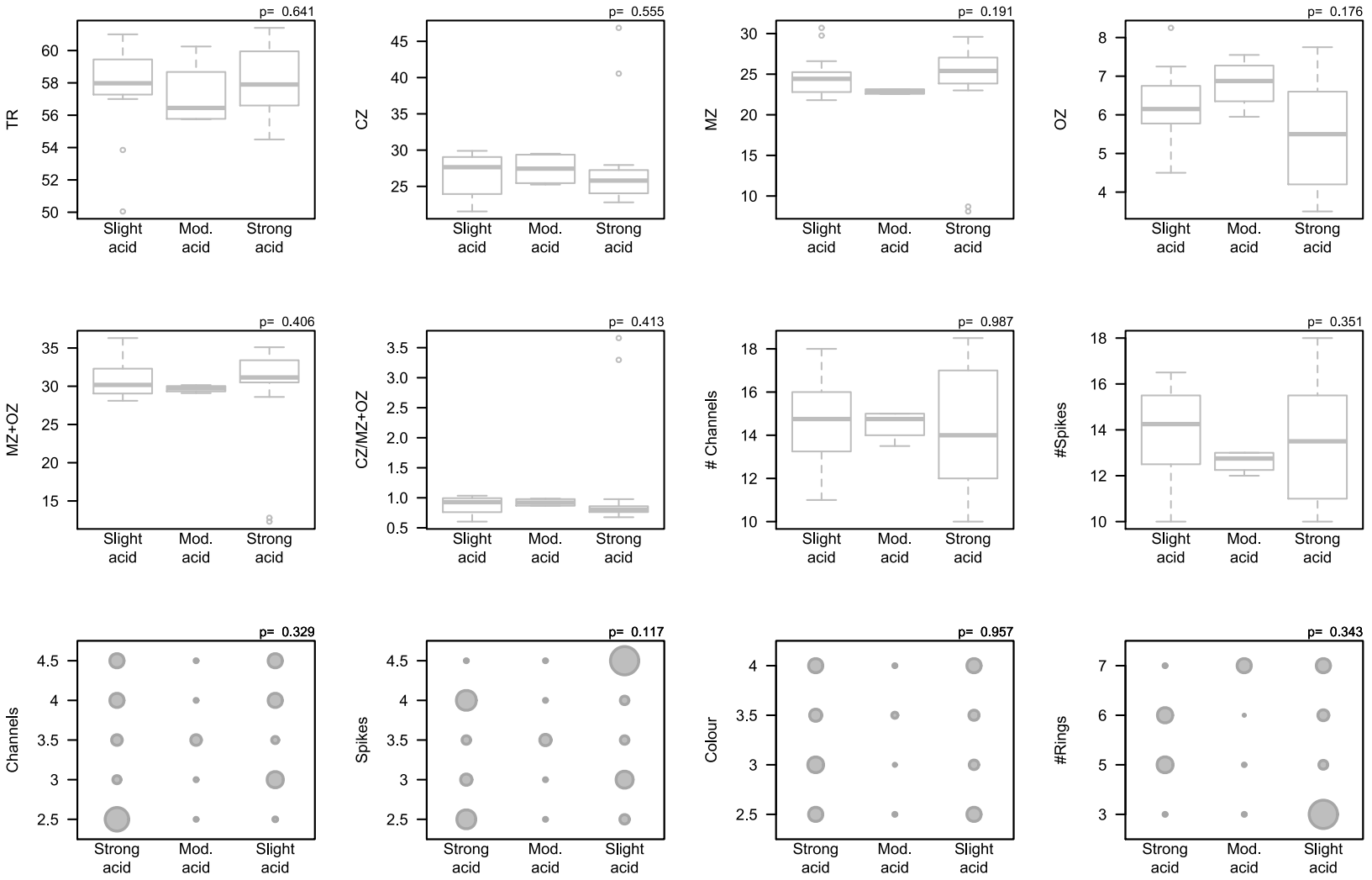

Figure 4.15. Distribution of chromatogram characteristics over soil quality parameters for pH from remnant vegetation samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

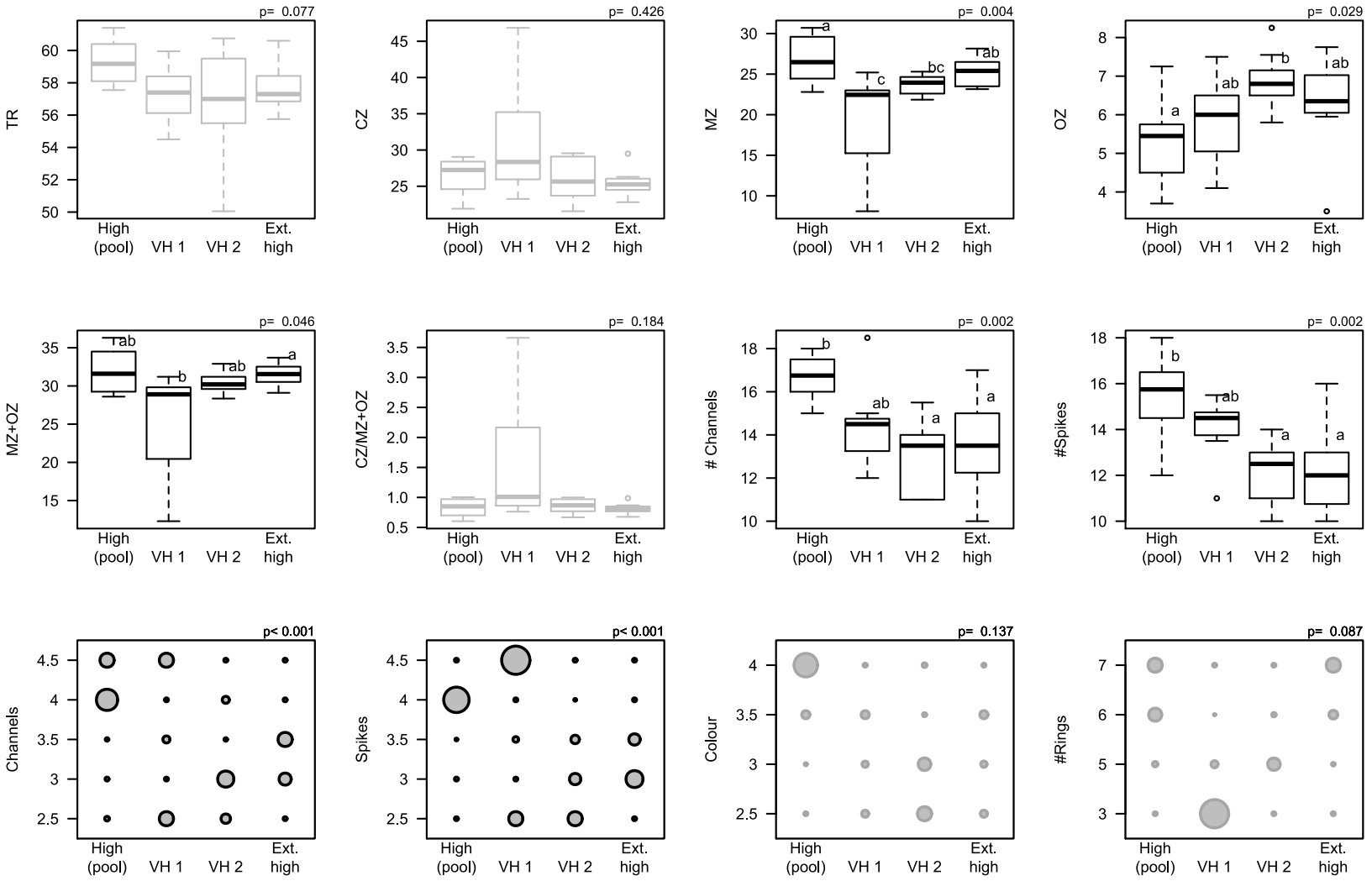

Figure 4.16. Distribution of chromatogram characteristics over soil quality parameters for total organic carbon (TOC) from remnant vegetation samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

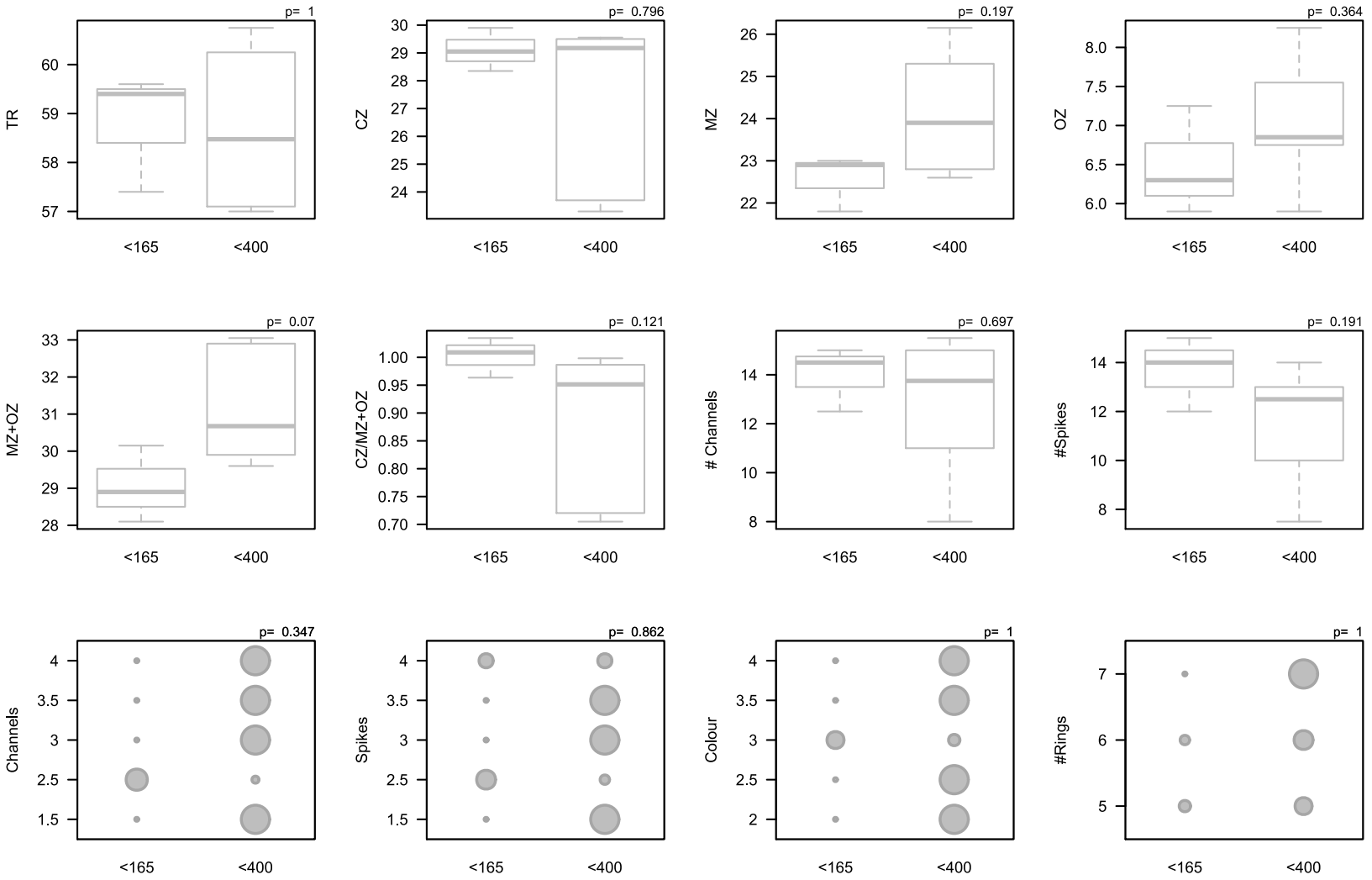

Figure 4.17. Distribution of chromatogram characteristics over soil quality parameters for CO_2 burst tests from remnant vegetation samples. Black box and circle borders represent significant differences in chromatogram characteristic among categories. Lower case letters indicate significant groups.

4.2.4 Conclusions

This study demonstrated that chromatograms can be used to assess soil health in southwestern Australia to some extent. Not all chromatogram features displayed consistent responses to soil variable gradients across differing soils, suggesting context dependent processes. Similarly, responses to gradients in soil health variables were only expressed in features of chromatograms from soils of only one land use type (Figure 4.18).

Chromatogram features appeared to be most sensitive to changes in TOC (Figure 4.18). Of interest is that channel, spike, and colour attributes all decrease with increasing TOC. Higher levels of these chromatogram features are thought to signify increased soil health (Kokornaczyk *et al.*, 2017). It would thus be expected to see an increase in these chromatogram features with increasing TOC. This result may be due to the high levels of TOC (75% of values were greater than 3.92% TOC). The studied cited in the report did not state TOC (%) levels of soils in their studies.

Chromatogram features also showed changes over gradients in soil pH, however these changes were often from only one soil type, or inconsistent among soil types (Figure 4.18). Similarly, chromatogram features showed responses to changes in soil EC, although the direction of change was not always consistent among soil types (Figure 4.18).

The channel, spike, and colour attributes of chromatograms appear to be the most useful as a tool for soil health assessment, however, the soil type should be taken into consideration when interpreting chromatogram features (Figure 4.18).

The overall conclusion from this study is that although chromatograms do show potential as a low-cost soil health assessment tool, responses of chromatogram features to soil health gradients are inconsistent. However, the type of soil the chromatogram was developed from should be taken into consideration when interpreting chromatograms. In addition, several features within chromatograms created from the same soil type often displayed correlated responses to soil health variable gradients. This has two implications, first, time could potentially be saved by measuring only one of the correlated chromatogram characteristics. However, there is a lot of 'noise' in chromatogram feature responses to gradients in soil health variables. Thus secondly, the correlated nature of chromatogram features could be used to provide greater certainty in interpreting soil health from chromatogram features. For example, a chromatogram from pasture soil will tend to show a lower number of spikes under acidic conditions (Figure 4.5). Chromatograms from pasture also demonstrated increased outer zone radius (OZ) with decreasing pH (Figure 4.5). Thus, a chromatogram from pasture soil displaying both low number of spikes, and a high "OZ" is likely to have come from a soil sample with low pH.

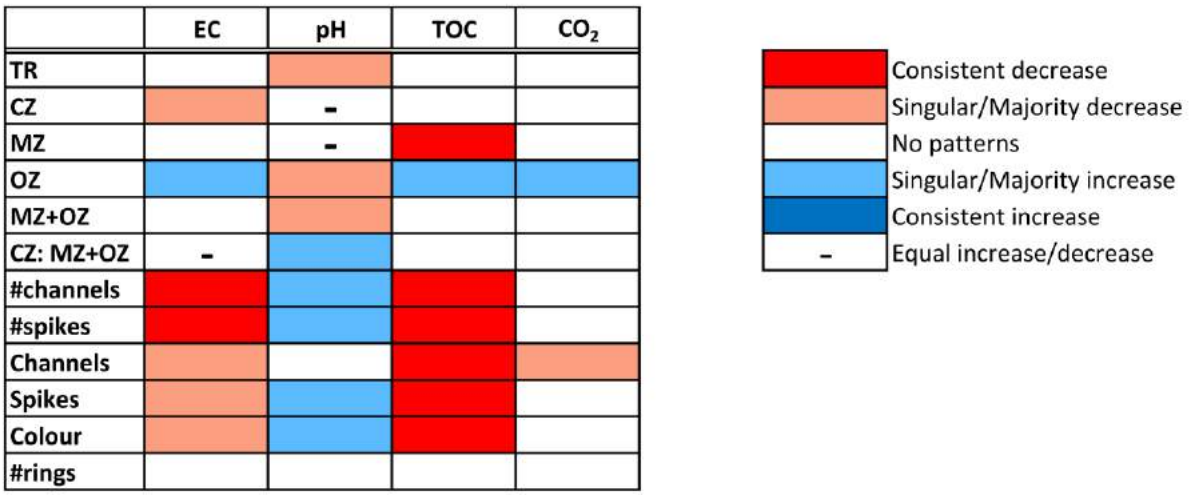

Figure 4.18. Summary of changes in chromatogram patterns over soil variable gradients. Decrease refers to a decrease in chromatogram feature with an increase in soil variable.

5 Acknowledgements

We would like to express our gratitude to the landowners who allowed us to carry out this research on their properties – without their consent and participation, this investigation could not have been undertaken. This project was supported by funding from the Western Australian Government's State NRM Program.

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7 Appendices

7.1 Data summaries

7.1.1 Pasture

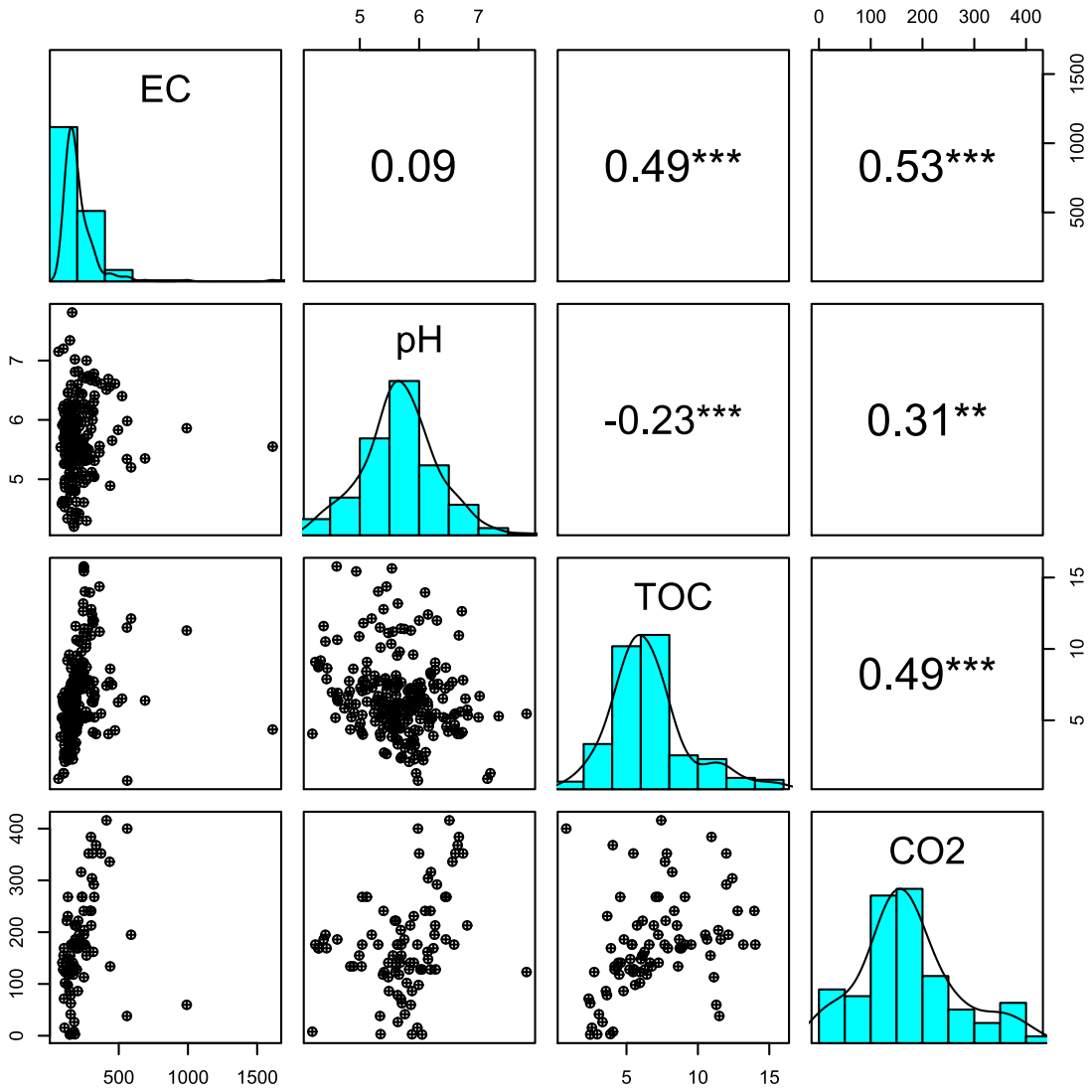

*Figure 7.1. Relationships among soil quality variables in samples from pasture. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

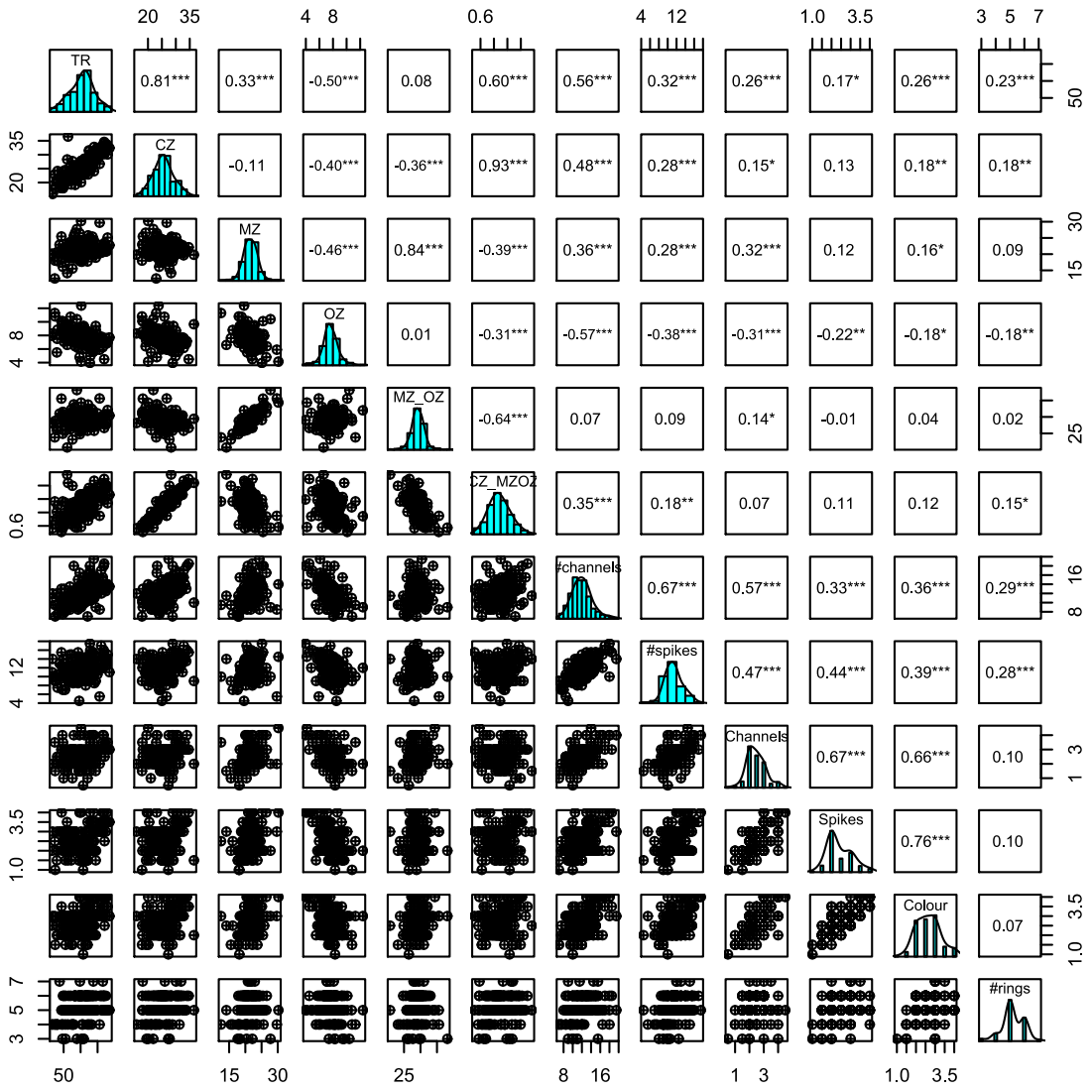

*Figure 7.2. Relationships among chromatogram features in samples from pasture. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

7.1.2 Orchards

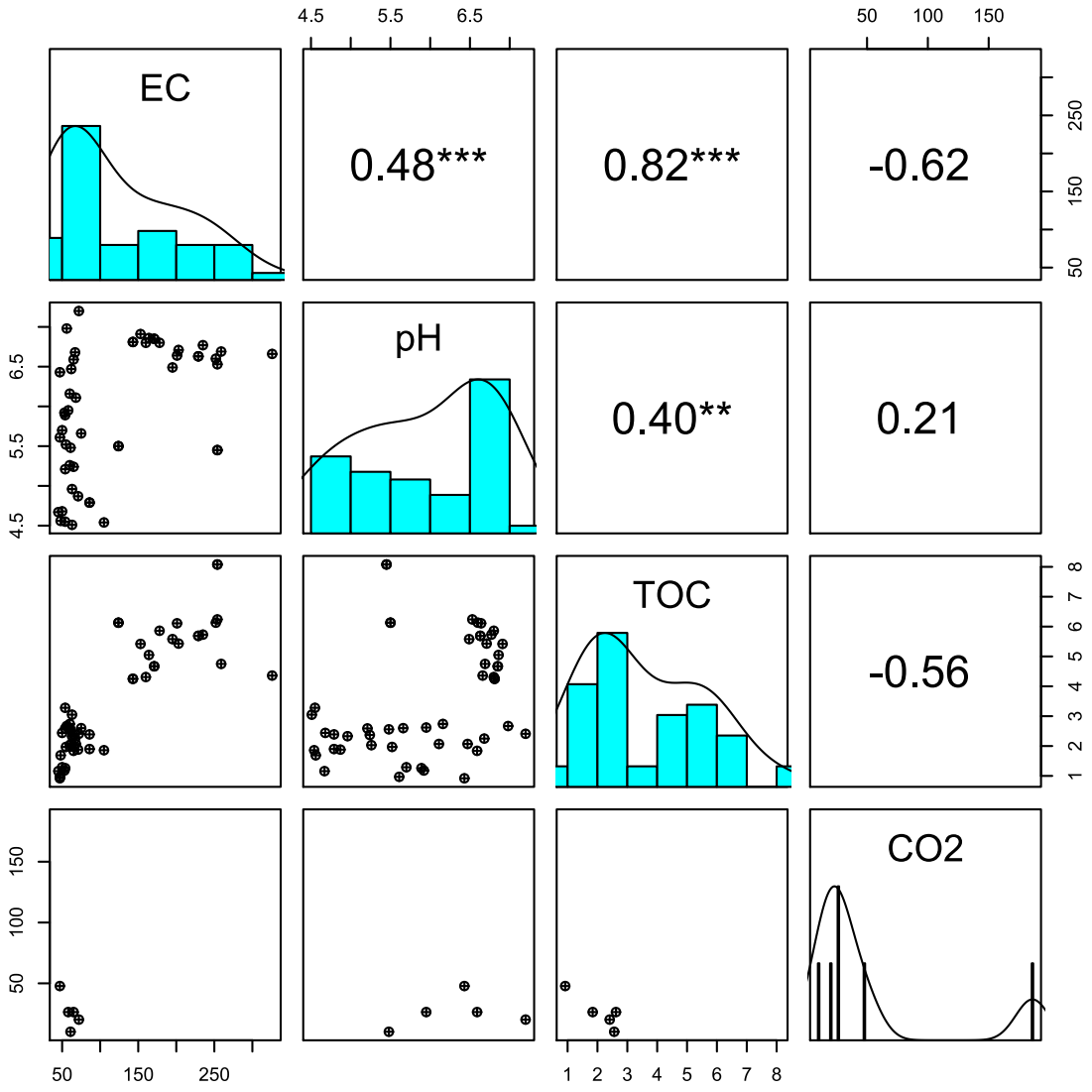

*Figure 7.3. Relationships among soil quality variables in samples from orchards. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

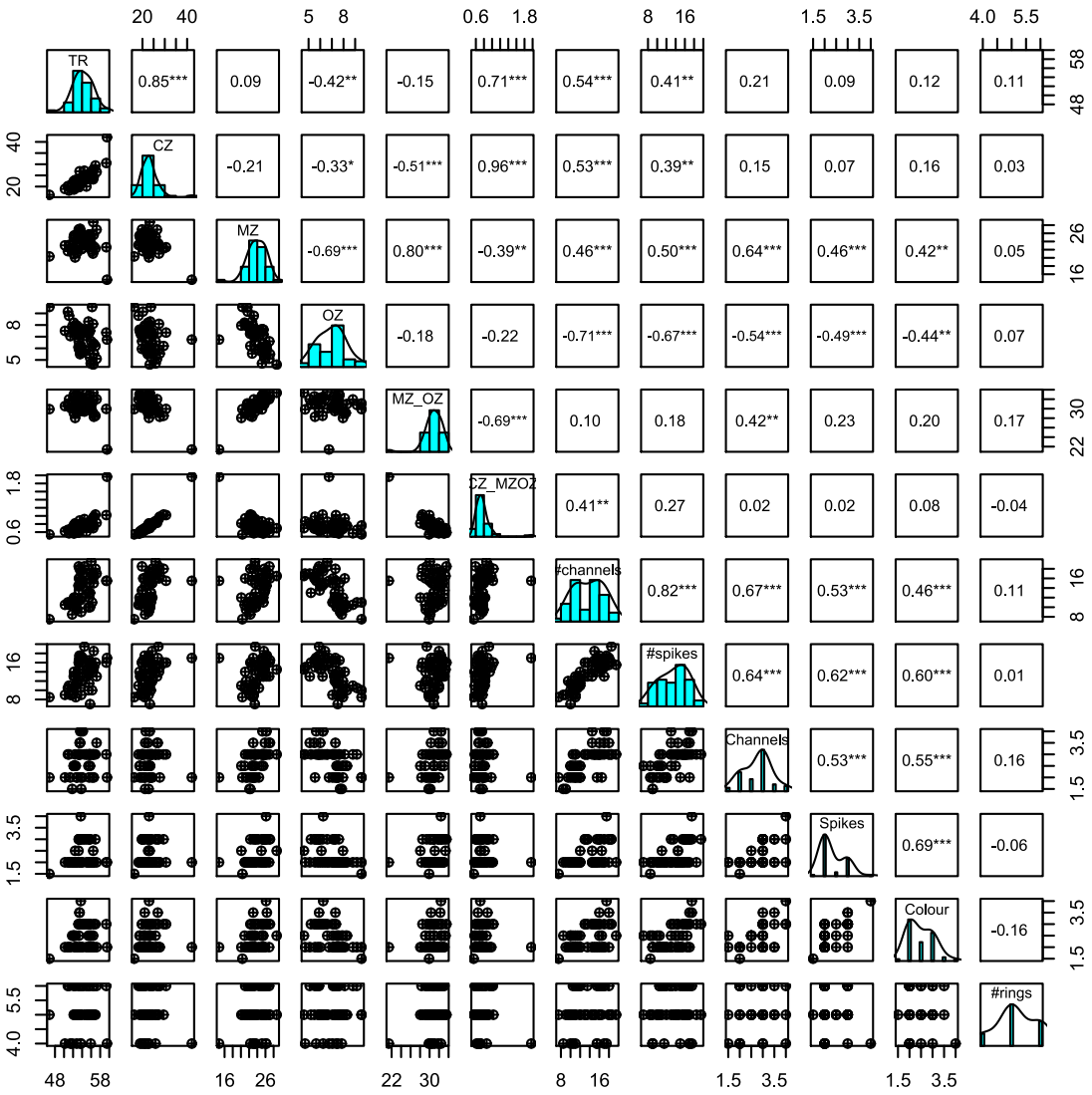

*Figure 7.4. Relationships among chromatogram features in samples from orchards. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

7.1.3 Gardens

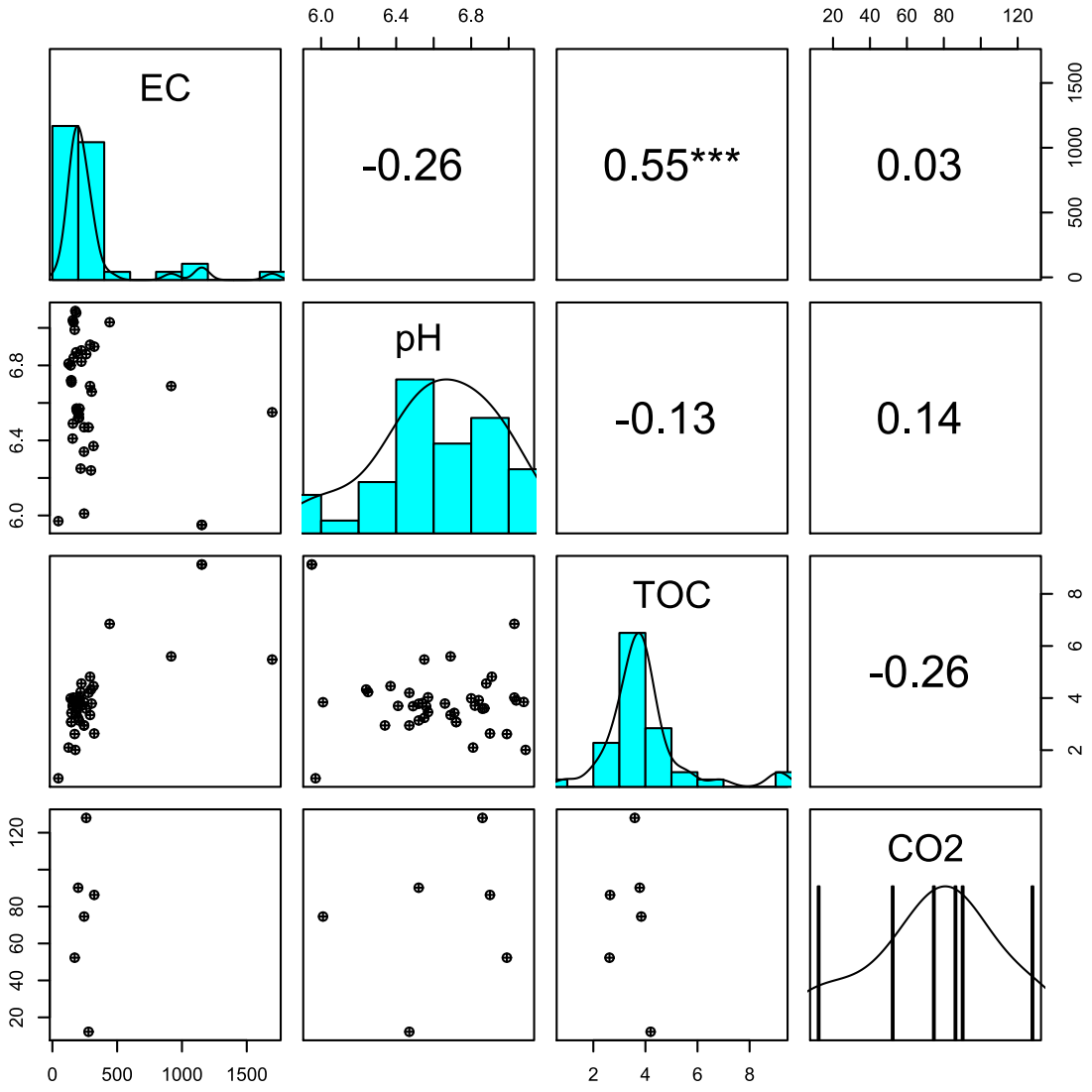

*Figure 7.5. Relationships among soil quality variables in samples from gardens. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

*Figure 7.6. Relationships among chromatogram features in samples from gardens. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

Remnant vegetation

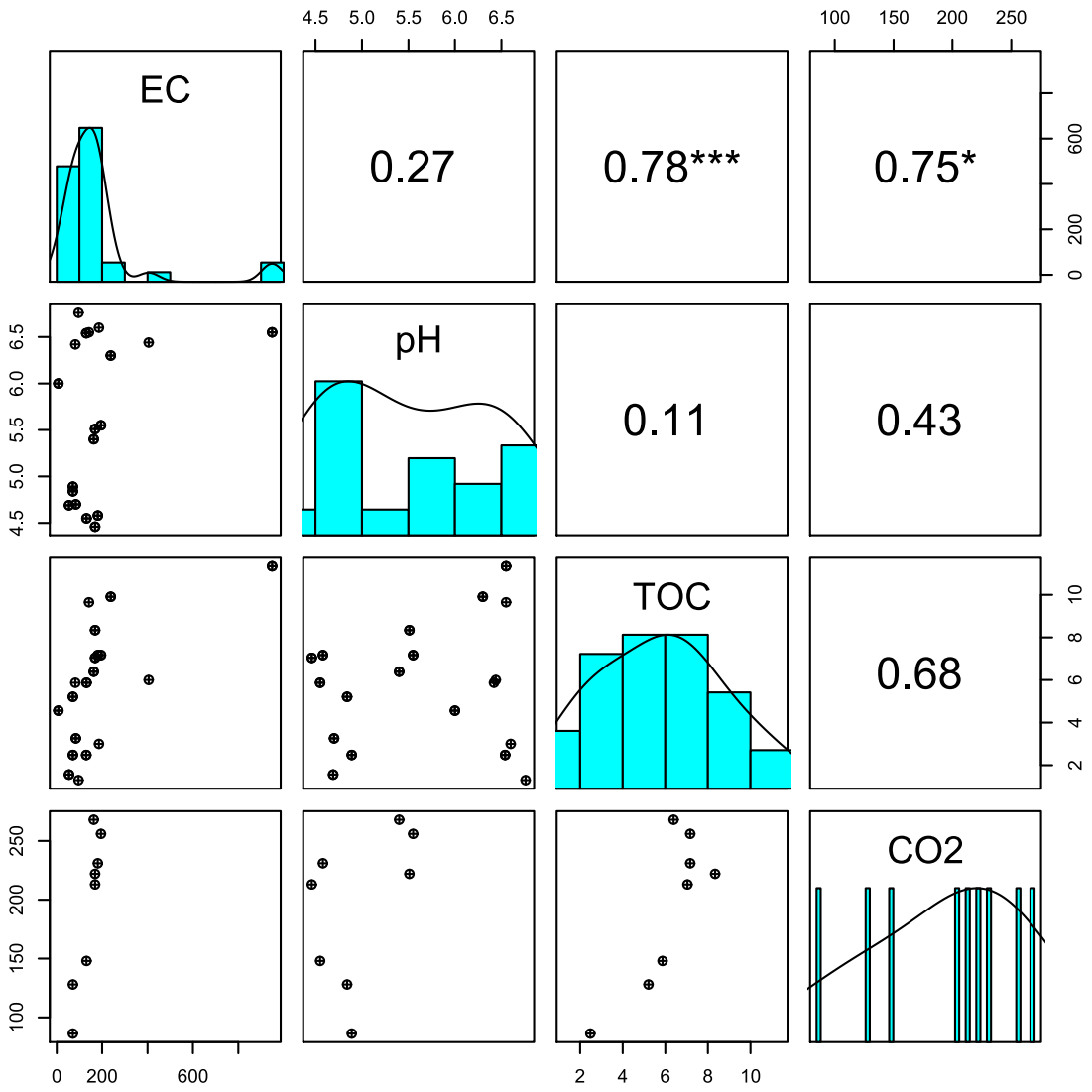

*Figure 7.7. Relationships among soil quality variables in samples from remnant vegetation soils. Histograms show distribution of values for each variable. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*

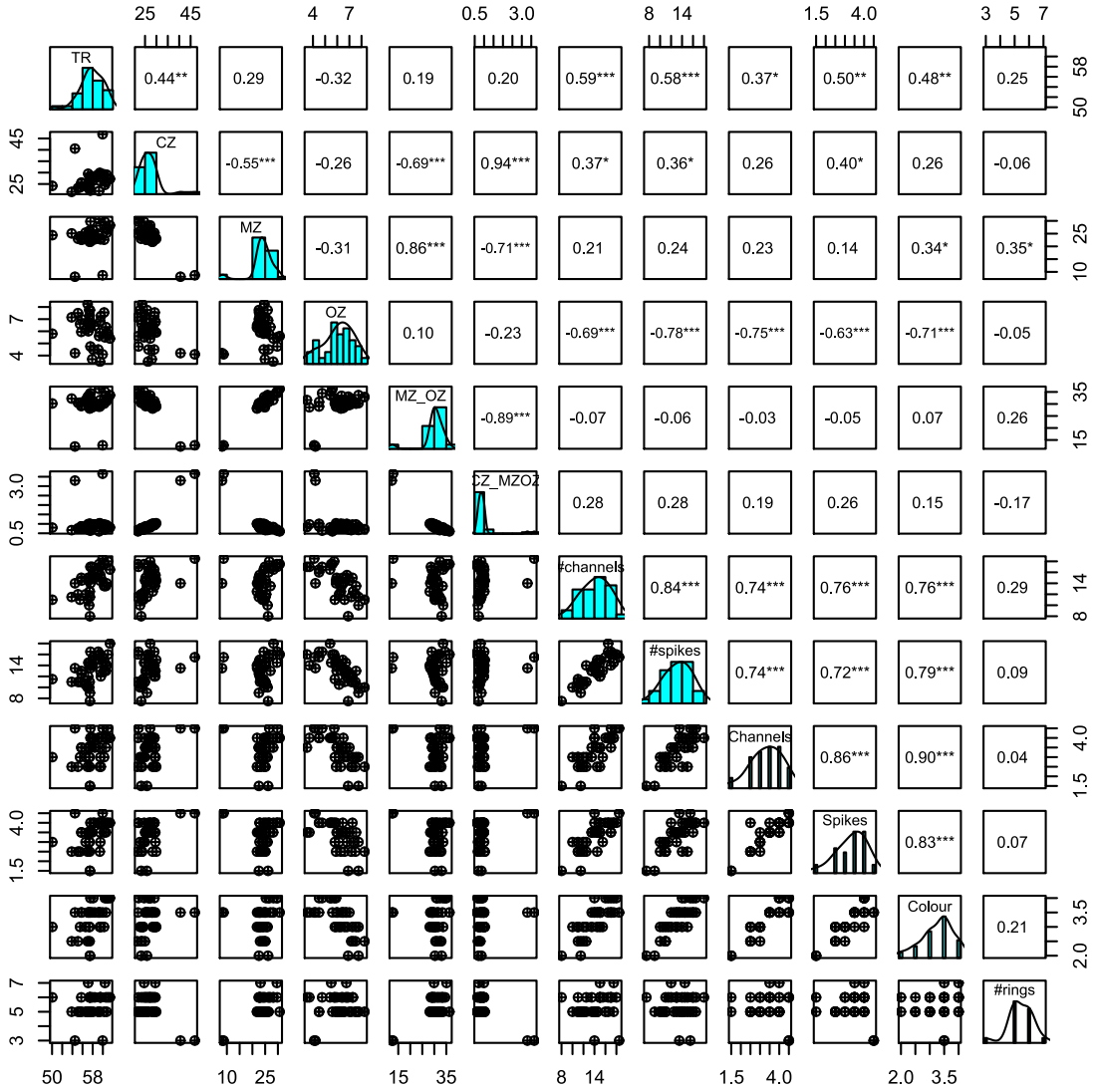

*Figure 7.8. Relationships among chromatogram features in samples from remnant vegetation soils. Numbers above diagonal are Spearman correlations. * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.*