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REMOTE SENSING OF BIOLOGICALLY REWORKED SEDIMENTS: A LABORATORY EXPERIMENT

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ABSTRACT

*The present study aims to test the application of remote sensing to address the impact of bioturbation on physical sediment properties. Therefore, a laboratory experiment was developed, using microcosms mimicking a marine intertidal water-sediment interface to test the influence of *Corophium volutator* densities on sediment properties. Three main variables (water content, clay content and mean grain size) were measured in three treatments (no *Corophium*, 5,000 *Corophium*/m² and 20,000 *Corophium*/m²) after 16 days of bioturbation. Results obtained with conventional – destructive – techniques showed a significant increase of water content and a significant, but small decrease of clay content in the presence of *Corophium*. The remote sensing technique detected the impact of *Corophium* on water content as an increase in absorption at 1,450 nm, but was not able to detect the animal impact on clay content. This study demonstrates that remote sensing data could be significantly modified by bioturbation activities and that remote sensing can be applied in the laboratory to address the impact of bioturbation on sediment properties. This possibly opens new perspectives for long term experiments concerning the role of bioturbation on sedimentary processes.*

KEY WORDS: clay content, *Corophium volutator*, grain size, hyperspectral measurements, remote sensing, water content

1. INTRODUCTION

Dependent on the surface materials, various fractions of electromagnetic radiation incident on the surface are reflected, absorbed and/or emitted (Lillesand and Kiefer, 2000). Some materials, such as the pigment chlorophyll *a*, water and clay absorb light at specific wavelengths due to molecular vibration and rotation, while other materials, such as vegetation, scatter near-infrared light due to the internal structure of plant leaves (Lillesand and Kiefer, 2000). Remote sensing devices capture the reflected radiation which can be used to identify and quantify surface materials. With the development of high spectral resolution laboratory, field and airborne sensors, the reflected signal can be used to predict surface properties qualitatively (Thomson *et al.*,

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1998; Adam *et al.*, 2006; Deronde *et al.*, 2006) and, to some extent, quantitatively (Rainey *et al.*, 2003; Carrère *et al.*, 2003). Remote sensing has the great advantage that it is a nondestructive method and less time consuming than conventional sampling.

In laboratory conditions, hyperspectral signals have been used to quantify mud (Kooistra *et al.*, 2003; Lagacherie *et al.*, 2008; Adam, 2009), organic matter (Ben-dor *et al.*, 2002; Kooistra *et al.*, 2003), chlorophyll *a* (Méléder *et al.*, 2003; Combe *et al.*, 2005) and moisture content (Ben-dor *et al.*, 2002; Adam, 2009) in sediments. In the field, good results have been obtained to estimate chlorophyll *a* content (Méléder *et al.*, 2003; Carrère, 2004; Combe *et al.*, 2005; Murphy *et al.*, 2005; Adam, 2009) and moderate results to quantify mud (Kooistra *et al.*, 2003; Rainey *et al.*, 2003; Lagacherie *et al.*, 2008) and moisture (Ben-dor *et al.*, 2002; Adam, 2009) content.

Few studies used remote sensing data to predict distribution of macrobenthos on an intertidal flat (Yates *et al.*, 2003; van der Wal *et al.*, 2008). However, the effect of bioturbation on the remotely sensed signal, and hence, the potential of remote sensing to detect bioturbation, have not been investigated. Nevertheless, bioturbation is recognised as one of the major processes that influence the structure and function of sediment environments (Lohrer *et al.*, 2004; Meysman *et al.*, 2006). Sediment reworking results from various activities, and strongly affects the physical, chemical and biological characteristics of marine sediments (Rhoads, 1974; Aller, 1982; Hall, 1994; Jones *et al.*, 1994; Rowden *et al.*, 1998). As such, bioturbation could potentially influence remote sensing analyses through changing sediment properties.

The mud shrimp, *Corophium volutator* (Crustacea, Amphipoda) is an abundant deposit and/or filter feeding species in intertidal mudflats, where it lives in U shaped burrows in the upper 5 cm of the sediment (Meadows and Reid, 1966). Population densities frequently reach > 20,000 ind./m² and in summer months densities can locally increase up to 100,000 – 140,000 ind./m² (Gerdol and Hughes, 1994b). The high population densities make *C. volutator* a critical species in many mudflat ecosystems, where it is a significant prey of migratory shore birds and juvenile flounder (Murdoch *et al.*, 1986; Boates *et al.*, 1995). Through its activities, *Corophium* alters sediment properties by changing porosity, water content, grain size distribution and the chemistry of the sediment (Jones and Jago, 1993; Gerdol and Hughes, 1994a; Pelegri and Blackburn, 1994b; Limia and Raffaelli, 1997; Mermillod-Blondin *et al.*, 2004). However,

contradicting results are often found and still no consensus on the real effect of *Corophium* on the physical characteristics of the sediment is reached.

The objectives of the present study were twofold:

- 1) to examine the physical impact of bioturbation by *C. volutator* on water content and grain size of the sediment with conventional, destructive techniques using microcosms in laboratory conditions, i.e. without any confounding factors such as the presence of microphytobenthos and differences in sediment characteristics,
- 2) to investigate whether physical changes caused by bioturbation of a macrobenthic species influence the hyperspectral signal

2. MATERIAL AND METHODS

2.1. Experimental setup

Sediment and *C. volutator* were collected in October 2007 in the Flemish nature reserve "IJzermending", a mudflat-saltmarsh area in Nieuwpoort (Belgium, 51°08'N, 2°44'E). The sediment was defaunated by three cycles of 24h freezing-24h thawing. Grain size analysis (Malvern Mastersizer 2000 laser diffraction) showed that freezing-thawing did not alter median grain size (t-test, $p=0.48$). To reduce natural heterogeneity and to obtain equal starting conditions, the sediment was homogenised by thorough mixing before use. Sediment microcosms were established by transferring the homogenised sediment into PVC cores (15 cm deep and 8 cm internal diameter) to a depth of 10 cm. Fifteen cores were placed in an aquarium (0.8 x 0.8 m) in a temperature controlled climate room ($16\pm 1^\circ\text{C}$) with a 12:12h light:dark regime, and subjected to a simulated tidal regime, resembling the natural tidal conditions (i.e. 3h of submersion and 9h of emersion, salinity= 32 psu). Three days after microcosm preparation, *C. volutator* was added to the PVC cores in different densities. Three treatments were set up (five replicates per treatment): (1) no *Corophium* i.e. control (C), (2) 25 *Corophium*, i.e. low density (5,000 ind./m²; LD), and (3) 100 *Corophium*, i.e. high density (20,000 ind./m²; HD). All treatments were placed randomly in the aquarium during 16 days¹³. On day 16, hyperspectral measurements of the sediment surface were performed 3h after emersion. Immediately thereafter, the surface sediment was frozen with liquid nitrogen (± 1 cm deep), based on the contact coring technique pioneered by Wiltshire *et al.*

¹³ Mortality was very low, on average $1\% \pm \text{SD } 1.2\%$.

(1997). This technique allows for undisturbed surface samples and avoids changes in water content by draining of the pore water. Afterwards the upper 0.5 cm of the core was used to determine water content and grain size in the surface layer.

2.2. Hyperspectral and physical measurements

Spectral reflectance measurements Hyperspectral measurements were acquired with the Analytical Spectral Device (ASD) spectrometer (FieldSpec® Pro FR, Analytical Spectral Devices Inc., Boulder, Colorado, USA), recording the reflectance from 350 till 2,500 nm, i.e. in the visible (VIS), near-infrared (NIR) and shortwave-infrared (SWIR) region of the spectrum. The spectral resolution is 3 nm for the 350-1,000 nm region and 10 nm for the 1,000-2,500 nm region. Spectral reflectance measurements were acquired at 50 cm height, nadir looking with a field of view of 1°, so that a small area of 0.6 cm² was sampled. The reflectance was measured at four positions in each microcosm on the sediment surface to include spatial heterogeneity. Black cloths, which absorb more than 95% of the light, were hung around the experimental setup and put on the table to avoid diffuse light entering the sensor. The light source was a tungsten halogen 50-W OSRAM lamp and calibration was performed every 10 min using a Spectralon® panel (0.30 x 0.30 m Labsphere, North Sutton, USA), which has a quasi-Lambertian reflectance higher than 98 % over a range from 400 to 1,500 nm and higher than 93 % from 1,500 to 2,500 nm. Hence, the Spectralon® panel is characterised by a reflectance factor for each wavelength. The relative reflectance from the sediment (radiant exitance from the sediment/radiant exitance from the Spectralon® panel) was multiplied by the Spectralon® reflectance factors to obtain absolute reflectance. In laboratory conditions, the instrument and the light source are stable enough for calibration to be performed with a 10 min time interval, since 10 min after the calibration, the reflectance of the Spectralon® panel was almost equal to 100%, except for small deviations situated at the extreme upper and lower ends of the spectrum.

In order to avoid measurements with an incorrect position of the sample under the ASD, causing reflectance from the PVC cores, a quality procedure based on the overall brightness of the spectrum was performed. One spectrum of the high density treatment had to be removed, because the difference in the visible light between this spectrum and the mean of the other high-density spectra was very large (more than five times the standard deviation of the correctly measured high-density spectra).

Water content Frozen sediment from 0 to 0.5 cm depth is weighed after removal of the animals to determine wet weight, subsequently freeze-dried for 12h, and weighed again. Water content¹⁴ was calculated as follows:

$$\text{Water content (\%)} = \frac{\text{Wet sample weight (g)} - \text{Dry sample weight (g)}}{\text{Wet sample weight (g)}} * 100$$

Grain size Grain size analysis was carried out on the freeze-dried samples (8 cm Ø) after determination of the water content. Grain size distribution (according to the Wentworth scale)¹⁵ and mean grain size (µm) were determined using a Malvern Mastersizer 2000 laser diffraction, capable of detecting the 0.02 µm to 2 mm grain size range.

2.3. Absorption features

Absorption can be quantified by 1) the ratio between minimal reflectance in the absorption feature and reflectance outside the absorption feature: R_b/R_c (R_b is reflectance at maximum absorption, R_c is reflectance out of absorption feature), 2) the scaled band depth after continuum removal, and 3) the scaled band area of the absorption feature after continuum removal (Fig. 1). Continuum removal is a normalisation technique in order to compare absorption features from a common baseline. This continuum consists of straight-line segments that connect local spectra maxima. It is then removed by dividing it into the actual spectrum (Clark and Roush, 1984). The continuum is calculated preferably between the local maxima of the absorption feature of interest. If there is no local maximum, the shoulder of the dip or a pre-defined wavelength is considered for continuum removal.

Specifically, we used the absorption dips at 1,450 nm and 2,206 nm as assessors for respectively water and clay content. A laboratory spectrum for dry fine sand with clay shows absorption features of clay at around 1,420 nm, 1,950 nm and 2,206 nm (Fig. 2; Adam *et al.*, 2008), where the latter is caused by Al-OH bonds in the clay mineral (Yang *et al.*, 2000). Absorption at around 1,420 nm and 1,950 nm is also due to clay molecular water absorbing light (Hunt, 1977; Yang *et al.*, 2000). Therefore, the best assessor for clay content is the absorption dip at 2,206 nm.

¹⁴ We measured bulk water content in the top 0.5 cm layer.

¹⁵ The clay fraction was defined as all particles < 4 µm.

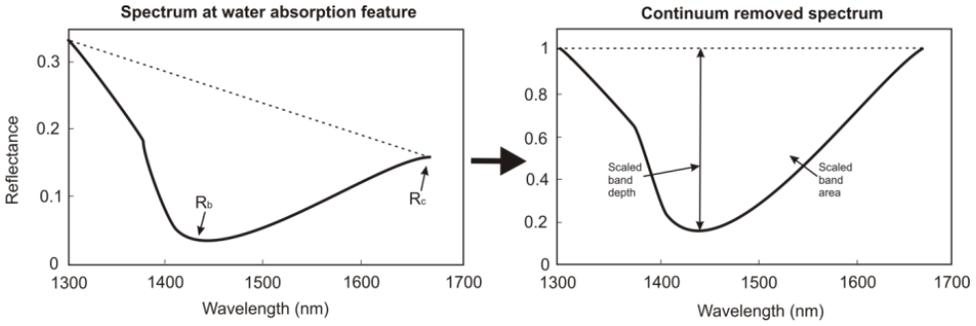


Figure 1: Measures that quantify absorption features. R_b is the reflectance at maximum absorption, R_c is the reflectance out of absorption feature. Example for the water absorption feature at 1450 nm.

The spectrum of moist fine sand shows water absorption features at 970 nm, 1,190 nm, 1,450 nm and 1,950 nm (Fig. 2; Adam *et al.*, 2008). The first two disappear when the sediment is drier, and the latter becomes saturated at relatively low moisture contents (measurements by S. Adam). Furthermore, the best prediction potential of relative moisture content in laboratory conditions was obtained using the scaled band area of the water absorption feature at 1,450 nm (measurements by S. Adam). For these reasons, the absorption at 1,450 nm was considered in the further analysis.

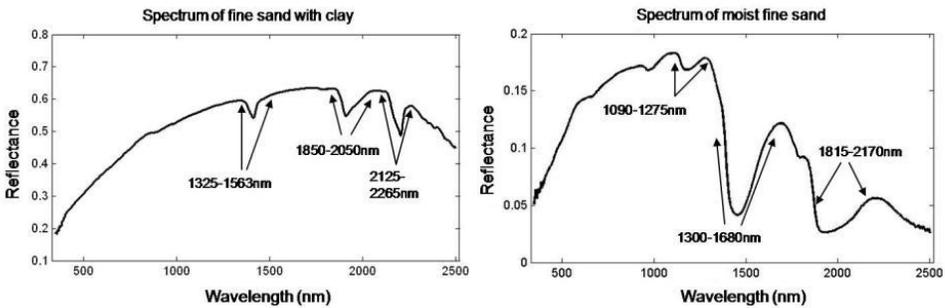


Figure 2: Spectrum of dry fine sand with clay (66% by weight) (left) and of moist fine sand (RMC=27%) (right) (from Adam *et al.*, 2008)

2.4. Data analysis

Differences in density treatments were tested using one-way ANOVA. Percentage data (clay, water content) were arcsine-square root transformed, reflectance (x10) and mean grain size data were log transformed to meet assumptions of normality (Shapiro Wilks' test) and homogeneity of variances (Cochran and Bartlett tests). Whenever appropriate, a Tukey's Post Hoc test was used to assess differences between treatments.

Furthermore, to investigate relationships between density, physical characteristics and spectral measurements, Spearman rank or Pearson correlations were performed.

3. RESULTS

3.1. Physical characteristics

The high-density treatment (HD) had a significantly ($p < 0.01$, Tukey's Post Hoc) lower percentage clay (mean \pm SD, $3.3 \pm 0.1\%$) in the upper layer than the low-density (LD; $3.6 \pm 0.2\%$) and the control treatment (C; $3.6 \pm 0.06\%$) (Fig. 3a). Furthermore, we found a negative correlation between density and clay percentage (Spearman $r = -0.70$, $p = 0.0037$). Although, mean grain size of HD ($179 \pm 3 \mu\text{m}$) was larger compared to C and LD (LD = $177 \pm 3 \mu\text{m}$, C = $176 \pm 2 \mu\text{m}$; Fig. 3b), no significant differences in mean grain sizes were found.

Table 1: One-way ANOVA table to determine significant differences for the different physical characteristics (clay percentage, mean grain size and water content) between the different treatments. Clay percentage and water content were arcsine-square root transformed, median grain size was log-transformed. Significant p-levels are highlighted.

	df	Clay % MS	Clay % F	p	Mean MS	Mean F	p	Water % MS	Water % MS	p
Treat	2	0.0001	8.9	0.004	0.00005	1	0.4	0.002	37.4	<0.001
Error	12	0.00002			0.00004			0.00005		

Water content increased significantly when *Corophium* was present, both for LD ($19 \pm 0.7\%$) and HD ($20 \pm 0.6\%$), compared to C ($17 \pm 0.4\%$; Fig. 3c, Table 1). No difference however, was found between LD and HD (Tukey's Post Hoc, $p > 0.05$). Water content correlated positively with density (Spearman $r = 0.74$, $p = 0.0017$).

3.2. Hyperspectral measurements

The reflectance in the visible region of the spectrum differed significantly between the density treatments, as shown in Table 2 for the reflectance at 545 nm and 630 nm and in Fig. 4. The continuum removed spectra at the absorption dip of water at 1,450 nm (Fig. 5) show that the dips are maximum for the treatments with *Corophium* and minimum for C. As expected, the scaled band area of the water absorption feature at 1,450 nm was positively correlated with water content (Pearson's $r = 0.8$, $p = 0.0006$). Differences between the three treatments (Table 2) were significant for the scaled band area at

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1,450 nm, where C differed significantly from LD and HD (Tukey's Post Hoc, resp. $p=0.0003$ and $p=0.005$; Fig. 6).

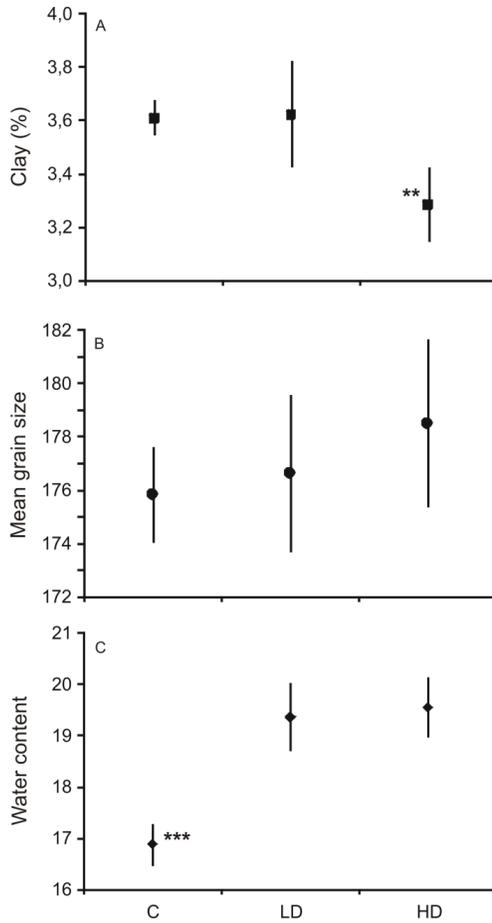


Figure 3: (A) Average percentage of clay (B) average mean grain size and (C) average water content (in %) for the different treatments ($n=5 \pm S.D.$) (C = Control, LD = Low Density, HD = High Density), * * and *** indicate significance levels of Tukey's Post Hoc

Table 2: One-way ANOVA table to determine significant differences for the hyperspectral measurements (reflectance at 545 nm, reflectance at 630 nm and scaled band area 1,450 nm) between the different treatments. All variables were log (x10) transformed. Significant p-levels are highlighted.

	df	Refl 545 MS	Refl 545 F	p	Refl 630 MS	Refl 630 F	p	Area 1450 MS	Area 1450 F	p
Treat	2	0.01	15.9	<0.001	0.01	16.8	<0.001	0.02	21	<0.001
Error	11	0.0009			0.0007			0.0008		

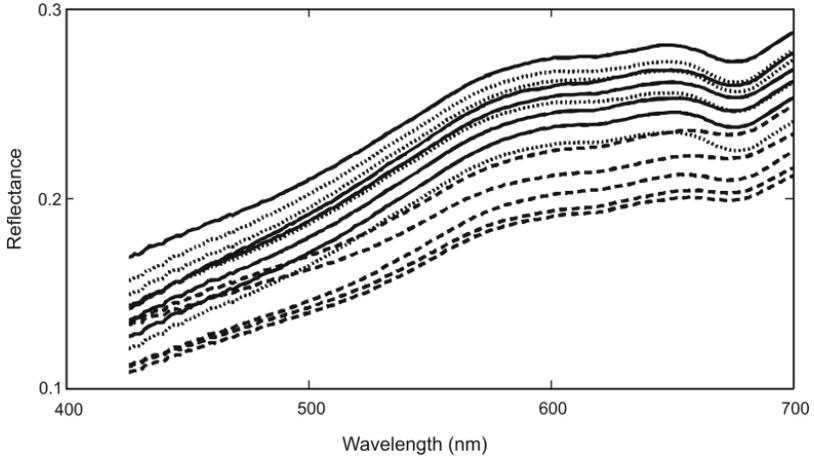


Figure 4: Detail of the reflectance spectra in the visible region of the light (545 nm and 630 nm) from all five replicates of the three different treatments. Control :dashed lines, low density: solid lines and high density: dotted lines.

For the clay absorption feature at 2,206 nm, scaled band area was zero for all treatments. The absence of a clay absorption feature was due to the absorption by water and the high signal noise in this region of the spectrum.

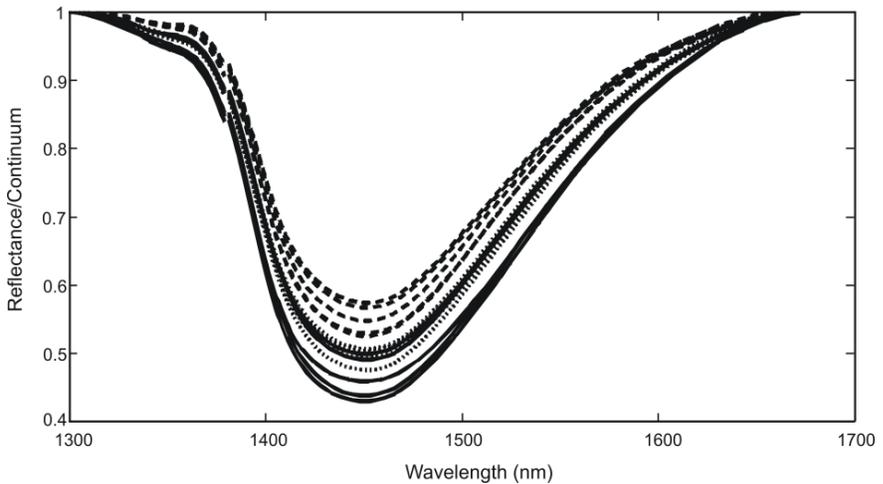


Figure 5: Continuum removed reflectance spectra at the water absorption feature at 1450 nm for all five replicates from the different density treatments. Control: dashed lines, low density: solid lines and high density: dotted lines.

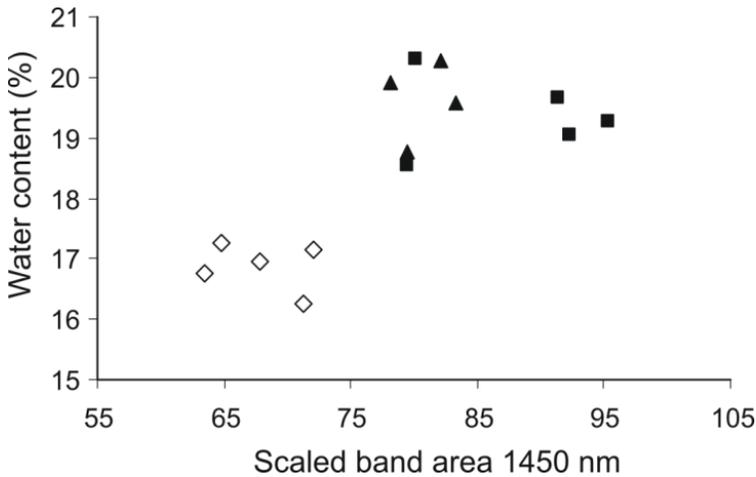


Figure 6: Scatterplot of scaled band area 1,450 nm versus water content (in %) for the different treatments (□ = Control, ■ = Low Density, ▲ = High Density)

4. DISCUSSION

4.1 Physical impact of *Corophium volutator*

Our experiment showed that whenever *Corophium* was present, both in low and high density, changes in water content of the surface sediment were visible, i.e. water content of the top half cm increased. No difference was detected between the two density treatments, but the difference between control sediment and sediment with *Corophium* was significant. Gerdol and Hughes (1994a) found results consistent with our study, while other studies found opposite results (Meadows and Tait, 1989) or no influence of *Corophium* on water content (Grant and Daborn, 1994; Limia and Raffaelli, 1997; de Deckere *et al.*, 2000). In our study, however, no biofilm was present (unlike in the above mentioned studies), to be able to focus on the effect caused solely by *C. volutator*. Thus, in our experiment, complexity was reduced by excluding confounding factors such as the presence of a biofilm, since it has been proven more than once that the presence of a biofilm increases water content of the sediment surface (Defew *et al.*, 2003; Orvain *et al.*, 2003). The presence of a biofilm might explain why in some studies no difference was found between a control with biofilm and treatments with *Corophium*. In a control treatment, the biofilm retains water, which increases water content. On the other hand, in the *Corophium* treatment, the biofilm is grazed upon by *Corophium* (Gerdol and Hughes, 1994b), so it does not retain water, but then again *Corophium* influences the

water content due to bioturbation. This indicates that the impact of the presence of a biofilm and the presence of *Corophium* on the water content counterbalance each other and no significant effects are found. On the other hand, the absence of a biofilm could possibly affect bioturbation rates, since food is absent. As is generally the case for infauna (Rhoads, 1974), the presence of *C. volutator* led probably to an increase in pore space. In addition, the saturation on emersion increased, caused by retention of water due to the secretion of burrow linings (Meadows *et al.*, 1990; Gerdol and Hughes, 1994a), and this caused an increase in water content in the sole presence of *Corophium*. It should be considered however, that the drainage in the laboratory was different from natural conditions, since groundwater level was not able to drain as in natural conditions, and there was also no drying due to sun or wind. Nevertheless, conditions were constant and standard for all treatments, so we can expect that observations made were consistent, but the water content for this top layer was higher than in natural conditions, both for control and density treatments.

Another physical change visible in the top half centimeter due to bioturbation of *C. volutator* was the increase in mean grain size at high density. This increase was mainly caused by the significant loss of the clay fraction in the top layer. *Corophium volutator* is known to actively resuspend particles during feeding and burrowing (de Deckere *et al.*, 2000), and moreover, *Corophium* was observed to flush its burrows immediately after submersion (De Backer *et al.*, 2010). Both activities probably contribute to the loss of the finest particles, since these smallest, and thus lightest, particles resuspend easier and get washed away with the tides, while the heavier and biggest particles remain on the sediment surface. Only at high density, the decrease in clay percentage was significant indicating that only at high densities *Corophium* considerably contributes to the sediment grain size distribution. This result is consistent with a previous study that quantified bioturbation effects of *Corophium* using luminophores (De Backer *et al.*, unpublished data= Chapter 3). In that study, density of *Corophium* was found an important factor affecting the magnitude of bioturbation, and mainly high densities of *C. volutator* significantly contributed to sediment mixing (De Backer *et al.*, unpublished data).

4.2 Physical changes influencing remote sensing

Results showed that the changes in water content due to bioturbation of *C. volutator* affected the spectral reflectance. The higher percentage of water content in the treatments with *Corophium*, increased the reflectance of the visible light (545 and 630 nm). At low sediment moisture conditions, the sediment becomes darker (decrease in reflectance) with increasing sediment moisture, but this relation is reversed at a certain water content which is dependent on the soil hydrodynamic properties (Weidong *et al.*, 2002). This means that, for the high water contents of our sediment samples, the reflectance increases for higher water content and the change in reflectance is the highest in the visible light for sediment with high water content (Weidong *et al.*, 2002). This increase in reflectance can be explained by a water film covering all the sediment particles and changing the scattering processes (Neema *et al.*, 1987). The difference in water content between control and density treatments was also noted in an increase in light absorption at around 1,450 nm by water. The light absorption was quantified by the scaled band area of the water absorption feature, which is a better measure than the scaled band depth to quantify water content, because at these high water contents, the absorption dip not only becomes deeper, but also wider (Adam *et al.*, 2008). No differences in water absorption between density treatments were measured, which was consistent with the analysed water contents.

The small, but significant decrease in clay content on the sediment surface for the high density treatment did not influence the hyperspectral measurements. The clay absorption dip at 2,206 nm has been used in laboratory conditions in dry soils to assess differences in clay content (Lagacherie *et al.*, 2008; Adam, 2009) with a root mean square error of prediction between 3.5 and 5.8 weight % (Lagacherie *et al.*, 2008) and between 1.3 and 1.4 weight % (Adam, 2009). Since the differences in clay content for the control, low-density and high-density treatments were smaller than these error values, the clay absorption dip was not successful to detect changes in clay content between the treatments. Furthermore, the absorption dip was not visible in the spectra, since clay absorption degrades with increasing moisture content due to water absorbing light in this region of the spectrum (Adam *et al.*, 2008; Adam, 2009). In general, quantification of clay content in moist sediments using hyperspectral remote sensing is very difficult (Rainey *et al.*, 2003; van der Wal and Herman, 2007; Adam, 2009).

4.3 Some considerations and conclusions

Our laboratory results showed that the bioturbation activity of a species can influence the hyperspectral signal. *Corophium volutator* was used to test this objective, and we observed an increase in water content in the presence of *Corophium*. This increase could be measured hyperspectrally, through an increased reflectance in the visible light and an increase of scaled band area of the water absorption feature at 1.450 nm. With conventional destructive sampling, no difference was found between the low-density and high-density treatment, and this was consistently measured hyperspectrally. However, generalisation to other macrobenthic species should be done with care, because each species alters physical, chemical and biological characteristics of marine sediments in a different way (Rhoads, 1974; Aller, 1982; Meadows and Meadows, 1991; Hall, 1994; Rowden *et al.*, 1998). These macrobenthos-mediated effects on sediment processes are strongly influenced by species-specific life modes (Mermillod-Blondin *et al.*, 2005). Nevertheless, the application of remote sensing in the laboratory seems promising to address the impact of bioturbation in terms of water content. For *Corophium*, there was no difference in water content between different densities, but the hyperspectral results indicate that, if for other species differences in water content would exist and also be large enough and consistent, hyperspectral laboratory measurements should be able to detect them.

A decrease in clay content was measured at high densities of *Corophium*, probably due to resuspension and wash out of this finest fraction by bioturbation. This decrease was too small to be detectable by remote sensing. Airborne spectral measurements have been used to determine in situ sediment properties in an undisturbed manner (Ben-dor *et al.*, 2002; Carrère *et al.*, 2003; Deronde *et al.*, 2006; Lagacherie *et al.*, 2008). But even though hyperspectral airborne sensors, such as HyMap, contain the spectral detail necessary to quantify water absorption, it is not possible to extrapolate the obtained results from this study to field conditions, because we used a simplified ecosystem in which only one mechanism was studied. Yet in natural intertidal sediments, these comparatively simple relationships disappear, being replaced with complex interactions between the biological and physical components which create considerable temporal and spatial variability, resulting in apparently site-specific responses in the properties of sediment (Riethmüller *et al.*, 1998; Defew *et al.*, 2002; Chapman and Tolhurst, 2004). Nevertheless, this study showed that bioturbation can significantly influence remote

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sensing analyses. Thus, remote sensing for the assessment of environmental variables needs to take into account the potential impact of bioturbation activities, because they significantly affect reflectance properties.

Furthermore, this study proved the usefulness of the application of remote sensing to study biologically induced changes in sediment properties in a nondestructive manner. Future laboratory experiments studying animal-sediment relationships can gain advantage in using hyperspectral measurements. Changes in physical surface properties can be measured without disturbing the sediment surface and it is possible to follow up biologically reworked sediments in time. This can, especially, open new perspectives for long term experiments concerning the role of bioturbation on sedimentary processes.

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