CrossMark

REGULAR ARTICLE

Compositional aspects of herbaceous litter decomposition in the freshwater marshes of the Florida Everglades

Oliva Pisani · Min Gao · Nagamitsu Maie ·
Toshikazu Miyoshi · Daniel L. Childers ·
Rudolf Jaffé ⓘ

Abstract

Aims Litter decomposition in wetlands is an important component of ecosystem function in these detrital systems. In oligotrophic wetlands, such as the Florida Everglades, litter decomposition processes are dependent on nutrient availability and litter quality. The aim of this study was to assess the differences and changes in chemical composition of above- and belowground plant tissues at different stages of decomposition, and to compare them to organic matter accumulating in wetland surface soils.

Methods To understand the chemical changes occurring during the early stages of litter decomposition in wetlands, short-term subaqueous decomposition patterns of above- and belowground tissues from *Cladium jamaicense* and *Eleocharis cellulosa* were investigated at two freshwater marsh sites in the Florida Everglades. The composition of litter at different stages of decomposition was compared to

that of the two end-members, namely fresh plant tissues and soil organic matter (SOM), in an effort to assess both the gradual transformation of this organic matter (OM) and the incorporation of above- vs. belowground biomass to wetland soils. The chemical composition of the litter and of surface soils was assessed using solid-state ^{13}C nuclear magnetic resonance spectroscopy.

Results Decomposition indices (alkyl/*O*-alkyl ratio, Aromaticity index) of *Cladium* and *Eleocharis* leaves varied during incubation likely reflecting physical leaching processes followed by a shift to microbial decomposition. Overall, *Eleocharis* leaves were more labile compared to *Cladium* leaves. Relative to aboveground litter, the belowground biomass of both species was more resistant to degradation, and roots were more resistant than rhizomes. Compared to the observed early diagenetic transformations of the plant litter, the SOM is at a more advanced stage of degradation, suggesting that

O. Pisani · M. Gao · R. Jaffé (✉)
Southeast Environmental Research Center, Florida International University, University Park Campus, OE-148, Miami, Florida 33199, USA
e-mail: jaffer@fiu.edu

M. Gao · R. Jaffé
Department of Chemistry & Biochemistry, Florida International University, Miami, FL 33199, USA

N. Maie
School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

T. Miyoshi
Department of Polymer Science, University of Akron, Akron, OH 44325, USA

D. L. Childers
School of Sustainability, Arizona State University, Tempe, AZ 85287, USA

Present Address:
O. Pisani
USDA-ARS, Southeast Watershed Research Laboratory, Tifton, GA, USA

the decomposition of litter and belowground biomass prior to its incorporation into wetland soils requires longer degradation times than those applied in this study.

Conclusions Litter decomposition in Everglades' freshwater marshes is driven by a combination of tissue quality and site characteristics such as hydroperiod and nutrient availability, ultimately leading to the accumulation of peat.

Keywords Litter decomposition · *Cladium jamaicense* · *Eleocharis cellulosa* · Soil organic matter · Soil formation · Florida Everglades

Introduction

The decomposition of litter and soil organic matter (SOM) in wetlands controls internal nutrient cycling, soil accumulation/loss and peat production (Newman et al. 2001), and is an important component of ecosystem function in these detrital systems (Mitsch and Gosselink 2015). Decomposition in wetlands involves aerobic and anaerobic processes and is often incomplete under anaerobic conditions that result from inundation (DeBusk and Reddy 2005; Kayranli et al. 2010). The slow decomposition of plant remains is a main factor in the formation of organic-rich peat soils (Mitsch and Gosselink 2015). Because wetlands contain about one-third of the global soil organic carbon pool (~450×10^{15} g C; Mitsch and Gosselink 2015), the relationship between SOM and litter decomposition is an important component of the C cycle in these ecosystems (Bridgham et al. 2006; Kayranli et al. 2010).

The initial stages of litter decomposition in wetlands are rapid and include processes such as litter fragmentation and leaching of soluble materials (Davis et al. 2006; Maie et al. 2006; Valiela et al. 1985). This is followed by longer-term and slower decomposition processes attributed to microbial degradation (Benner et al. 1990; Benner et al. 1991; Tremblay and Benner 2006). Litter breakdown is affected by the tissue nutrient and fiber content because high nutrient litter decomposes faster than low nutrient litter (Melillo et al. 1982; Rubio and Childers 2006; Serna et al. 2013). In addition, some tissue components, such as lignin, are more resistant to microbial degradation compared to polysaccharides (Melillo et al. 1982). Consequently, lignin becomes increasingly enriched in decomposing plant

tissues after the more labile components, such as polysaccharides, have been broken down (Benner et al. 1987). Other environmental factors and climatic conditions, including nutrient availability, temperature, hydrology, and light availability, are also important regulators of litter decomposition rates in wetland ecosystems (Gingerich et al. 2014; Poi de Neiff et al. 2006).

In oligotrophic wetland ecosystems litter decomposition processes are extremely sensitive to subtle environmental changes (Rubio and Childers 2006). The Florida Everglades is a subtropical wetland that is characteristically oligotrophic and phosphorus-limited (Noe et al. 2001). Typical freshwater marsh plant communities include extensive areas of sawgrass (*Cladium*offset *jamaicense* Crantz.) growing on higher elevation ridges and deeper wet prairie and slough communities dominated by Gulfcoast spikerush (*Eleocharis cellulosa* Torr.; Rubio and Childers 2006; Todd et al. 2010). These macrophytes are major contributors to the organic peats that underlie much of this ecosystem (McVoy et al. 2011), and their biomass, productivity, and decomposition have been linked to the formation of the typical ridge-and-slough landscape of the Everglades (Larsen and Harvey 2010). Anthropogenic activities in the form of nutrient loading and hydrologic changes have been shown to cause changes in vegetation quality and quantity (Noe and Childers 2007; Saunders et al. 2015) and in the loss of the characteristic ridge-and-slough landscape (Larsen et al. 2007). In addition, even small changes in nutrient availability have been shown to increase litter and organic matter (OM) decomposition rates (Corstanje et al. 2006; Davis 1991; Qualls and Richardson 2000; Newman et al. 2001; Pisani et al. 2015; Serna et al. 2013).

The rates of decomposition of *Cladium* and *Eleocharis* aboveground litter in the Florida Everglades are dependent on nutrient availability and litter quality (Rubio and Childers 2006). Using litterbag deployments for up to 18 months, these authors found that *Cladium* leaf litter decomposed faster at sites with higher water levels (longer hydroperiods) and higher soil total phosphorus (TP) content, while *Eleocharis* litter decomposed faster at sites with higher nitrogen (N) concentrations. In addition, the litter quality (the C:N and C:P molar ratios of the initial plant tissue) of *Eleocharis* was higher than that of *Cladium*, resulting in a generally faster decomposition of the former compared to the latter. Although the relationships among *Cladium* and *Eleocharis* litter decomposition rates, litter quality, and nutrient availability

are now well established (Corstanje et al. 2006; Davis 1991; Newman et al. 2001; Rubio and Childers 2006; Serna et al. 2013), not much is known about how the chemical composition of plant tissues affects early decomposition stages of either the above- or belowground biomass of these two macrophyte species. As such, the objective of this study was to determine changes in litter composition during a short-term litterbag decomposition experiment (12 months) performed on aboveground and belowground biomass of both *Cladium jamaicense* and *Eleocharis cellulosa*. An attempt was made to compare rates of litter decomposition between long and short hydroperiod sites, to assess how certain hydrological drivers can affect these rates. It is important to note that periphyton mats are also abundant at both study sites, but are more productive at the long hydroperiod site (Ewe et al. 2006). Their presence may influence SOM accumulation rates and composition, including OM co-metabolism. While periphyton-derived OM has been found to accumulate in Everglades soils (Neto et al. 2006), its influence on the accumulation of emergent vegetation-derived SOM remains unknown. Because the two macrophytes studied are major contributors of OM to Everglades' soils (McVoy et al. 2011), this study is expected to lead to a better understanding of the biogeochemical controls leading to the gradual transformation of and the relative contributions of above- vs. belowground macrophyte litter incorporation to wetland soils.

Methods

Site description and sample collection

The litterbag experiment (Rubio and Childers 2006) was conducted at two freshwater marsh sites in the Florida Everglades—one in the Shark River Slough (SRS) and one in the Taylor Slough (TS; Table 1). These two sites are part of the ongoing Florida Coastal Everglades-Long Term Ecological Research program (FCE LTER; http://fcelter.fiu.edu). Both sites are located in freshwater marsh environments, but differ in their hydrology, nutrient availability, soil type, and plant biomass and productivity patterns. The SRS site is characterized by a long hydroperiod (9–12 months inundation time), while TS is defined as short hydroperiod (< 8 months; Gunderson 1994). The peat soils of SRS marshes are characterized by their high organic content while TS marshes feature carbonate-rich

marl soils. As to nutrient levels, P and N availability are higher at the SRS site compared to the TS site (Childers et al. 2003, 2006a; Chambers RM and Pederson 2006). The dominant emergent vegetation at both sites is sawgrass (*Cladium jamaicense*) at higher elevations and spikerush (*Eleocharis cellulosa*) in the wet prairies. *Cladium* favors shallow water depths and typically grows on ridges while *Eleocharis* is better adapted to the deep water levels of sloughs (Gunderson 1994; Todd et al. 2010). *Cladium* productivity is typically higher at SRS compared to TS (Childers et al. 2006b; Ewe et al. 2006) while *Eleocharis* productivity is positively related to water level and hydroperiod (Childers et al. 2006b). Both sites are also characterized by abundant periphyton mats that have been shown to contribute detrital OM to the underlying soils (Neto et al. 2006; Pisani et al. 2013).

Senesced leaves that had not been shed by the plant were clipped above the water surface, air dried and cut up (Rubio and Childers 2006). Fiberglass mesh litterbags (15 cm^2) containing ~5 g of *Cladium* and *Eleocharis* leaf material were prepared in December 2001 and deployed in February 2002 as part of a previously published study (Rubio and Childers 2006). Triplicate samples of the litterbags were placed on the soil surface of the site where the litter was collected and were retrieved in triplicate after 2, 4, 6, and 12 months. Upon collection, all bags were rinsed gently with deionized water, dried at 70 °C, and ground in a Wiley Mill for further analysis. At the beginning of the study, a subset of litter from each site was also characterized for initial conditions.

Since the roots and rhizomes of plants likely follow different decomposition patterns compared to the aboveground biomass, litterbag experiments for these belowground tissues were also conducted. Duplicate samples of the roots and rhizomes of *Cladium* and *Eleocharis* were collected by hand at SRS and TS at the end of July 2005 and stored on ice for transport to the laboratory. Senescent roots, rhizomes and other debris were carefully separated from live belowground biomass to provide uniform starting materials. Litterbags (10 cm × 5 cm) were prepared from nylon fiber material and were filled with oven-dried (3 days at 50 °C) *Cladium* (~ 1 g) or *Eleocharis* (0.1–0.45 g) roots and rhizomes. The bag mesh size was 1.0 mm to minimize the intrusion of roots from the surrounding soil environment. The litterbags were buried vertically 10 cm below the soil surface in August 2005 at the site where the

Table 1 Main properties of the studied sites located in the Shark River Slough (SRS3) and Taylor Slough (TS3), including hydroperiod, soil type, soil total phosphorus (TP), soil total nitrogen (TN), water TP, and water TN

Site	Hydroperiod	Soil type	Soil TP (mg g^{-1})*	Soil TN (mg g^{-1})*	Water TP (μM)*	Water TN (μM)*
SRS	Long (9–12 months)	Peat	0.60	39.51	0.3	69.6
TS	Short (< 8 months)	Marl	0.14	7.47	0.1	29.3

*Values obtained from Rubio and Childers (2006)

belowground tissues were collected. The replicate bags were collected after 4 and 12 months from each site and for each tissue type. Litterbags were stored on ice in ziplock® plastic bags after collection, and brought back to the laboratory. The litterbags were opened and any intruded new roots were picked out manually. The remaining material was washed carefully with distilled water, then dried at 50 °C for 3 days. The dry samples were ground using a Wig-l-Bug for further analysis.

Two soil cores were collected at each site: one was collected in an area dominated by *Cladium* and the other was collected in an area dominated by *Eleocharis*. The cores were taken by manually pushing butyrate coring sleeves (25 cm × 7.2 cm i.d.) into the soil. Upon retrieval, the cores were capped and kept on ice during transport to the laboratory where they were extruded and sectioned into 2 or 3 cm sub-samples. The individual sections were placed into clean glass jars and kept at −10 °C. The top 3.5 cm section of the surface soils were used for this study. The soils were freeze-dried, ground, and passed through a No. 32 mesh (500 μm) sieve to remove coarse materials such as small rocks and plant tissues prior to analysis.

Solid-state ^{13}C nuclear magnetic resonance spectroscopy

The dried and ground leaf, root, and rhizome material, as well as the surface soil samples were analyzed by ^{13}C cross polarization with magic angle spinning nuclear magnetic resonance (CP-MAS NMR) spectroscopy (Bruker Avance). The plant material was analyzed without any pretreatment while the soil samples were treated with 1 N HCl to eliminate carbonates (Ertel and Hedges 1984). Each sample was packed into a 4 mm zirconium rotor and the spectra were acquired on a Bruker DSX300 spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 4 mm CP-MAS probe and using a ramp-CP pulse program with a spinning rate of

13 kHz and a ramp-CP contact time of 1 ms and 2 s recycle delay. The spectra were processed using a Lorentzian line broadening of 100 Hz. The spectra were integrated into four chemical shift regions corresponding to: alkyl carbon (0–45 ppm) from cutin, suberin, aliphatic side-chains and lipids; *O*-alkyl carbon (45–110 ppm) including oxygen and nitrogen-substituted aliphatic constituents from carbohydrates, peptides and the methoxy carbon in lignin; aromatic and phenolic carbon (110–160 ppm) from lignin, amino acids in peptides and black carbon; and carboxyl and carbonyl carbon (160–210 ppm) from phenolic acids, fatty acids, and amino acids in peptides (Baldock et al. 1992; Simpson et al. 2008).

All chemical shifts were expressed with respect to tetramethylsilane by using the carbonyl carbon of glycine as an external reference (176.03 ppm). The total NMR signal (0–210 ppm) was normalized to 100% for comparisons of the relative contents of each type of carbon. In order to assess early diagenetic transformations of the plant biomass, two ^{13}C NMR spectra-based proxies, the alkyl/*O*-alkyl ratio (Baldock et al. 1992; Simpson et al. 2008) and the Aromaticity index (Lu et al. 2003; Maie et al. 2005, 2006) were calculated for the leaves, roots and rhizomes, and for the surface soils. The alkyl/*O*-alkyl ratios were calculated by dividing the areas of the alkyl and the *O*-alkyl regions of the spectra. This ratio can be used to estimate the relative degree of OM degradation because *O*-alkyl compounds are labile and more easily degraded compared to alkyl compounds, resulting in increased ratios with progressive degradation (Baldock et al. 1992; Simpson et al. 2008). The Aromaticity index was calculated by dividing the area of the aromatic region of the spectrum by the sum of the aromatic, *O*-alkyl and alkyl regions. The Aromaticity index has been used to characterize the extent of dissolved OM degradation in the Everglades, under the assumption that aromaticity increases during decomposition (Lu et al. 2003; Maie et al. 2005, 2006).

Results and discussion

Litterbag mass loss rates of above and belowground biomass

The data for the percent weight remaining of the *Cladium* and *Eleocharis* aboveground tissues were taken from Rubio and Childers (2006). Those results are briefly presented here and further interpreted for a complete comparison with the mass loss rates of the belowground biomass (roots and rhizomes). The above- (Rubio and Childers 2006) and belowground litter lost weight gradually during the 12-month litterbag experiment (Fig. 1a-c). However, the aboveground tissues of both plant species lost weight more rapidly than did their corresponding roots and rhizomes. Compared to aboveground herbaceous tissues, belowground biomass typically contains more recalcitrant material such as the long-chain aliphatic structures of suberin and the large polyphenolic structures of lignin (Kögel-Knabner 2002; Otto and Simpson 2006; Rasse et al. 2005). These compounds are generally more resistant to degradation (Rasse et al. 2005; Thevenot et al. 2010), leading to slower decomposition rates for roots and rhizomes.

Eleocharis leaf litter decomposed more rapidly compared to *Cladium* leaves at both sites (Fig. 1a; Rubio and Childers 2006). During decomposition, *Eleocharis* leaves have been reported to release larger amounts of organic carbon compared to *Cladium* leaves (Maie et al. 2006), suggesting this more rapid weight loss included the leaching of dissolved OM. In addition, the higher nutrient content of *Eleocharis* tissues compared to *Cladium* (Rubio and Childers 2006; Serna et al. 2013) likely contributed to more rapid degradation of tissues of the former species. In comparison, the belowground biomass showed slower decomposition rates compared to aboveground biomass. *Eleocharis* roots showed a faster decomposition rate compared to *Cladium* roots at both sites (Fig. 1b) likely due to the higher tissue nutrient content of the former. The decomposition rates of rhizomes showed a similar pattern when compared to leaf litter decomposition rates (Fig. 1c), except that no clear differences were observed in the rhizomes decomposition rate of *Eleocharis* versus *Cladium*. The lack of a difference in decomposition rate between the rhizomes of *Cladium* and *Eleocharis* suggests that these tissues may be compositionally similar between the two macrophyte species compared to the leaves and the roots.

When comparing long versus short hydroperiod effects (SRS vs. TS) on decomposition rates, the above- (Rubio and Childers 2006) and belowground tissues of both plant species lost weight more rapidly at SRS compared to TS (Fig. 1a–c). This is likely due to the more rapid leaching of soluble compounds from plant material at the long hydroperiod site (SRS) compared to the short hydroperiod site (TS), due to continuous inundation of the litter. Slower decomposition rates at the short hydroperiod site may have been associated with litter and soil desiccation during the dry season (Battle and Golladay 2007; Rubio and Childers 2006). These shifts from inundated to dry conditions likely reduced the invertebrate and microbial decomposer communities at this site, slowing litter decomposition. In addition to hydroperiod, higher nutrient availability (particularly P) at SRS compared to TS (Childers et al. 2003) likely contributed to more rapid litter decomposition at this site. Thus, herbaceous litter decomposition in freshwater
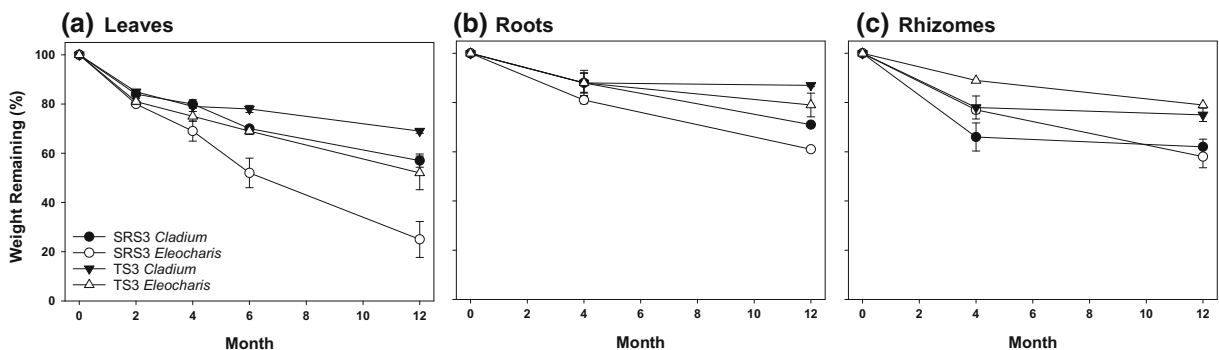

Fig. 1 Percent weight (%) remaining after one year of incubation of the leaves (**a**), roots (**b**) and rhizomes (**c**) of *Cladium* and *Eleocharis* at SRS and TS. The data used to generate this figure was taken from Gao (2007) and Rubio and Childers (2006). Standard deviations for triplicate measurements for the leaf incubations are indicated in (**a**); Ranges for duplicate measurements are indicated for root and rhizome incubations in (**b**) and (**c**)

Everglades' marshes appears to be influenced by litter chemistry and quality as well as by environmental conditions such as nutrient availability and hydroperiod.

Since the OC concentration of SRS soils is higher than TS soils (peat vs. marl), these results might at first seem unexpected. However, note that this litterbag incubation experiment was aimed to unveil early diagenetic changes of plant litter from major vegetation of the Everglades (1-year incubation study), where physical leaching of soluble compounds followed by microbial breakdown seem to be the two dominant degradation processes. Lignin degradation by white-rot fungi under oxidative conditions has been reported, but at later stages of decomposition than the time-scale of this study (Virzo De Santo et al. 2009). Therefore, if considering a longer-term decomposition scenario, plant litter at TS characterized by shorter hydroperiod and seasonal dry-out, might in fact experience more degradation compared to that at SRS due to seasonal open-air conditions.

Molecular characterization of litter composition during decomposition

The ^{13}C CP-MAS NMR spectra of plant leaves at time 0, after 12 months of incubation and for the surface soils are presented in Fig. 2. At both sites, the most intense region of the spectrum for both *Cladium* and *Eleocharis* fresh above- and belowground material was the *O*-alkyl carbon region (Tables 2 and 3), containing signals from oxygen-substituted carbon bonds of carbohydrates and methoxy groups of lignin (Simpson et al. 2008). The carbohydrate region of the spectrum of the fresh plant material is characterized by an intense signal at ~72–74 ppm (Fig. 2) that represents cellulose and hemicellulose. The signal originating from the anomeric carbon of cellulose and hemicellulose appears at ~95–105 ppm (Preston et al. 2000) while lignin is represented by the methoxy peak at ~56 ppm. The NMR spectra of dissolved OM leached from *Cladium* and *Eleocharis* leaves from the Everglades are also characterized by an intense *O*-alkyl carbon region (Lu et al. 2003; Maie et al. 2006), suggesting that carbohydrates and lignin-derived phenols are important components of plant leachates in this ecosystem. Similar to degradation studies of terrestrial grasses (Nelson and Baldock 2005; Preston et al. 2000), the intensity of the alkyl carbon region originating from cutin, suberin, aliphatic side-chains, and lipids, was not very intense in the NMR spectrum of both *Cladium* and *Eleocharis*. The higher

intensity of the *O*-alkyl region of the NMR spectrum relative to the alkyl region, resulted in relatively low alkyl/*O*-alkyl ratios for the fresh leaves of both species (Tables 2 and 3). Although the vegetation studied here are not classified as grasses, the alkyl/*O*-alkyl ratios reported here are similar to values for fresh above- and belowground biomass of perennial grasses (Mathers et al. 2007).

Some minor differences were observed in the chemical composition of the two plant species. At both sites, the NMR spectra of the *Eleocharis* above- and belowground fresh tissues had a slightly higher intensity of *O*-alkyl carbon compared to *Cladium* tissues (Tables 2 and 3). The higher relative intensity of the *O*-alkyl region in the *Eleocharis* spectrum may explain its more rapid decomposition rate compared to *Cladium* (Fig. 1a–c), presuming that leaching of *O*-alkyl compounds is involved. In fact, *Eleocharis* leachates have been found to contain more total sugars compared to *Cladium* leachates after 36 days of incubation (Maie et al. 2006).

In the fresh leaves, roots, and rhizomes of both plant species, the second most intense region of the NMR spectrum was the aromatic and phenolic carbon region (Tables 2 and 3) which includes signals from polyphenols and lignin (Simpson et al. 2008). The aromatic and phenolic region of the NMR spectrum was more intense for the *Cladium* above- and belowground tissues compared to the *Eleocharis* tissues (prior to decomposition), resulting in a higher Aromaticity index for the former at both sites (Tables 2 and 3). Because aromatic compounds are considered to be more resistant to degradation compared to polysaccharides (Benner et al. 1990; Richardson et al. 1999), a higher Aromaticity index for the *Cladium* tissues suggests an enhanced stability of this OM. The Aromaticity index was found to be consistently and significantly higher for the roots compared to leaves and rhizomes for *Cladium* at both study sites. In contrast, the Aromaticity index of the *Eleocharis* roots was only slightly higher when compared to the corresponding leaf and rhizome litter at SRS, while the roots and rhizomes were higher than the leaves at TS. Finally, the carboxylic and carbonyl carbon region from aliphatic and phenolic acids, esters, and amino acids in peptides (Simpson et al. 2008), was the least intense region of the spectrum for the fresh plant material for both species. The presence of these carbon moieties increases with progressive oxidative decomposition of OM and their low abundance in the fresh plant material suggests less extent of decomposition.

Fig. 2 Solid-state ^{13}C nuclear magnetic resonance (NMR) spectra of the fresh litter (0 months), decomposing litter (12 months) and surface soil for *Cladium* at SRS (**a**), *Eleocharis* at SRS (**b**), *Cladium* at TS (**c**) and *Eleocharis* at TS (**d**)

Top: Soil
Middle: Decomposing litter (12 months)
Bottom: Fresh litter (0 months)
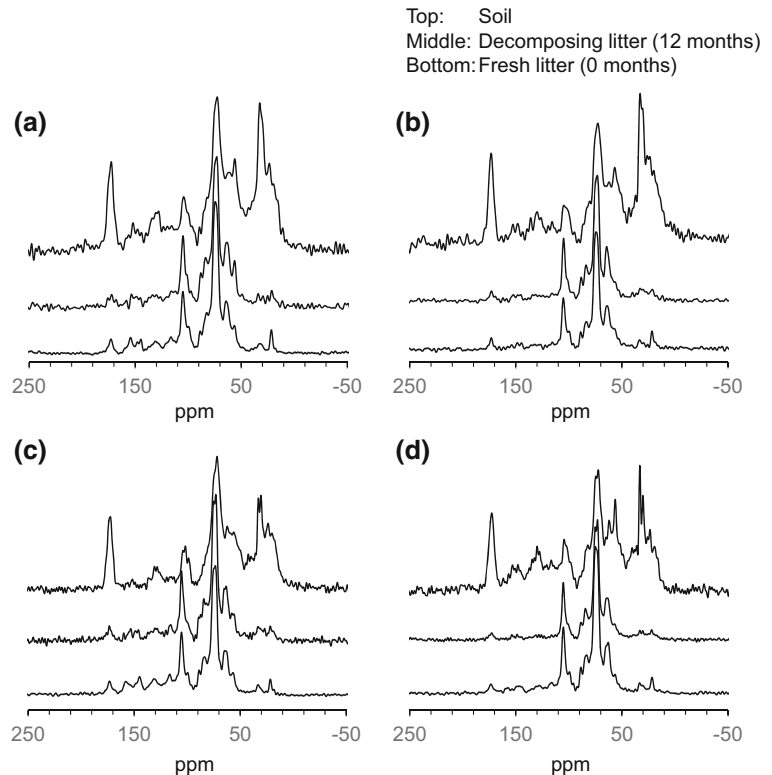
Changes in the composition of *Cladium* and *Eleocharis* litter during the decomposition process were assessed using the alkyl/*O*-alkyl ratio and the Aromaticity index. The alkyl/*O*-alkyl ratios for *Cladium* and *Eleocharis* leaves at SRS and TS, although quite variable, generally increased with incubation time, likely due to the loss during leaching and through the degradation of *O*-alkyl components, leading to an enrichment in the alkyl carbon signal (Maie et al. 2006). However, the alkyl/*O*-alkyl ratios increased to a different extent between the sites and the plant species: For the *Cladium* leaf litter a maximum value was reached after 4 months, then decreased at SRS while it initially decreased and then increased again at TS (Tables 2 and 3). The alkyl/*O*-alkyl ratio of the *Eleocharis* leaf litter initially decreased and then increased at SRS, while it increased gradually at TS (Tables 2 and 3). The smaller overall increase in the alkyl/*O*-alkyl ratio for *Cladium* compared to *Eleocharis* may reflect the refractory nature of *Cladium* under low nutrient conditions.

The Aromaticity index of the *Cladium* and *Eleocharis* litter varied only by a few % (Tables 2 and 3). *Cladium* leaves, roots and rhizomes showed a reduction in Aromaticity index during decomposition. In contrast, *Eleocharis* showed no change in Aromaticity index during decomposition of leaves and roots, but an increase in the rhizome tissue. While these variations generally represented only a few % points of change, they reflected differences in composition between the two plant types and between leaves, roots, and rhizomes within a species.

The root and rhizome experiments showed an increase in the alkyl/*O*-alkyl ratio for *Eleocharis* while the net change was higher for SRS compared to TS (Tables 2 and 3). This increase in alkyl/*O*-alkyl ratio was due to the preferential loss of *O*-alkyl components via leaching which was more pronounced at the long hydroperiod site. *Cladium* roots and rhizomes, on the other hand, showed no significant change at SRS, and only a slight decrease in the alkyl/*O*-alkyl ratio at TS. These patterns suggest that belowground tissues are generally more refractory than aboveground litter. The Aromaticity index of the *Cladium* root biomass decreased at both sites during the 12-month incubation. The *Eleocharis* root biomass showed an initial decrease in Aromaticity index followed by an increase at both sites (Tables 2 and 3), possibly due to a combination of loss of polar aromatics through leaching, followed by an enrichment due to loss of more labile components during microbial reworking.

Table 2 Relative contribution (%) of the four main carbon structures to the solid-state ^{13}C CP-MAS NMR spectra after integration and the resulting alkyl/O-alkyl ratios and Aromaticity index (%) for the leaf, root and rhizome litterbags and for the surface soils at SRS

Vegetation	Sample	Time (months)	Alkyl (0–45 ppm)	O-Alkyl (45–110 ppm)	Aromatic + Phenolic (110–160 ppm)	Carboxylic + (160–210 ppm)	Alkyl/O-Alkyl	Aromaticity (%)
Cladium	Leaves	0	5	75	15	5	0.07	16
		2	6	76	14	4	0.08	15
		4	10	71	15	5	0.14	15
		6	10	76	11	3	0.13	12
		12	7	76	13	4	0.09	14
	Roots	0	6	69	20	5	0.08	21
		4	7	73	16	4	0.10	17
		12	7	71	17	5	0.09	18
	Rhizomes	0	6	75	15	4	0.09	16
		4	7	78	11	3	0.09	12
		12	7	74	14	5	0.09	15
	Soil		30	47	14	10	0.63	15
Eleocharis	Leaves	0	8	79	8	5	0.10	8
		2	6	81	9	4	0.07	9
		4	8	78	9	5	0.11	9
		6	10	78	9	3	0.12	9
		12	10	80	8	2	0.12	8
	Roots	0	8	79	10	4	0.10	10
		4	9	80	8	3	0.11	8
		12	12	72	10	5	0.17	11
	Rhizomes	0	8	79	9	4	0.10	9
		4	8	77	11	4	0.10	12
		12	9	72	13	6	0.13	14
	Soil		35	44	12	8	0.79	14

As with the biomass litter, the ^{13}C NMR spectra of the soils (Fig. 2) were dominated by signals originating from O-alkyl carbon (Tables 2 and 3). However, the intensity of this signal was much lower in the soils compared to the plant tissues at the corresponding sites. This was accompanied by an increase in alkyl components in soils, resulting in elevated alkyl/O-alkyl ratios at both sites (3 to 5 times higher than in the biomass). The higher alkyl/O-alkyl ratios of the SOM compared to the decomposing plant tissues suggested enrichment in alkyl carbon and/or further degradation of the biomass-derived OM after incorporation into the soil. The SOM alkyl/O-alkyl ratios were higher in SRS soils compared to those from TS (Tables 2 and 3), suggesting the selective accumulation of more refractory OM at the long hydroperiod site. This is in agreement with the higher alkyl/O-alkyl ratios for *Eleocharis* litter compared to *Cladium* after 12 months of

decomposition, indicating the likely contribution of the former to the formation of organic-rich peat soils in this area. At SRS, the *Eleocharis*-dominated soil had a higher alkyl/O-alkyl ratio compared to the *Cladium*-dominated soil (Table 2), suggesting that the more labile and nutrient-rich content of *Eleocharis* tissues decompose more quickly and contribute less to the SOM pool. The opposite trend was observed at TS, where the *Cladium*-dominated soil had a higher alkyl/O-alkyl ratio compared to the *Eleocharis*-derived soil (Table 3). Notably, the soils at both sites also receive significant OM inputs from abundant periphyton mats (Neto et al. 2006; Pisani et al. 2013), and these inputs may be influencing this ratio by contributing O-alkyl constituents, such as carbohydrates, to the underlying soil. It is important to note that, while the longer hydroperiod SRS site is subject to longer inundation periods, TS marshes are commonly dry for several

Table 3 Relative contribution (%) of the four main carbon structures to the solid-state ^{13}C CP-MAS NMR spectra after integration and the resulting alkyl/*O*-alkyl ratios and Aromaticity index (%) for the leaf, root and rhizome litterbags and for the surface soils at TS

Vegetation	Sample	Time (months)	Alkyl (0–45 ppm)	*O*-Alkyl (45–110 ppm)	Aromatic + Phenolic (110–160 ppm)	Carboxylic + Carbonyl (160–210 ppm)	Alkyl/*O*-Alkyl	Aromaticity (%)
Cladium	Leaves	0	7	72	16	5	0.10	17
		2	6	72	16	6	0.08	17
		4	9	69	17	6	0.13	18
		6	10	74	12	4	0.14	13
		12	9	73	14	5	0.12	14
	Roots	0	7	64	24	5	0.11	25
		4	7	71	17	5	0.10	18
		12	7	74	15	4	0.09	16
	Rhizomes	0	8	74	12	6	0.11	13
		4	6	77	13	4	0.08	13
		12	7	78	11	4	0.09	11
	Soil		30	52	8	10	0.57	9
Eleocharis	Leaves	0	7	83	7	3	0.08	7
		2	8	83	6	3	0.10	6
		4	8	79	9	4	0.10	10
		6	8	77	10	5	0.11	11
		12	9	79	8	4	0.11	8
	Roots	0	7	79	11	3	0.09	11
		4	9	80	8	3	0.12	8
		12	10	76	10	5	0.13	10
	Rhizomes	0	7	78	10	4	0.09	11
		4	8	76	12	4	0.11	12
		12	8	75	12	5	0.11	13
	Soil		24	47	18	11	0.50	21

months of a typical dry season. This difference in hydrology means that SRS soils are always reduced, which slows decomposition rates, while the TS soils are aerobic for several months each year, accelerating the relative oxidation rate of SOM in these marshes.

The Aromaticity index of the SOM was different between the two sites and between the soils collected in *Cladium*- and *Eleocharis*-dominated areas. This was particularly the case at the short hydroperiod TS site, where the Aromaticity index of the *Cladium*-derived soil was much lower compared to that of the *Eleocharis*-derived soil (Table 3). These patterns may be influenced by the environmental characteristics preferred by *Eleocharis,* which is adapted to grow in deep-water sloughs (Gunderson 1994; Todd et al. 2010), where the main decomposers of lignin (white-rot fungi) are not found, and lignin decomposition may be suppressed under these conditions (Thevenot et al. 2010; Virzo De Santo et al. 2009). At the long hydroperiod SRS site, the Aromaticity index of the SOM was similar between *Cladium*- and *Eleocharis*-derived soils (Table 2). Based on these results, and in agreement with the alkyl/*O*-alkyl ratio, the long-hydroperiod site (SRS) seemed to accumulate more degraded, and thus refractory SOM, which are conditions that favor the formation of peat. Finally, the surface soils at both sites showed higher carboxyl and carbonyl carbon signals compared to the plant biomass. The intensity of the carboxyl and carbonyl carbon signals was slightly lower for the *Eleocharis*-derived soil compared to the *Cladium*-derived soil at SRS (Table 2) while it was not different between the two soils at the short hydroperiod site (Table 3). These patterns provided molecular-level evidence that wetland plant litter and belowground tissues undergo significant additional alterations, likely linked to

white-rot fungi oxidative degradation, at later stages of decomposition than the time-scale of this study (12 months), prior to incorporation into soils.

Conclusions

Wetland litterbag experiments showed changes in the molecular composition of both dominant plant species at both short and long hydroperiod sites over a 12-month period. The NMR spectra of *Cladium* and *Eleocharis* leaves suggested a balance between physical leaching processes (Maie et al. 2006) and microbial processing early in the decomposition process. At both study sites, *Eleocharis*-derived material decomposed more quickly than *Cladium* tissues, partly because *Eleocharis* has a higher initial nutrient content (Rubio and Childers 2006; Serna et al. 2013), because of differences in the OM molecular character, and as a result of differences in inundation regimes. Relative to the early diagenesis of litter, the SOM was present at a more advanced stage of degradation suggesting that the litter is more extensively reworked before incorporation into the soil compared to our litterbag experiments. This is likely due to the fact that the time-scale of this study was too short to observe changes in OM composition as a result of degradation by white rot fungi. The differences in decomposition patterns between litter (above- and below-ground after a 12 month period) and SOM, was greater at the long hydroperiod site (SRS), suggesting the accumulation of recalcitrant litter components such as aliphatic and aromatic structures, and oxidized lignin, which contribute to the formation of peat in this environment. In contrast, OM at the short hydroperiod site, and seasonally exposed to open-air conditions, results in a higher rate of respiration and less accumulation of degraded OM in soils. Although belowground biomass was more refractory early in the decomposition process, the geochemical proxies used in this study did not allow for a quantitative assessment of the sources of OM that make up Everglades soils. This suggests that leaves, roots, and rhizomes should be considered separately when evaluating their role in plant-SOM feedbacks (Ma et al. 2016).

Environmental implications

Most wetlands that accrete peat in the long term do so because the balance between OM production and decomposition is skewed to the former process, either because of long hydroperiods, oligotrophic conditions, or both (Mitsch and Gosselink 2015). Many parts of the Everglades landscape fit this model well. Past studies have demonstrated the importance of hydroperiod, nutrient availability, and source tissue quality as drivers of plant decomposition in Everglades' wetlands (Corstanje et al. 2006; Davis et al. 2006; Maie et al. 2006; Rubio and Childers 2006; Serna et al. 2013). This study supports those findings using novel organic geochemical techniques and proxies for OM lability and recalcitrance. Plant-derived OM in *Eleocharis* sloughs decomposes more quickly than *Cladium* tissues, leading to more rapid accumulation of SOM on *Cladium* ridges. Additionally, belowground tissues of *Cladium* are more resistant to decomposition than either *Cladium* leaves or *Eleocharis* roots and rhizomes. *Cladium* roots and rhizomes are important stabilizers of peat soils, and their slow rates of decomposition contribute to peat accretion in *Cladium* soils. These differential decomposition rates between shallow water ridges and deeper water sloughs contribute to the topographic equilibrium between these two habitats, and probably to the formation of the ridge-and-slough "corrugated" topography in the first place. These findings have implications for Everglades Restoration: Any hydrologic changes associated with this restoration that reduce the ridge-slough disparity in plant and SOM decomposition rates will likely contribute to the continuing loss of this characteristic topographic landscape feature.

References

Baldock JA, Oades JM, Waters AG, Peng X, Vassallo AM, Wilson MA (1992) Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biogeochemistry 16:1–42

Battle JM, Golladay SW (2007) How hydrology, habitat type, and litter quality affect leaf breakdown in wetlands on the Gulf Coastal Plain of Georgia. Wetlands 27:251–260

Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329:708–710

Benner R, Hatcher PG, Hedges JI (1990) Early diagenesis of mangrove leaves in a tropical estuary: bulk chemical characterization using solid-state ^{13}C NMR and elemental analyses. Geochim Cosmochim Ac 54:2003–2013

Benner R, Fogel ML, Sprague EK (1991) Diagenesis of below-ground biomass of Spartina alterniflora in salt-marsh sediments. Limnol Oceanogr 36:1358–1374

Bridgham SD, Megonigal JP, Keller JK, Bliss NB, Trettin C (2006) The carbon balance of North American wetlands. Wetlands 26:889–916

Chambers RM, Pederson KA (2006) Variation in soil phosphorus, sulfur, and iron pools among south Florida wetlands. Hydrobiologia 569:63–70

Childers DL, Doren RF, Jones R, Noe GB, Rugge M, Scinto LJ (2003) Decadal change in vegetation and soil phosphorus pattern across the Everglades landscape. J Environ Qual 32: 344–362

Childers DL, Boyer JN, Davis SE, Madden CJ, Rudnick DT, Sklar FH (2006a) Relating precipitation and water management to nutrient concentrations in the oligotrophic "upside-down" estuaries of the Florida Everglades. Limnol Oceanogr 51: 602–616

Childers DL, Iwaniec D, Rondeau D, Rubio G, Verdon E, Madden CJ (2006b) Responses of sawgrass and spikerush to variation in hydrologic drivers and salinity in Southern Everglades marshes. Hydrobiologia 569:273–292

Corstanje R, Reddy KR, Portier KM (2006) Typha latifolia and Cladium jamaicense litter decay in response to exogenous nutrient enrichment. Aquat Bot 84:70–78

Davis SM (1991) Growth, decomposition and nutrient retention of Cladium jamaicense Crantz and Typha domingensis Pers. in the Florida Everglades. Aquat Bot 40:203–224

Davis S, Childers DL, Noe G (2006) The contribution of leaching to the rapid release of nutrients and carbon in the early decay of wetland vegetation. Hydrobiologia 569:87–97

DeBusk WF, Reddy KR (2005) Litter decomposition and nutrient dynamics in a phosphorus enriched Everglades marsh. Biogeochemistry 75:217–240

Ertel JR, Hedges JI (1984) The lignin component of humic substances: distribution among soil and sedimentary humic, fulvic and base-insoluble fractions. Geochim Cosmochim Ac 48:2065–2074

Ewe SML, Gaiser EE, Childers DL, Iwaniec D, Rivera-Monroy VH, Twilley RR (2006) Spatial and temporal patterns of aboveground net primary productivity (ANPP) along two freshwater-estuarine transects in the Florida Coastal Everglades. Hydrobiologia 569:459–474

Gao M (2007) Chemical characterization of soil organic matter in an oligotrophic, subtropical, freshwater wetland system: sources, diagenesis and preservation. Florida International University, Dissertation

Gingerich RT, Merovich G, Anderson JT (2014) Influence of environmental parameters on litter decomposition in wetlands in West Virginia, USA. J Freshw Ecol 29:535–549

Gunderson LH (1994) Vegetation of the Everglades: determinants of community composition. In: Davis SM, Ogden JC (eds) Everglades: the ecosystem and its restoration, St. Lucie press, Delray Beach, Florida, pp 323–340

Hajje N, Jaffé R (2006) Molecular characterization of Cladium peat from the Florida Everglades: biomarker associations with humic fractions. Hydrobiologia 569:99–112

Kayranli B, Scholz M, Mustafa A, Hedmard Å (2010) Carbon storage and fluxes within freshwater wetlands: a critical review. Wetlands 30:111–124

Kögel-Knabner I (2002) The macromolecular organic composition of plant material residues as inputs to soil organic matter. Soil Bio Biochemist 34:139–162

Larsen LG, Harvey JW (2010) How vegetation and sediment transport feedbacks drive landscape change in the Everglades and wetland worldwide. Am Nat 176:E66–E79

Larsen LG, Harvey JW, Crimaldi JP (2007) A delicate balance: Ecohydrological feedbacks governing landscape morphology in a lotic peatland. Ecol Monogr 77:591–614

Lu XQ, Maie N, Hanna JV, Childers DL, Jaffè R (2003) Molecular characterization of dissolved organic matter in freshwater wetlands of the Florida Everglades. Water Res 37:2599–2606

Ma C, Xiong Y, Li L, Guo D (2016) Root and leaf decomposition become decoupled over time: implications for below- and above-ground relationships. Funct Ecol. https://doi.org/10.1111/1365-2435.12619

Maie N, Yang CY, Miyoshi T, Parish K, Jaffè R (2005) Chemical characteristics of dissolved organic matter in an oligotrophic subtropical wetland/estuarine ecosystem. Limnol Oceanogr 50:23–35

Maie N, Jaffè R, Miyoshi T, Childers DL (2006) Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland. Biogeochemistry 78:285–314

Mathers NJ, Jalota RK, Dalal RC, Boyd SE (2007) ^{13}C-NMR analysis of decomposing litter and fine roots in the semi-arid Mulga Lands of southern Queensland. Soil Bio Biochemist 39:993–1006

McVoy CW, Said WP, Obeysekera J, VanArman JA, Dreschel TW (2011) Landscapes and hydrology of the predrainage Everglades. University Press of Florida, Gainesville

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626

Mitsch WJ, Gosselink JG (2015) Wetlands. Wiley, New York

Nelson PN, Baldock JA (2005) Estimating the molecular composition of a diverse range of natural organic materials from solid-state 13C NMR and elemental analyses. Biogeochemistry 72: 1–34

Neto RR, Mead RN, Louda JW, Jaffé R (2006) Organic biogeochemistry of detrital flocculent material (floc) in a subtropical, coastal wetland. Biogeochemistry 77:283–304

Newman S, Kumpf H, Laing JA, Kennedy WC (2001) Decomposition responses to phosphorus enrichment in an Everglades (United States of America) slough. Biogeochemistry 54:229–250

Noe GB, Childers DL (2007) Phosphorus budgets in Everglades wetland ecosystems: the effects of hydrology and nutrient enrichment. Wetl Ecol Manag 15:189–205

Noe GB, Childers DL, Jones RD (2001) Phosphorus biogeochemistry and the impact of P enrichment: why is the Everglades so unique? Ecosystems 4:603–624

Otto A, Simpson MJ (2006) Evaluation of CuO oxidation parameters for determining the source and stage of lignin degradation in soil. Biogeochemistry 80:121–142

Pisani O, Louda JW, Jaffé R (2013) Biomarker assessment of spatial and temporal changes in the composition of flocculent material (floc) in the subtropical wetland of the Florida Coastal Everglades. Environ Chem 10:424–436

Pisani O, Scinto LJ, Munyon JW, Jaffé R (2015) The respiration of flocculent detrital organic matter (floc) is driven by phosphorus limitation and substrate quality in a subtropical wetland. Geoderma 241-242:272–278

Poi de Neiff A, Neiff JJ, Casco SL (2006) Leaf litter decomposition in three wetland types of the Paraná River floodplain. Wetlands 26:558–566

Preston CM, Trofymow JA, CIDEW Group (2000) Variability in litter quality and its relationship to litter decay in Canadian forests. Can J Bot 78:1269–1287

Qualls RG, Richardson CJ (2000) Phosphorus enrichment affects litter decomposition, immobilization, and soil microbial phosphorus in wetland mesocosms. Soil Sci Soc Am J 64: 799–808

Rasse DP, Rumpel C, Dignac MF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. Plant Soil 269:341–356

Richardson CJ, Ferrell GM, Vaithiyanathan P (1999) Nutrient effects on stand structure, resorption efficiency, and secondary compounds in Everglades sawgrass. Ecology 80:2182–2192

Rubio GA, Childers DL (2006) Controls on herbaceous litter decomposition in the estuarine ecotones of the Florida Everglades. Estuar Coasts 29:257–268

Saunders CJ, Gao M, Lynch JA, Jaffé R, Childers DL (2006) Using soil profiles of seeds and molecular markers as proxies for sawgrass and wet prairie slough vegetation in shark slough, Everglades National Park. Hydrobiologia 569:475–492

Saunders CJ, Gao M, Jaffé R (2015) Environmental assessment of vegetation and hydrological conditions in Everglades freshwater marshes using multiple geochemical proxies. Aquat Sci 77:271–291

Serna A, Richards JH, Scinto LJ (2013) Plant decomposition in wetlands: effects of hydrologic variation in a re-created Everglades. J Environ Qual 42:562–572

Simpson MJ, Otto A, Feng X (2008) Comparison of solid-state carbon-13 nuclear magnetic resonance and organic matter biomarkers for assessing soil organic matter degradation. Soil Sci Soc Am J 72:268–276

Thevenot M, Dignac MF, Rumpel C (2010) Fate of lignins in soil: a review. Soil Bio Biochemist 42:1200–1211

Todd MJ, Muneepeerakul R, Pumo D, Azaele S, Miralles-Wilhelm F, Rinaldo A, Rodriguez-Iturbe I (2010) Hydrological drivers of wetland vegetation community distribution within Everglades National Park, Florida. Adv Water Resour 33: 1279–1289

Tremblay L, Benner R (2006) Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus. Geochim Cosmochim Ac 70:133–146

Valiela I, Teal JM, Allen SD, Van Etten R, Goehringer D, Volkmann S (1985) Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. J Exp Mar Biol Ecol 89:29–54

Virzo De Santo A, De Marco A, Fierro A, Berg B, Rutigliano FA (2009) Factors regulating litter mass loss and lignin degradation in late decomposition stages. Plant Soil 318:217–228