FTIR spectral band shifts explained by OM–cation interactions

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Abstract

Background: The organic matter (OM) in soils interacts with polyvalent cations such as Ca^{2+} through hydroxyl (OH), carboxylic acid, ester, keto, aldehyde (summarized as C=O), and carboxylate (COO^-) functional groups. Such interactions affect the bonding strength of the double bond between the C and the O atom in the functional groups, which is assumed to shift the wavenumber (WN) region of O–H (hydroxyl), C=O, COO^-, and OM_{cat} (*i.e.*, C=O interacting with cations *plus* O–H groups) absorption band maxima in the Fourier transform infrared (FTIR) spectra. Such band shifts limit the evaluation of spectral information on OM in soil samples.

Aims: The objective of this study was to analyze the extent of band shifts and the changes in absorption band intensities for relations with cation concentrations, and to estimate effects of band shifts on OM properties such as potential wettability of OM evaluated from FTIR absorption band ratios.

Methods: Polygalacturonic acid (PGA) solutions were mixed with a $CaCl_2$ solution at different relations. The freeze-dried mixtures were analyzed with FTIR spectroscopy in the mid-infrared spectral range by using KBr-technique. The FTIR spectra were interpreted with respect to O–H, C=O, COO^-, and OM_{cat} bands, the latter reflecting the formation of PGA-Ca^{2+} complexes.

Results: The FTIR spectra of the PGA–Ca mixtures compared to that of pure PGA indicate band shift effects by $CaCl_2$ addition on both, intensity and WN value of the OM_{cat}, C=O and COO^- absorption band maxima. The COO^-/C–O–C ratio increased with Ca^{2+} concentration while the C–H/C–O–C ratio decreased. Furthermore, the C=O and COO^- absorption band maxima were shifted towards lower WN values, while the OM_{cat} absorption band was shifted towards higher WN values. The shift of OM_{cat} band maxima was two times higher than that of the C=O band maximum and increased with Ca^{2+} concentration.

Conclusion: Spectral band shifts depend on polyvalent cation concentration and limit automated interpretations of FTIR spectra without prior soil-specific spectral corrections.

Key words: band ratios / band shift / FTIR / PGA-cation interactions

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1 Introduction

Polyvalent cations like Ca^{2+} are able to (1) interact with organic matter (OM) in soils (*Mimmo* et al., 2005; *Dyab* et al., 2016), and to form bridges (2) between different organic matter (OM) molecules (*i.e.*, polysaccharides; *Maloviková* et al., 1994), as well as (3) between OM and mineral surfaces in soils (*Cai* et al., 2006). For interactions with cations, the OM is mostly using functional groups that contain double bonds between C and O atoms (*Aquino* et al., 2008) such as carboxylate anions, carboxylic acids, esters, amides (proteins), ketones, and aldehydes. These groups are either fully negatively charged (*i.e.*, carboxylate anion; Fig. 1a) or partially negatively charged (*i.e.*, carboxylic acids, esters, amides, ketones, aldehydes; Fig. 1b), and thus contribute to the cation exchange (*Kaiser* et al., 2008) among other processes.

The double bonds between C and O atoms (C=O-double bond) show characteristic absorption bands in Fourier transform infrared (FTIR) spectra (*i.e.*, mid infrared), characterized by the wavenumber (WN), and the intensity of the band maxi-

ma (*Hesse* et al., 2005). While the C=O double bond of carboxylic acid, ester, aldehyde, and keto groups (summarized here as C=O band) show absorption bands within WN 1650 to 1800 cm^{-1}, the carboxylate groups (COO^- band) show absorption bands at lower WN (*i.e.*, 1550 to 1620 cm^{-1}) (Tab. 1).

The strength of the bond between C and O is affected by the molecular structure, the kind of functional groups, and the molecular size. Binding strength may additionally be affected by the presence of cations since interactions between OM and cations can cause a proton-cation exchange at carboxylic acid groups resulting in carboxylate groups (Fig. 1a; red letters) and ionic interactions. This interaction causes a decrease in the intensity of the COOH band at the WN range of 1730 to 1710 cm^{-1} (Tab. 1), while a COO^- band appears at WN range of 1620 to 1550 cm^{-1} (*Hesse* et al., 2005). Interactions between the free electron pairs of the oxygen within the C=O-double bond and the cation can form uncharged OM-cation complexes (Fig. 1b), which result in absorption

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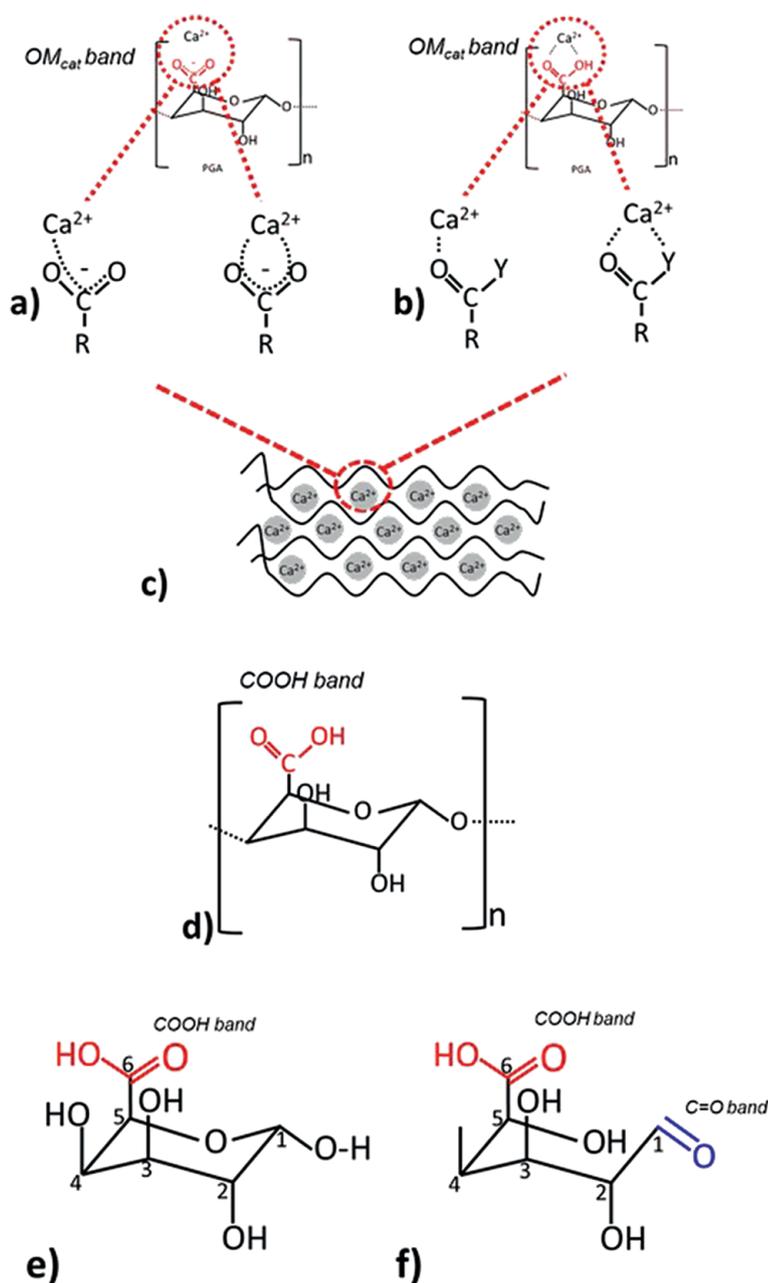
Figure 1: Schemes visualizing the interactions between cations (*i.e.*, Ca^{2+}) and (a) charged (COO^-) and (b) un-charged groups (COOH) in OM (adopted from *Lu* and *Miller*, 2002; in red the bond affected by the OM-cation interaction; R, the backbone of OM), as well as (c) the egg-box model illustrating the structure resulting from polysaccharide-cation interactions (adopted from *Grant* et al., 1973), and the molecular structures of (d) polygalacturonic acid, (e) the ring structure and (f) the open ring of galacturonic acid (adopted from *Falbe* and *Regitz*, 1998) including the respective C=O, COOH and OM_{cat} bands in FTIR.

C=O-double bonds within OM are able to interact with cations forming either charged (Fig. 1a) or uncharged complexes (Fig. 1b) such that polymers like polysaccharides can interact with a number of cations. This and the fact that polyvalent cations like Ca^{2+} can use up to six binding sites for the interaction (*Tipping*, 2005), can explain the egg-box model (Fig. 1c) suggested by *Grant* et al. (1973) for describing the structure of complexes formed between polysaccharides and cations. Polygalacturonic acid (PGA; Fig. 1d) is often used as a model for mucilage because of its high content of uronic acid (*Morel* et al., 1986) and the similarities between mucilage and PGA-Ca networks (*Gessa* and *Deiana*, 1992). The galacturonic acid (Fig. 1e) can undergo a ring-opening *via* a proton transfer from the O–H group at C atom 1 to the O within the pyranose ring thereby forming a carbonyl group (C=O) at the C atom 1 (*Falbe* and *Regitz*, 1998). Only galacturonic acid located at the end of the PGA chain can form an open ring (Fig. 1f). Thus, PGA contains in addition to the COOH groups (Fig. 1e, f; red letters) also some carbonyl (C=O) groups (blue letters).

Changes in the binding strength within the C=O-double bond caused by interaction with cations are related to a shift of the maximum of the absorption bands in FTIR (*Hesse* et al., 2005). Shifts in absorption bands caused by OM–cation interactions are documented for pectate gels (*Mimmo* et al., 2005), fatty acids (*Lu* and *Miller*, 2002), PGA (*Mimmo* et al., 2003), OM extracted from forest floor litter layers (*Scheel* et al., 2008; *Eusterhues* et al., 2014), and for humic acids (*Dyab* et al., 2016) as well as for OM–soil mineral associates (*Cai* et al., 2006). Most studies found the OM_{cat} bands to be shifted towards higher WN (Tab. 2). For humic acids and OM extracted from forest litter, the cation addition was found to shift the maxima of the COO^- band towards lower WN (*Scheel* et al., 2008; *Dyab* et al., 2016). In contrast, *Eusterhues* et al. (2014) and *Carrasquero-Durán* and *Flores* (2004) found the COO^- band shifted towards higher WN by adding ferrihydrite and Cd to forest floor extracts and humic acids, respectively. The difference in those findings may be explained by difference in the added cations. *Mimmo* et al. (2003) also found a shift towards higher WN but studied the sorption of Al to a PGA-Ca gel. Additionally, *Mimmo* et al. (2005) reported the intensity of the absorption bands to be affected by the cations concentration which is also shown by *Alvarez-Puebla* et al. (2005) for the addition of Co to humic acids. The above-mentioned studies indicate that WN positions and intensities of mid-infrared bands are affected by associated ions and other functional groups, but the quantitative relations are still unknown, especially for OM of complex soil systems.

Spectral interpretations that are used to determine OM composition (*e.g.*, *Ellerbrock* and *Gerke*, 2013) or to predict soil bands at WN 1380 to 1400 cm^{-1} (*Alvarez-Puebla* et al., 2005) called here OM_{cat} band. Note that also chloride could absorb to nitrogen containing organic matter (*e.g.*, amine), which is most effective for N–H and less for C=O adsorption bands (*Zhuang* et al., 2020).

In both cases (Fig. 1a, b), the OM can act as a mono-dentate or a bi-dentate ligand (*Lu* and *Miller*, 2002). Note that all

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Table 1: WN regions (right and left limits) of absorption bands in FTIR bands caused by C=O-double bonds (C=O band) within carboxylic acid (COOH), ester (COOR), aldehyde (COH), keto (COR), and carboxylate (COO⁻) groups in FTIR spectra, adopted from *Hesse* et al. (2005).

Group	WN range (cm^{-1})
COOH	1710–1730
COOR	1715–1800
COH	1650–1750
COR	1650–1730
COO⁻	1550–1620

properties (*e.g.*, *Ludwig* et al., 2008; *Reeves III*, 2012) are mostly based on (1) WN ranges given in textbooks on FTIR spectroscopy (*e.g.*, *Hesse* et al., 2005) or on (2) a set of single WN identified by statistical approaches (*e.g.*, *Ludwig* et al., 2008). In both cases, a shift of the absorption bands caused by OM-cation interactions may limit the evaluation of spectral information in yet unknown ways because OM-cation interaction may shift relevant absorption bands. Thus, cations within soil samples may lead to a "masking" of absorption bands as a result of the shift. The "masking" of absorption bands may potentially explain why for each set of soil samples a new calibration curve is needed, *e.g.*, when using FTIR spectra of soil samples to determine SOC contents (*Ludwig* et al., 2008). Thus, the knowledge on the shifts of OM absorption bands in FTIR spectra and their correlation with cation concentration can be important for qualitative spectral interpretations. However, to the best of our knowledge, systematic investigations on the relation between OM-cation interactions on FTIR band shifts are missing to date.

We hypothesize that (1) interactions between PGA and Ca^{2+} especially affect the strength of the double bond between C and O (*Aquino* et al., 2008); (2) adding a CaCl$_2$ solution causes the formation of COO⁻ groups (*Lu* and *Miller*, 2002) resulting in a decrease of the intensity of the COOH band and an increase of the COO⁻ absorption band in FTIR (*Mimmo* et al., 2005), (3) the WN of C=O and COO⁻ absorption band maxima is shifted towards lower WN and those of the OM$_{cat}$ absorption bands towards higher WN (*Scheel* et al., 2008; *Eusterhues* et al., 2014), and (4) the band shifts (*Dyab* et al., 2016) and changes in intensity (*Mimmo* et al., 2005) of C=O, COO⁻, and OM$_{cat}$ absorption band maxima increase with Ca^{2+} concentration.

The objective of this study is to test the hypotheses on relations between cation concentrations and band shifts in FTIR considering basic physico-chemical principles. Specific aims are to (1) identify the band shift for a model substance, (2) determine its magnitude, (3) evaluate the relation between band shift and cation concentration, and (4) discuss the impact on soil properties obtained from FTIR spectra such as the potential wettability and cation exchange capacity. The analysis will be carried out with Polygalacturonic acid (PGA) as a model for mucilage mainly because of its high content of uronic acid (*Morel* et al., 1986) and the similarities between mucilage and PGA-Ca networks (*Gessa* and *Deiana*, 1992).

2 Material and methods

2.1 OM–cation solutions

The molecular weight of the galacturonic acid unit (Fig. 1c) within PGA that each contain a single COOH group is 176 (*Falbe* and *Regitz*, 1998). To obtain a PGA solution that contains 0.001 M of COOH group per litre (*i.e.*, 1×10^{-6} mol of COOH groups per mL) 176 mg of PGA were suspended in 1000 mL of deionized water. The 0.001 M CaCl$_2$ solution was

Table 2: Wave numbers of the absorption bands (cm^{-1}) in FTIR spectra of organic components (Original) and respective associations resulting from deprotonation and cation adsorption (Result) that are caused by C=O bonds in carboxylic acid anions (COO⁻) and in COOH, COOR; COO⁻, keto- or aldehyde groups that form complexes with cations (C=O$_{cat}$).

Components	COO⁻ band Wave numbers (cm^{-1})		OM$_{cat}$ band			References
	Original	Result	Original		Result	
Humic acid *plus* Zn(II)	1617	1583	1385	→	1384	*Dyab* et al. (2016)
Forest floor extract *plus* ferrihydrite	1622 →	1632				*Eusterhues* et al. (2014)
DOM forest floor litter layer (Oh) *plus* Al(III) at pH 3.8	1626 →	1618	1383	→	1401	*Scheel* et al. (2008)
Oh *plus* Al(III) at pH 4.5	→	1618		→	1401	
Humic acid *plus* Cd(II) *plus* Co	1600 →	1610	1325	→	1340 and 1381	*Carrasquero-Durán* and *Flores* (2004); *Alvarez-Puebla* et al. (2005)
Ca-PGA gel *plus* Al	1609	1630	1425		1429	*Mimmo* et al. (2003, 2005)

prepared by adding 111 mg $CaCl_2$ per 1000 mL de-ionized water (*i.e.*, 1×10^{-6} mol Ca^{2+} per mL). Here highly diluted solutions were used to avoid the formation of PGA-Ca networks (Fig. 1f; *Maloviková* et al., 1994; *Brax* et al., 2019) as good as possible when adding the $CaCl_2$ to the PGA solution.

The PGA-Ca^{2+} mixtures were prepared as follows: a defined volume of the 0.001 M PGA solution (Tab. 3) was filled into an Erlenmeyer flask, and stirred using a magnetic stirrer (Velp Scientific, Italy). To the PGA solution a respective volume (Tab. 3) of the 0.001 M $CaCl_2$ solution was added dropwise. This procedure was replicated with different stoichiometric $COOH:Ca^{2+}$ ratios (*i.e.*, 3:1, 6:1, 9:1, and 12:1; Tab. 3) to consider that Ca^{2+} can interact with up to six C=O-double bonds within PGA (*Tipping*, 2005; *Ellerbrock* et al., 2019). The resulting PGA-Ca^{2+} mixtures were stirred for three hours at room temperature (20°C) and freeze dried. Each mixture was prepared in nine replicates ($n = 9$). The pH effect on the protonation status of the functional groups was not considered here because the pH values of the highly diluted mixtures were ranging only between 5.5 and 6.0.

2.2 FTIR analyses

The freeze-dried PGA-Ca mixtures were analyzed with FTIR spectroscopy by using KBr technique (*Ellerbrock* and *Gerke*, 2013), here described briefly: 1 mg of the PGA-Ca mixture was mixed with 100 mg potassium bromide (KBr), stored overnight in an exsiccator, finely grinded using an agate mortar and pressed into pellets (*Ellerbrock* et al., 1999). The pellets were analyzed using a FTS135 (BioRad Corp, Hercules, CA, USA). All spectra were recorded under the same conditions: in absorption mode, at a resolution of 1 cm^{-1} by using 16 scans (= 16 co-added single spectra; *Ellerbrock* et al., 1999), corrected against ambient air as background (*Haberhauer* and *Gerzabek*, 1999) and smoothed using a "boxcar"-function (f = 105).

The baseline-corrected spectra (*e.g.*, *Ellerbrock* et al., 1999) were analyzed for the WN and the intensity of the absorption band maxima of the C=O bonds within (1) carboxylic acids, esters, amides, ketones and aldehydes at WN 1750 to 1650 cm^{-1} (C=O band; Fig. 2), (2) carboxylate anions at WN 1675 to 1610 cm^{-1} (COO$^-$ band; *Celi* et al., 1997), and (3) OM-cation associates at WN 1450 to 1390 cm^{-1} (OM$_{cat}$ band). The maxima of the O–H, C–H, C=O, COO$^-$, OM$_{cat}$, and C–O–C bands were identified using an automated identification procedure of the BioRad WINIREZ Software (BioRad Corp, Krefeld, Germany) as follows: the left and right limits of the WN region characteristic for each band (Tab. 4; *Hesse* et al., 2005) were used to construct so called "def"-files (offered by WINIREZ) that are than applied within the automated BioRad WINIREZ procedure.

The heights of O–H, C–H, C=O, COO$^-$, OM$_{cat}$, and C–O–C band maxima as a measure for the band intensity were computed by using the BioRad WINIREZ (BioRad Corp., Krefeld, Germany) software as follows: the height of the C–H band (characteristic for hydrophobic alkyl groups) was measured as a vertical distance (*i.e.*, as height) from a local baseline plotted between tangential points in the spectral regions between WN 3016–2804 cm^{-1}. The heights of the O–H, C=O, COO$^-$, OM$_{cat}$, and C–O–C bands were measured as a vertical distance from the maximum of the band to the global baseline (*Kaiser* et al., 2008) within the regions given in Tab. 4. Differences in the band intensities (y-axis) indicate in general differences in the contents of the respective functional groups but were also affected by an extinction coefficient

Table 4: Left and right limits of WN regions used for the automated search of the O–H, C–H; C=O, COO$^-$, OM$_{cat}$ (resulting from interactions between the free electron pairs of the oxygen within the C=O-double bond and the cation) and C–O–C band maxima in the FTIR spectra of PGA and PGA-Ca mixtures.

Band	Limits for WN region of maxima		
	Left	Right	REF
	(cm^{-1})	(cm^{-1})	
O–H	3500	3350	*Ellerbrock* et al. (2019)
CH	3016	2804	*Hesse* et al. (2005)
C=O	1750	1719	*Ellerbrock* et al. (1999)
COO$^-$	1656	1610	*Ellerbrock* et al. (1999)
OM$_{cat}$	1450	1382	*Alvarez-Puebla* et al. (2005)
C–O–C	1120	1000	*Ellerbrock* et al. (1999)

Table 3: Composition of PGA:calcium (PGA:Ca) mixtures: Volume of the polygalacturonic acid (PGA) and the calcium chloride ($CaCl_2$) solutions, the molar concentration of carbocxyl acid groups (COOH; 10^{-6} mol mL^{-1}) and calcium cations (Ca^{2+}; 10^{-6} mol mL^{-1}) in water, the stoichiometric ratios between COOH and Ca^{2+} (COOH:Ca), and the Ca^{2+} concentration within the PGA-Ca^{2+} mixture.

PGA	$CaCl_2$	COOH	Ca^{2+}	COOH:Ca	Ca^{2+} concentration
(mL)	(mL)	(10^{-6} mol L^{-1})			(10^{-6} mol L^{-1})
30	10	30	10	3:1	2.5
60	10	60	10	6:1	1.4
45	5	45	5	9:1	1.0
60	5	60	5	12:1	0.8
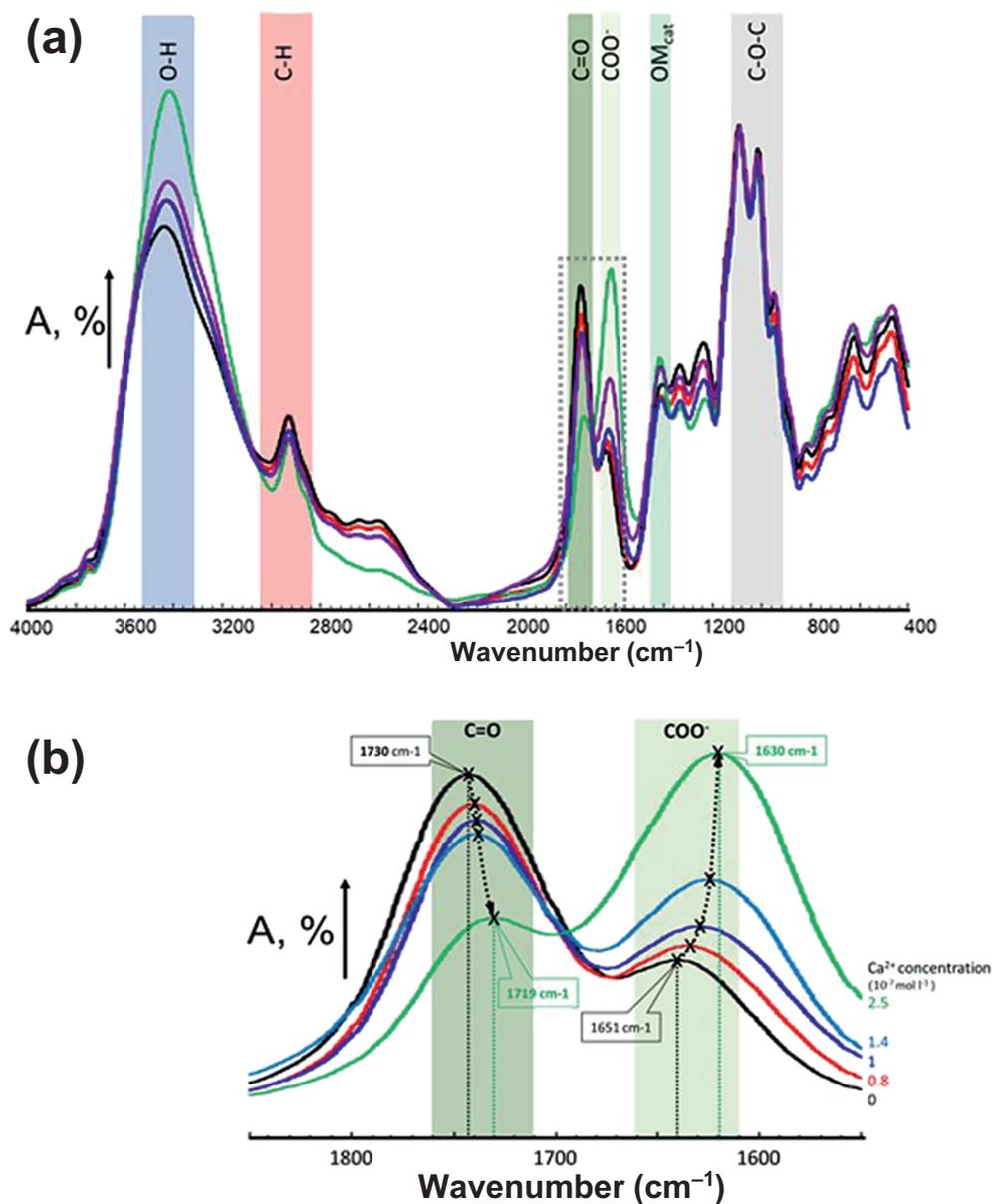
Figure 2: (a) FTIR spectra of PGA and PGA-Ca mixtures (with different Ca^{2+} concentrations; 10^{-6} mol L^{-1}) and (b) enlargement of WN region from 1850 to 1550 cm^{-1}. The grey rectangles indicate the WN region of C=O and COO⁻ bands. Insert in Fig. 2a FTIR of $CaCl_2$.

which is characteristic for the studied components (Lambert Beers Law; *Hesse* et al., 2005). Therefore, the intensity of the C–O–C band at WN 1120 to 1000 cm^{-1} (characteristic for polysaccharide groups; Tab. 4) was used as an internal reference such that the ratio between the intensity of different bands and that of the C–O–C band reflect on the content of respective functional groups relative to that of the C–O–C group (*Ellerbrock* and *Gerke*, 2013). When interpreting the spectra, we focus on C=O, COO⁻, and OM_{cat} bands which were assumed to be mostly affected by interactions with cations like Ca^{2+} (*Aquino* et al., 2008). The band of the hydroxyl groups at WN 3500 to 3350 cm^{-1} (O–H band) was also analyzed since the O–H groups are also able to interact with the Ca^{2+} which may affect intensity and location of the O–H band maximum.

The WN of the band maxima in the FTIR spectra of PGA-Ca mixtures were compared with the ones in the spectra of PGA [*i.e.*, WN(PGA-Ca mixture) *minus* WN(PGA)] to determine the shifts of the absorption bands in the spectra of the PGA-Ca mixtures relative to those in PGA (band shift). The ratio of the band intensities in the FTIR spectra of the PGA-Ca mixtures were related to the ones in the FTIR spectra of PGA (*e.g.*, C=O/C–O–C ratio of the PGA-Ca mixture divided by the C=O/C–O–C ratio of PGA) to determine the relative changes (band ratios). The spectral data (relative band shift as well as band ratios) were tested for outliers using the Nalimov test. The differences of the mean values between the PGA-Ca mixtures and PGA were compared with Student's t-test (*Kaiser* and *Gottschalk*, 1977).

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3 Results

3.1 Spectral band shifts

The spectra of PGA and PGA-Ca mixtures (Fig. 2a) show O–H, C–H, C=O, COO⁻, OM$_{cat}$, and C–O–C absorption bands. The FTIR spectra of the PGA-Ca mixtures show for the C=O and COO⁻ band maxima a shift towards lower WN (Fig. 2b), and for the OM$_{cat}$ band maxima a shift towards higher WN (Tab. 5) as compared to pure PGA. The shift of both, OM$_{cat}$ and C=O bands, was found to increase with the Ca^{2+} concentration. While the shift in the OM$_{cat}$ band was mostly larger than the standard deviation, the shift of the C=O band was smaller or equal to the standard deviation (Tab. 6a) except for the 3:1 PGA-Ca mixture. For the O–H and C–H bands, the band shifts are smaller than the standard deviation. The negative shift of the COO⁻ band was mostly the same for all PGA-Ca mixtures as compared to pure PGA (Fig. 3). The positive shift in the OM$_{cat}$ band maximum is up to 3-times higher than the negative shift of the C=O band (Tab. 6a).

Table 5: Range of the band shifts of the O–H, C–H, C=O, COO⁻, OM$_{cat}$ and C–O–C absorption band maxima in FTIR spectra of PGA-Ca mixtures as compared to those in FTIR of PGA [WN band shift: WN (PGA-Ca mixture) minus WN (PGA)].

Band	WN band shift (cm⁻¹)		
OM$_{cat}$	11.51	to	34.01
COO⁻	−20.42	to	14.27
C=O	−9.35	to	−1.72
O–H	−2.72	to	5.05
C–H	−0.93	to	10.83
C–O–C	0.06	to	11.04
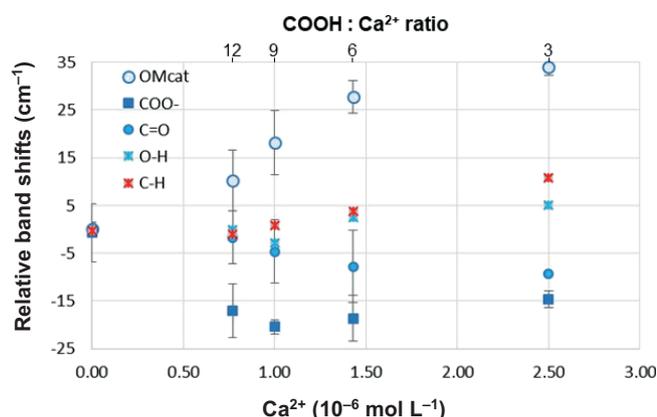
Figure 3: Shifts of the C–H, O–H, C=O, COO⁻, and OM$_{cat}$ band maxima in FTIR spectra of the PGA-Ca mixtures relative to the ones in the FTIR spectra of PGA (relative band shifts) *versus* Ca^{2+} concentration (bottom x-axis) and *versus* the COOH : Ca ratios of the mixtures (second x-axis at the top).

However, a Nalimov test indicated up to three outliers for the shifts in the OM$_{cat}$, COO⁻, C=O, and C–H band maxima and up to four outliers for that of the O–H band maxima (Tab. 6a). The t-test indicated the WN of the OM$_{cat}$ band maxima for the PGA-Ca mixtures (3:1, 6:1, 9:1, and 12:1) to be significantly larger at the 99.9% level than that of PGA (Tab. 6a). The WN of the COO⁻ band maxima for the 9:1 and 6:1 PGA-Ca mixtures were lower at the 99.9% level as compared to those of PGA, while the differences for 12:1 and 3:1 mixture were not statistically significant; and shifts in the C=O, CH, O–H, and C–O–C absorption band maxima were not statistically different as compared to PGA except for the 3:1 PGA-Ca mixture (Tab. 6a).

3.2 Band ratios

The COO⁻/C–O–C band intensity ratios in the FTIR spectra of the PGA-Ca mixtures relative to those of PGA (band ratio) increase with Ca concentration (Fig. 4). The increase in the mean COO⁻/C–O–C band ratio with Ca^{2+} concentration is mostly larger than the standard deviation (Tab. 6b). The decrease in the mean C–H/C–O–C band ratio is larger than the standard deviation but the changes in the mean OM$_{cat}$/C–O–C, C=O/C–O–C, and O–H/C–O–C band ratios are smaller than the corresponding standard deviations (Tab. 6b). For the OM$_{cat}$/C–O–C, COO⁻/C–O–C, C=O/C–O–C, and C–H/C–O–C band ratios the Nalimov test indicated only a single outlier but up to four outliers for the O–H/C–O–C ratios. The t-test indicated the COO⁻/C–O–C band ratios of the PGA-Ca mixtures to be significantly higher at the 99.9% level as comapred to that of pure PGA (Tab. 6b). The PGA-Ca mixtures did not show statistical differences in the other band ratios compared to those of PGA, except for the OM$_{cat}$/C–O–C and the C–H/C–O–C ratios of the 6:1 and 3:1 PGA-Ca mixtures (Tab. 6b).

4 Discussion

4.1 Adsorption band shifts

The FTIR of the PGA-Ca mixtures show in comparison to that of the PGA changes in the intensity and the WN especially with respect to the maxima of the C=O and COO⁻ bands (Fig. 2b). This finding suggests that Ca addition affected both the intensity and the WN region of the C=O and COO⁻ absorption bands, which is in accordance with data reported for CaCl$_2$ addition to pectate gels (*Mimmo* et al., 2005) and Al sorption on PGA-Ca networks (*Mimmo* et al., 2003). Note that in contrast to the highly diluted PGA suspension used here, *Mimmo* et al. (2005) characterized effects of Ca sorption to higher concentrated pectate gels.

The OM$_{cat}$ band of the PGA-Ca mixtures were found to be shifted towards higher WN (Fig. 3), which is also reported by *Scheel* et al. (2008) and *Eusterhues* et al. (2014) for forest DOM and by *Dyab* et al. (2016) and *Carrasquero-Durán* and *Flores* (2004) for humic acids (Tab. 2). The COO⁻ band was shifted towards lower WN (Fig. 3) by Ca addition which is in contrast to *Mimmo* et al. (2003), who reported a band shift toward higher WN when adding Al (Tab. 2). This can be

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Table 6: Mean values (a) of the shift of the O–H, C–H, C=O, COO⁻, OM_{cat} and C–O–C absorption band maxima [Band shift: WN(PGA-Ca mixture) *minus* WN(PGA)] and (b) of the OM_{cat}/C–O–C, COO⁻/C–O–C, C=O/C–O–C, O/C–O–C, O–H/C–O–C, and C–H/C–O–C ratios of PGA-Ca mixtures as compared to PGA (band ratios), with s the standard deviation and *n* the number of replicates without outliers (Nalimov test).

(a) Band shifts

Ca	$OM_{cat.}$			COO⁻			C=O			O–H			C–H			COC		
(10⁻⁶ mol mL⁻¹)	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s
PGA	8	0.12	0.27	8	–0.72	6.13	8	0.00	1.51	8	0.00	2.05	8	–0.30	0.80	8	0.00	1.95
12:1	8	**11.51***	5.42	9	–17.06	5.68	9	–1.72	5.50	9	0.00	3.02	9	–0.93	3.68	9	0.93	3.62
9:1	7	**18.12***	6.77	7	**–20.42***	1.50	7	–4.67	6.61	7	–2.82	1.69	8	0.74	3.33	8	0.06	5.12
6:1	9	**27.72***	3.38	9	**–18.67***	4.81	9	–7.75	7.58	9	2.58	6.77	9	3.88	5.48	9	7.09	5.33
3:1	8	**34.01***	1.76	7	–14.27	1.35	8	**–9.35+**	0.00	6	5.05	1.66	8	**10.83+**	0.60	8	**11.04#**	5.64

(b) Band ratios

Ca	OM_{cat}/COC			COO⁻/COC			C=O/COC			O–H/COC			C–H/COC		
(10⁻⁶ mol mL⁻¹)	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s	n	Mean	s
0.00	9	1.00	0.049	8	0.99	0.047	9	1.02	0.067	6	0.99	0.064	8	1.00	0.077
0.77	8	1.05	0.064	8	**1.24***	0.108	8	0.93	0.086	6	0.94	0.031	8	0.98	0.023
1.00	8	1.07	0.018	8	**1.27***	0.123	8	0.98	0.154	4	0.82	0.013	8	0.92	0.134
1.43	9	**1.10***	0.124	9	**1.57***	0.214	9	0.98	0.243	8	0.96	0.248	9	**0.85+**	0.106
2.50	9	**1.17#**	0.073	8	**2.08***	0.156	9	1.06	0.135	8	1.21	0.224	8	**0.68***	0.137

*: highly significant at 99.9% level from PGA;

#: significant at 99% level from PGA;

+: significant at 95% level from PGA.

explained by the fact that sorption of Al on a PGA-Ca gel (*Mimmo* et al., 2003) is different from the addition of highly diluted Ca solution to PGA solution studied here. The increasing shift of OM$_{cat}$ and C=O absorption band maxima with Ca^{2+} concentration (Fig. 3) as reported in *Mimmo* et al. (2003) for Al sorption on Ca-pectate gels is corresponding to the hypothesis that the Ca^{2+} concentration affects especially the shift of OM$_{cat}$ and C=O bands.

Although the maximum of the CO$^-$ band is shifted to smaller WN as compared to PGA, it did not change with Ca concentration (Fig. 3). An explanation is that the COO$^-$ is not only formed during the interaction with Ca^{2+} but also during the preparation of the KBr pellet prior to the FTIR analysis. During sample preparation, the surplus of KBr at a KBr:sample ratio of 99:1 can cause a deprotonation of the COOH groups in PGA (*Hesse* et al., 2005) such that the COO$^-$ could be formed independent of the PGA-Ca^{2+} ratio.

The shift in the maximum of the OM$_{cat}$ band is two times higher as compared to that of the C=O absorption band (Fig. 3). This can be explained by the highly diluted solutions used here. The amount of PGA-Ca complexes formed by interactions between uncharged C=O-double bonds and Ca^{2+} (Fig. 1d; OM$_{cat}$ band) may be much smaller than that between carboxylate ions in PGA and Ca^{2+} (Fig. 1c; COO$^-$ band) resulting in less intense shifts of the C=O bands (Fig. 3). This finding can be explained with the larger number of COOH and COO$^-$ groups within PGA as compared to the number of the C=O groups because the latter ones can only be formed at the end of the PGA chains. The increase in band shift with Ca^{2+} concentration is mostly larger than the standard deviation (Tab. 6a) when comparing the different PGA-Ca mixtures; however, the t-test only characterized the data of the 3:1 PGA-Ca mixture to be statistically different at the 99% level from that of the 12:1 PGA-Ca mixture.

4.2 Band ratios

In accordance with hypothesis (A), the intensity of the COO$^-$-absorption bands in the FTIR spectra of the PGA-Ca^{2+} mixtures relative to the ones of PGA (band ratio) increases with Ca^{2+} concentration, which is reflected by an increase of the COO$^-$/C–O–C band ratio (Fig. 4). At a first glance, the gain in COO$^-$/C–O–C band ratio should range in the same magnitude as the loss in C=O/C–O–C band ratio. However, the change in the C=O/C–O–C band ratio is mostly smaller than the standard deviation (Fig. 4). This discrepancy can be explained by changes in the extinction coefficient, ε, in the Lambert Beer's law:

$$A = \varepsilon \times d \times c, \tag{1}$$

with A the absorption intensity, c the concentration characterizing the amount of functional groups, and d the sample thickness. Note that ε depends on (1) the type of OM and (2) the wavenumber of the absorption band (*Hesse* et al., 2005), such that ε may be higher for the WN region of the COO$^-$ band as compared to that of the C=O band. Additionally, the increasing formation of PGA-Ca complexes with Ca^{2+} concentration may change ε of the CH bands which can explain
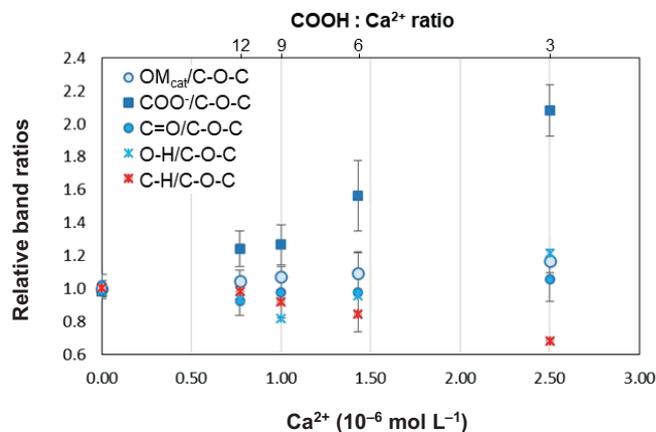
Figure 4: The COO$^-$/C–O–C, OM$_{cat}$/C–O–C, O–H/C–O–C, C=O/C–O–C, and C–H/C–O–C, ratios in FTIR spectra of PGA-Ca mixtures relative to the ones in FTIR spectra of PGA (y-axis; relative band ratios) *versus* the Ca concentration of the mixtures (x-axis). The second x-axis (on top of the figure) reflect on the respective COOH:Ca ratios of the mixtures.

why the CH/C–O–C ratio decrease with increasing Ca^{2+} concentration (Fig. 4, Tab. 6b).

The increase in the COO$^-$/C–O–C ratio with Ca concentration (Tab. 6b) found here for the PGA-Ca^{2+} mixtures is in accordance with *Alvarez-Puebla* et al. (2004). However, in contrast to *Alvarez-Puebla* et al. (2004, 2005), the O–H/C–O–C, C=O/C–O–C, and OM$_{cat}$/C–O–C ratios of the PGA-Ca^{2+} mixtures did not change significantly with Ca concentrations (Fig. 4, Tab. 6b). This discrepancy may be explained by the differences in the OM and cations used by *Alvarez-Puebla* et al. (2005) as compared to present study. An explanation why the increase in the COO$^-$/C–O–C ratio is five times higher than the increase in the OM$_{cat}$/C–O–C ratio (Tab. 6b) is that the amount of uncharged PGA-Ca-complexes (Fig. 1b) (*i.e.*, OM$_{cat}$ band) is possibly formed to a much smaller extent as compared to that of COO$^-$ groups (*i.e.*, COO$^-$ band). However, an analysis of the ratio between the charged (Fig. 1a) and uncharged (Fig. 1b) PGA-Ca^{2+} complexes was beyond the scope of this study.

Since Ca^{2+} addition was found to affect intensity and WN of the OM$_{cat}$, COO$^-$, and C=O bands characteristic for OM composition, OM-cation interactions caused changes in the spectral feature can affect the interpretation of the spectra. These band shifts caused by OM–Ca interaction can affect the prediction of soil properties from FTIR spectra that are based on a set of single wavenumbers (*Ludwig* et al., 2008). In addition to PGA–Ca, interactions between OM and anions (*i.e.*, Cl$^-$) may also affect the absorption bands in FTIR (*Zhuang* et al., 2020). However, OM–anion interactions need to be considered mainly for N containing OM. The PGA studied here contains only traces of amines and imines such that the anion effects on absorption bands in FTIR of PGA-Ca mixtures was not relevant and beyond the scope of the present study. Cation dependent changes in spectral feature may hide absorption bands within the chosen set of single wavenumbers, depending on cation concentration and composition in the samples. This means that both, calibration procedure and

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selection of the single set of WN, need to be adopted to consider effects of OM–cation interactions on the WN relevant for absorption bands of OM functional groups.

5 Conclusions

The FTIR spectrum of PGA was compared with FTIR spectra of PGA-Ca mixtures to identify potential shifts in absorption band maxima and their magnitude, to evaluate the relation between band shift and Ca^{2+} concentration, and to estimate potential effects of Ca^{2+} addition on OM composition dependent properties. For the PGA-Ca mixtures, the magnitude of the band shifts and absorption intensities was found related to the Ca^{2+} concentration. The results confirmed that Ca addition led to a decresase in the intensity of the C=O band and to an increase in the intensity of the COO^- band. The shift of the C=O and COO^- band maxima towards lower and of those of OM_{cat} towards higher WNs confirmed the hypothesis that the spectral properties of PGA are affected by polyvalent cations such as Ca^{2+}. The results confirmed the relation between band shifts and changes in band height intensity and the Ca^{2+} concentration. As expected, the effect is most pronounced for C=O, COO^- and OM_{cat} bands. With respect to the potential wettability of OM (C–H/C–O–C-ratio), the results of the cation concentration dependent changes in absorption band intensities suggest a seemingly decrease in the C–H group content as a result of a changed extinction coefficient.

The observed effects of OM-Ca^{2+} interactions on shift and intensity of the C=O, COO^-, and OM_{cat} band maxima suggest that differences in soil polyvalent cation concentrations should be considered for characterizing OM composition and properties such as potential OM wettability and cation exchange capacity based on the interpretation of FTIR spectra. These band shifts limit the interpretation of FTIR spectra and suggest the need for soil-specific spectral corrections and the development of a spectral calibration procedure.

Acknowledgments

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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