



Uncovering the Fungal Community Composition of Alive and Dead *Posidonia oceanica* Matte

Sara Frasca^{1,2} · Annamaria Alabiso² · Marco Maria D'Andrea² · Luciana Migliore^{2,3} 

Received: 2 December 2024 / Accepted: 6 January 2025
© The Author(s) 2025

Abstract

Posidonia oceanica retains a large amount of carbon within its belowground recalcitrant structure, the ‘matte,’ which is characterized by low oxygen availability and biodegradation. Fungi may play a pivotal role in carbon sequestration within the matte, even if little/no information is available. To fill this gap, we profiled fungal communities from the upper and lower layers of alive and dead matte, by using an ITS2-5.8S rDNA metabarcoding approach. The study was conducted in a shallow coastal stretch of the Aegean Sea (Crete). Then, 184 operational taxonomic units were identified, predominantly belonging to Ascomycota, in alive and dead matte. Nevertheless, their composition significantly differed: the host-specific *Posidoniomyces atricolor* was dominant in alive but not in dead matte, while fast-growing saprotrophs, potentially accelerating the decomposition rate, increased in dead matte. These findings lay the groundwork for future investigations on the possible increase of biodegradation under the changing environmental conditions.

Keywords *Posidonia oceanica* · Alive matte · Dead matte · Fungal community · DNA metabarcoding

The Mediterranean seagrass *Posidonia oceanica* is regarded as an ecosystem engineer, able to stabilize sediments and sequester CO₂ as *blue carbon* [1, 2]. It exhibits a remarkable carbon storage capacity through its dense leafy canopy and its extensive belowground structures known as ‘matte’ (Supplementary data, Fig. S1). Indeed, approximately 21% of the annual CO₂ fixed by *P. oceanica* is sequestered within the matte [3], which can persist for thousands of years maintaining its integrity even after the meadows die [4, 5]. The long-term persistence is mainly due to the reduced biodegradation of the recalcitrant tissues and the anoxic conditions of the deep layers [6, 7], where the ability of microorganisms in decomposition processes is reduced, although their role is still understudied. Fungi are known to be able to decompose plant material via ligninolytic enzymes, breaking down recalcitrant compounds abundant in seagrass [8–10];

however, marine fungi are known to produce a wide range of hydrolytic and/or oxidative enzymes [11]. Moreover, recent studies revealed that fungal by-products—exudates and melanin—may reduce biodegradation and enhance soil carbon sequestration by stabilizing organic carbon [12–14]. However, environmental changes may modify fungal communities, reducing belowground carbon storage [15, 16].

We profiled the fungal community associated with *P. oceanica* matte in a living meadow (alive matte) and in nearby dead matte (dead matte), examining both the upper (at water–sediment interface) and lower layer (the deepest layer). Samples were collected in July 2021 at the Aegean Sea (35.335700 N, 25.281518 E) at 3–5 m depth, in three replicates (5-cm diameter cores, 8–13 cm in length, according to matte thicknesses), with the aim to determine fungal colonizer changes and their putative biodegradation activities. The matte samples were subjected to ITS2-5.8S rDNA metabarcoding by using an Illumina MiSeq platform. Sample preparation, extraction, sequencing, and bioinformatics analyses, according to Frasca et al. [17], are detailed in Supplementary data, Method section.

A total of 25,696 sequences (9583 in alive and 16,113 in dead matte), clustered in 184 OTUs with 97% sequence similarity (Supplementary data), were obtained after quality control/filtering steps. Nineteen OTUs had relative

✉ Luciana Migliore
luciana.migliore@uniroma2.it

¹ PhD Program in Evolutionary Biology and Ecology, Tor Vergata University of Rome, 00133 Rome, Italy

² Department of Biology, University of Rome Tor Vergata, 00133 Rome, Italy

³ eCampus University, 22060 Novedrate (CO), Italy

Fig. 1 Dominant fungal genera in alive/dead *P. oceanica* matte upper/lower layers (A = alive; D = dead; U = upper; Lo = lower); the genera are stacked in the same order in the figure as in the key

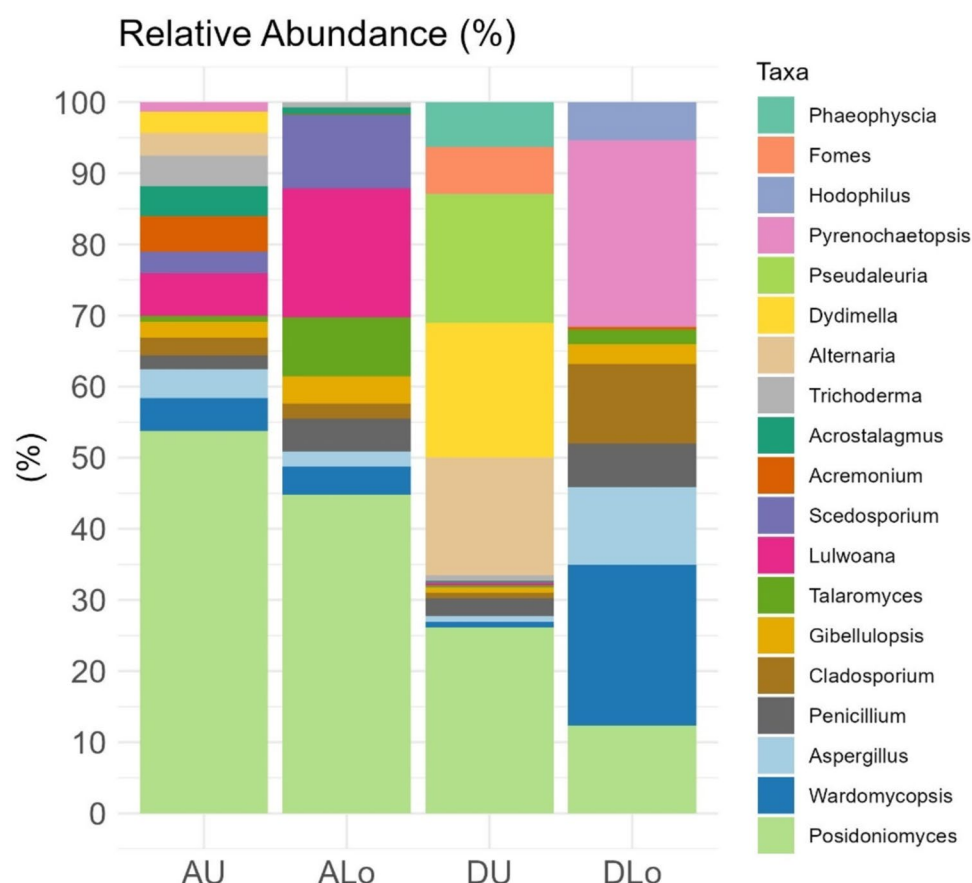


Table 1 Shannon index (H') in alive/dead *P. oceanica* matte upper/lower layers

<i>P. oceanica</i> 'matte'	Layers	H'
Alive	Upper	2.03 ± 0.64
	Lower	2.15 ± 0.76
Dead	Upper	2.41 ± 0.16
	Lower	2.06 ± 0.36

Table 2 Comparison of fungal colonizers in alive/dead *P. oceanica* matte upper/lower layers (PERMANOVA test, 999 permutations by using two distance metrics)

Comparison	Metrics	Test statistic	<i>p</i> -value
Alive/dead	Bray–Curtis	1.131	0.009
	UniFrac unweighted	1.470	0.027
Alive upper/lower	Bray–Curtis	1.016	ns
	UniFrac unweighted	0.842	ns
Dead upper/lower	Bray–Curtis	1.349	ns
	UniFrac unweighted	1.160	ns

abundance > 3% and were differently distributed across matte samples (Fig. 1).

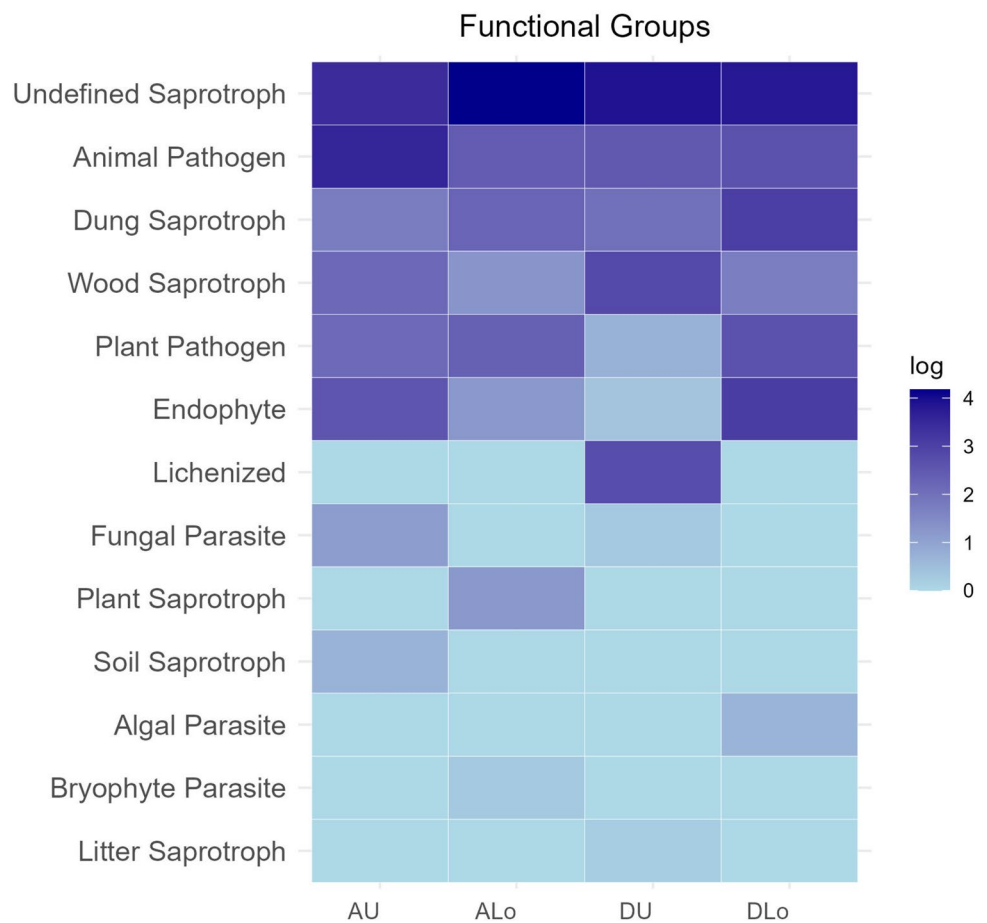
Alpha-diversity resulted quite similar in all samples, with not significant differences between the upper and lower layers of the matte and between the alive and dead matte (Kruskal–Wallis test; $p = \text{ns}$; Table 1). The highest diversity was found in the upper layer of dead matte, which is directly exposed to oxygenated seawater, allowing the recruitment of a great deal of microorganisms from water column.

Conversely, β -diversity resulted significantly different between alive/dead matte fungal colonizers, but not between upper/lower layers (Table 2). This heterogeneity related also to functional groups identified by FUNGuild analysis, which was significantly different between alive/dead matte (two-way PERMANOVA test; $F = 1.21$; $p = 0.03$). As expected,

saprotrophy was the dominant function (Fig. 2). The 131 OTUs classified based on their putative function through FUNGuild are reported in detail in Supplementary data, Table S2.

The taxonomic identification showed 97% of Ascomycota and only 3% of Basidiomycota. Ascomycota prefer generally aerobic environments, but their ability to utilize different compounds also under anoxic or sub-oxic conditions [18] allowed the colonization of both upper and lower layers, by displacing and/or increasing the mitochondrial number in oxygen deficiency conditions [19, 20]. This interpretation may explain the similarity of fungal community in the upper/

Fig. 2 Functional groups of fungi in alive/dead and upper/lower layers of *P. oceanica* matte (A=alive; D=dead; U=upper; Lo=lower)



lower samples, notwithstanding the possible oxygenation of the upper layer (due to living plants and hydrodynamics), absent in the lower layer. The Basidiomycota OTUs found in the matte were only 11 and exclusively present in dead matte (*Fomes inzengae* and *Hodophilus variabilipes* present > 2%, relative abundance); they were absent in alive matte. Some of them are efficient degraders of plant components and decaying material, putatively in line with the role of “late-stage” degraders in terrestrial systems [21]. Their absence or low relative abundance in matte may suggest a slow and weak decomposition rate.

The peculiar *P. oceanica* colonizer, *Posidonimyces atricolor* [22, 23], was found in all samples: it was the most abundant taxa in alive upper layer (50%) but decreased in the alive lower (42%); the decrease was more pronounced in dead matte (from 25% in dead upper to 11% in dead lower layer). *P. atricolor* is a slow growing aerobic pleosporalean fungus, which seems to be specifically associated to *P. oceanica* roots, where it forms intracellular resting and storage structures, known as microsclerotia, supposed to be responsible for the putative dark septate endophyte colonization pattern [22–24]. *P. atricolor* hyphae and microsclerotia are melanized and, in soil, melanized structures protect against extreme temperatures, UV irradiation, and

microbial degradation [13, 25, 26]. In a wider sense, this let us to hypothesize that root-symbiotic fungi may stabilize and sequester blue carbon in seagrass beds, as proposed by Vohník and Josefiová [24]. Furthermore, the highest relative abundance in both upper sections confirms that *P. atricolor* is more likely associated with *Posidonia* alive roots [27], the traces of its DNA may remain in dead roots and sediment, but progressively reducing after the plant death.

Beside *P. atricolor*, common colonizers of both alive and dead matte were the fast-growing saprotrophic *Aspergillus*, *Cladosporium*, *Penicillium*, and *Wardomyces*, showing the highest abundance in dead lower layer. There, they may contribute to matte degradation as, among these genera, there are degraders known to produce laccases, peroxidases, and tannases in the marine environment [9, 10]. *P. oceanica* has a high content of lignin which is refractory to bacterial digestion [8] and produces polyphenolic compounds, especially in stressful conditions [28, 29]. It is possible that part of the detected fast-growing saprobe DNA may belong to spores accumulated over time in the dead matte without significantly contributing to decomposition.

Some taxa were unique for specific matte layers: *Lulwoana* spp. were only found in alive matte and are supposed to be strictly associated with healthy *P. oceanica* roots [30],

although their association and beneficial relationship with *P. oceanica* remains is still discussed [23]. Anyway, the order Lulworthiales, to which *Lulwoana* belongs, is known to include saprobic genera [31], as indicated by the FUNGuild analysis. Furthermore, a novel insight into the roles of fungi in marine environment comes from the occurrence of *Phaeophyscia orbicularis*, a putative lichenicolous fungus, only found in the upper layer of dead matte. Submarine lichens are very rare: among the few reports, the fungus *Chadefaudia corallinarum* was found associated with red algae on seagrass leaves in the Mediterranean Sea [32].

In conclusion, the taxonomic composition of fungal communities associated with alive/dead *P. oceanica* matte significantly differed. *P. atricolor* confirmed its strong association with the living meadows, while in dead matte an enrichment of fast-growing saprotrophic groups could contribute to its degradation. The slow matte breakdown rate grants the carbon burial over time, but environmental change could alter this equilibrium. By increasing oxygenation (due to high hydrodynamics) and consequent biodegradation rate, the *P. oceanica* matte carbon sink capacity may be hampered.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00248-025-02492-6>.

Acknowledgements We thank Chiara Conte for field collection. We also thank CINECA award under the ISCRA initiative, for the availability of high-performance computing resources and support: the ISCRA CINECA Project Class C on the Galileo100 server, titled ‘FUN-SEA’ were granted to Sara Frasca (HP10CQP3QE project) and enabled the data elaboration. Sara Frasca held a PON doctorate in collaboration with GT50 s.r.l., while Annamaria Alabiso obtained an industrial doctorate in partnership with AlgaRes s.r.l.

Author Contribution S.F., A.A., M.M.D. and L.M. designed the study; S.F., A.A. performed data analysis; L.M. and M.M.D. supervised the statistical analyses; S.F. and L.M. drafted the manuscript. All authors reviewed the manuscript.

Funding Open access funding provided by Università degli Studi di Roma Tor Vergata within the CRUI-CARE Agreement. Sara Frasca and Annamaria Alabiso (from the XXXVII and XXXVI cycle, respectively) were funded by a three-year fellowship from the Ph.D. School of Evolutionary Biology and Ecology at Tor Vergata Rome University.

Data Availability The fungal DNA sequences have been deposited in the Sequence Read Archive (SRA) database of NCBI under BioProject ID: PRJNA1186200.

Declarations

Competing Interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated

otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Nellemann C, Corcoran E. (2009) Blue carbon: the role of healthy oceans in binding carbon: a rapid response assessment, UNEP/Earthprint
- Fourqurean JW, Duarte CM, Kennedy H, Marbà N, Holmer M, Mateo MA, Serrano O (2012) Seagrass ecosystems as a globally significant carbon stock. *Nat Geo* 5:505–509
- Pergent-Martini C, Pergent G, Monnier B, Boudouresque CF, Mori C, Valette-Sansevin A (2021) Contribution of *Posidonia oceanica* meadows in the context of climate change mitigation in the Mediterranean Sea. *Mar Environ Res* 165:105236
- Telesca L, Belluscio A, Criscoli A, Ardizzone G, Apostolaki ET, Frascetti S, Salomidi M (2015) Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. *Sci Rep* 5:12505
- Pedersen MØ, Serrano O, Mateo MÁ, Holmer M (2011) Temperature effects on decomposition of a *Posidonia oceanica* mat. *Aquat Microb Ecol* 65:169–182
- Canfield DE (1994) Factors influencing organic carbon preservation in marine sediments. *Chem Geol* 114:315–329
- Pedersen MF, Filbee-Dexter K, Frisk NL, Sárossy Z, Wernberg T (2021) Carbon sequestration potential increased by incomplete anaerobic decomposition of kelp detritus. *Mar Ecol Prog Ser* 660:53–67
- Klap VA, Hemminga MA, Boon JJ (2000) Retention of lignin in seagrasses: angiosperms that returned to the sea. *Mar Ecol Prog Ser* 194:1–11
- Panno L, Bruno M, Voyron S, Anastasi A, Gnani G, Miserere L, Varese GC (2013) Diversity, ecological role and potential biotechnological applications of marine fungi associated to the seagrass *Posidonia oceanica*. *N Biotechnol* 30:685–694
- Florio Furno M, Ferrero D, Poli A, Prigione V, Tuohy M, Oliva M, Varese GC (2022) Fungi from the sediments of the harbour of Livorno as potential bioremediation agents. In: Firenze University Press (ed) Ninth International Symposium “Monitoring of Mediterranean coastal areas: problems and measurement techniques”, pp 667–676.
- Bonugli-Santos RC, dos Santos Vasconcelos MR, Passarini MR, Vieira GA, Lopes VC, Mainardi PH, dos Santos JA, de Azevedo DL, Otero IVR, de Silva Yoshida AM, Feitosa VA, Pessoa A, Sette LD (2015) Marine-derived fungi: diversity of enzymes and biotechnological applications. *Front microbiol* 6:269
- Fernandez CW, Koide RT (2012) The role of chitin in the decomposition of ectomycorrhizal fungal litter. *Ecol* 93:24–28
- Fernandez CW, Heckman K, Kolka R, Kennedy PG (2019) Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming. *Ecol Lett* 22:498–505
- Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol Biochem* 34:139–162
- Drigo B, Kowalchuk GA, Knapp BA, Pijl AS, Boschker HT, Van Veen JA (2013) Impacts of 3 years of elevated atmospheric CO₂ on rhizosphere carbon flow and microbial community dynamics. *Glob Change Biol* 19:621–636
- Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD (2015) Carbon sequestration is related to mycorrhizal

- fungal community shifts during long-term succession in boreal forests. *New Phytol* 205:1525–1536
17. Frasca S, Alabiso A, D'Andrea MM, Cattaneo R, Migliore L (2024) Diversity and composition of *Posidonia oceanica*-associated bacterial and fungal communities: effect of boat-induced mechanical stress in the Villefranche-sur-Mer Bay (France). *Diversity* 16:604
 18. Cathrine SJ, Raghukumar C (2009) Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. *Mycol Res* 113:100–109
 19. Vödisch M, Scherlach K, Winkler R, Hertweck C, Braun HP, Roth M, Kniemeyer O (2011) Analysis of the *Aspergillus fumigatus* proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia. *J Proteome Res* 10:2508–2524
 20. Zain Ul Arifeen M, Chu C, Yang X, Liu J, Huang X, Ma Y, Liu C (2021) The anaerobic survival mechanism of *Schizophyllum commune* 20R-7-F01, isolated from deep sediment 2 km below the seafloor. *Environ Microbiol* 23:1174–1185
 21. Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Riedel K (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME J* 6:1749–1762
 22. Vohník M, Borovec O, Kolaříková Z, Sudová R, Réblová M (2019) Extensive sampling and high-throughput sequencing reveal *Posidoniomyces atricolor* gen. et sp. nov. (Aigialaceae, Pleosporales) as the dominant root mycobiont of the dominant Mediterranean seagrass *Posidonia oceanica*. *Mycoskeys* 55:59
 23. Vohník M (2022) Are lulworthioid fungi dark septate endophytes of the dominant Mediterranean seagrass *Posidonia oceanica*? *Plant Biol* 24:127–133
 24. Vohník M, Josefiová J (2024) Novel epiphytic root-fungus symbiosis in the Indo-Pacific seagrass *Thalassodendron ciliatum* from the Red Sea. *Mycorrhiza* 34:447–461
 25. Fan R, Gong X, Gao L, Shang W, Hu X, Xu X (2020) Temporal dynamics of the survival of *Verticillium dahliae* microsclerotia with or without melanin in soils amended with biocontrol agents. *Eur J Plant Pathol* 157:521–531
 26. Butler MJ, Day AW (1998) Destruction of fungal melanins by ligninases of *Phanerochaete chrysosporium* and other white rot fungi. *Int J Plant Sci* 159:989–995
 27. Lefebvre L, Compère P, Gobert S (2023) The formation of aegagropiles from the Mediterranean seagrass *Posidonia oceanica* (L.) Delile (1813): plant tissue sources and colonisation by melanised fungal mycelium. *Mar Biol* 170:19
 28. Migliore L, Rotini A, Randazzo D, Albanese NN, Giallongo A (2007) Phenols content and 2-D electrophoresis protein pattern: a promising tool to monitor *Posidonia* meadows health state. *BMC Ecol* 7:1–8
 29. Rotini A, Anello L, Di Bernardo M, Giallongo A, Valiante L, Migliore L (2013) Comparative analysis of bed density, total phenol content and protein expression pattern in *Posidonia oceanica* (L.) Delile. *Open J Ecol* 3:438–444
 30. Torta L, Burrano S, Giambra S, Conigliaro G, Piazza G, Mirabile G, Tomasello A (2022) Cultivable fungal endophytes in roots, rhizomes and leaves of *Posidonia oceanica* (L.) Delile along the coast of Sicily, Italy. *Plants* 11:1139
 31. Kohlmeyer J, Spatafora JW, Volkmann-Kohlmeyer B (2000) Lulworthiales, a new order of marine Ascomycota. *Mycologia* 92:453–458
 32. Kohlmeyer J, Kohlmeyer E (1979) Marine mycology. The higher fungi. Academic Press, New York, pp 70–78

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.