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PAPER

PATHOLOGY/BIOLOGY

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Vegetation Dynamics as a Tool for Detecting Clandestine Graves

ABSTRACT: The burial of a body can affect plant communities through mechanical disturbance and nutrient balance alteration. We performed an experimental trial using five swine carcasses buried in an open site in Italy. Vegetation dynamics was monitored recording monthly every plant individual on a regular sampling grid during 1 year on the graves, on an empty control grave, and on an undisturbed plot. Plant species composition and cover were significantly different between the disturbed and the undisturbed plots. Disturbed plots showed the increase in ruderal species and the reduction in stress-tolerant ones. Graves and the control grave could not be distinguished from each other. Disturbance was the main factor affecting plant cover, while the presence of a buried body did not affect vegetation dynamics. However, disturbance could be easily detected; the functional approach seems promising for the identification of dynamic patterns to be used in different biogeographic and ecological contexts.

KEYWORDS: forensic science, forensic botany, clandestine graves, CSR theory, plant succession, carcass burial

The research of clandestine burial sites is not an infrequent event. From an academic point of view, the type of experts and disciplines involved is many and varied: from aerial photography to canine units, geophysics and geosciences in general, archeology, entomology, and botany (1–6); in practice, however, such multidisciplinary approaches are seldom used. The role of botany in particular, which has been recognized and widely tested in other fields of forensic sciences (7), has probably been underestimated in the process of searching for a clandestine grave. Very few reports actually exist concerning trials set up for these purposes.

In the analysis of clandestine burials, the combined use of soil and plant science, considered as an example of "environmental profiling" (8), has focused mostly on pollen analysis, aimed at linking one or more suspects to a crime scene (8,9). However, vegetation traits can evidence changes imposed by the burial to the environment (5–7,10). The excavation of graves and the occurrence of a decomposing body can be viewed as peculiar aspects of environmental factors (disturbance, alteration of the nutrient balance) that normally influence plant communities triggering specific responses; thus, plant species succession could have an important role in detecting sites affected by such environmental pressures. An obstacle to the wide use of evidence resulting from plant cover is the specificity of each site: local flora and different ecological conditions make every location somewhat unique, and useful information can seldom be shared.

This study was part of a wider interdisciplinary project, which aims at studying the decomposition of buried swine carcasses and the effects of their burial on soil and surface vegetation. In the northwest of Italy, on average, a dozen forensic cases every year reach the attention of forensic research units (Police, Carabinieri, and Universities) and usually concern the burial of homicide victims of organized crime killed from 5 to 30 years before. Thus, verifying the role of different disciplines and their applicability in the search for clandestine graves is becoming more and more crucial.

The goal of this study was therefore the evaluation of vegetation dynamics following the burial of the carcasses to detect the effects of mechanical disturbance and carcass decomposition on vegetation structure and specific composition. Although investigated in parallel, other evidence such as chemical soil analyses was not taken into account to verify whether and how vegetation alone could provide evidence of the occurrence of burials. Another goal was to outline the general mechanisms underpinning plant colonization and succession to obtain useful information to be applied in different ecological and biogeographic contexts. For this purpose, the ecological profile of the investigated plant communities was evaluated through Landolt's ecological indices (11); such indices give each species an indicator value for the main ecological factors (temperature, soil moisture, soil pH, etc.) and are widely used in ecological studies (12). The functional profile was evaluated using Grime's CSR model (13). CSR theory predicts that the strategies of plant species are an adaptive response to a three-way trade-off in the investment of resources between the ability to compete with neighbors (competitive strategy—C), tolerate stress (stress-tolerant strategy—S), or survive disturbance (ruderal strategy—R). Functional traits of each species can be used to assess its life strategy under the form of coordinates on C, S, and R axes (14). This theory has proved to provide a functional interpretation of plant communities in different ecological conditions and can give general information beyond the site-specific floristic context (e.g., [15,16]).

Materials and Methods

Study Area

The study was carried out in the Ticino River Regional Park in northern Italy (45°23′N 8°50′E) at 95 m above sea level. The area

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is characterized by coarse fluvial deposits composed mainly of crystalline rocks, with poorly developed and highly permeable soil (*Dystric Leptosols*); soil pH is low, about 4.5–5.5 at the surface, rising to 5.6–6.6 at 50 cm depth (17). The climate is suboceanic with highest rainfall in spring and autumn; the average rainfall is 1050 mm/year. The vegetation cover is represented by acidophilic peduncolate oak (*Quercus robur*) woodlands, often replaced by degraded communities dominated by *Robinia pseudoacacia* and by highly invasive *Prunus serotina.* Open areas are occupied by dry grasslands dominated by grasses and sedges like *Bromus sterilis, Aira caryophyllea, Koeleria pyramidata, Carex caryophyllea*, and *Vulpia myuros*.

Experimental Design

The experiment was carried out in an open site with herbaceous vegetation. Swine (*Sus domesticus*) carcasses were used to model the behavior of human body decomposition. Swine are considered an acceptable model because they are similar to human bodies in their weight, fat–to-muscle ratio, and hair coverage (3,18). The average weight of the carcasses was about 80 kg to simulate an adult human body. Detailed information about the carcasses was recorded for further research involving other disciplines; these data are not reported here.

Five graves were excavated and filled with a carcass on May 21, 2009; a control grave (excavated and backfilled without any carcass) and a completely undisturbed control plot were also prepared to evaluate the role of mechanical disturbance from that of carcass burial. The depth of the graves ranged from 80 to 90 cm (mean 83 ± 2 cm) to have a soil layer approximately 40 cm thick on the top of the carcass. Such depth represents an average value encountered in forensic scenarios (e.g., intermediate between "shallow" values of 50–60 cm and "deep" values of 100–110 cm [19]).

Vegetation Sampling

Vegetation sampling was performed through point-quadrat analysis. Seven plots (five graves, one control grave, and one fully undisturbed plot) were established: each plot consisted of a 1 × 1 m quadrat with a 10-cm grid. At each node of the grid, we recorded the plant species and the number of contacts with the vertical line passing through the node, ultimately used as an indicator of the relative abundance of each species. Sampling was performed immediately before and after the burial, and then monthly from May 2009 to May 2010, resulting in 14 visits. Vascular plant species were recognized on the field; problematic individuals (i.e., those at vegetative stage) and bryophytes were identified with the aid of identification texts (20–23). The sampling was performed trying to minimize the impact on vegetation.

Data Analysis

The temporal trends of the total number of contacts and of species number were assessed for each plot. Analysis of variance (ANOVA) was employed to assess the difference among graves, control grave, and undisturbed plot as well as between disturbed (graves and control grave) and undisturbed plots. Exclusive or preferential species were evaluated for each of the carcass graves, the control grave, and the undisturbed control plot, and for different time spans, that is, the whole year of sampling and the period following quantitative vegetation recovery on the disturbed plots (November 2009–May 2010).

The ecological profile of the communities was assessed through Landolt's ecological indices: we evaluated the frequency of species belonging to each value class of indices N (soil nutrients) and R (soil pH). We outlined the functional profile building a triangular diagram for each species assemblage using the CSR coordinates from the data set provided by Cerabolini et al. (24) and Grime et al. (25). Bryophytes were not included in the CSR analysis, even if generally they are supposed to show an overall S strategy (25), as standardized methods to assess the strategy for these species are still lacking.

Results

Quantitative Variation in Plant Cover

The burial led, as expected, to the complete destruction of the vegetation cover. The recovery was very slow for every plot throughout the summer and the early autumn. During this period, even the undisturbed plot showed an important regression of plant cover concerning both species number and the overall number of contacts, decreasing to three species and 13 contacts, respectively (Figs 1 and 2). This is because of the disappearance of annual species during the driest months (see next paragraph). A quantitative recovery started in November and accelerated from March. At the end of the trial, the number of species reached again at every plot the predisturbance values; on the other hand, the number of contacts, representative of the overall vegetation cover, never recovered the predisturbance values except for the undisturbed control plot.

The number of contacts was significantly higher at the undisturbed control plot than at the graves (both the control grave and the filled graves) (ANOVA test and Tukey's Honestly Significant Difference (HSD) *post hoc* test: $p < 0.001$); the control grave and the filled graves were not significantly different from each other ($p = 0.634$). The number of species of the undisturbed control plot was significantly higher than that of the filled graves ($p = 0.005$). Considering only the visits performed after vegetation recovery, the number of species did not show any significant difference between control, control grave, and filled graves (ANOVA test: $p = 0.141$), while the number of contacts was higher for the undisturbed control plot ($p = 0.001$). Again, disturbed plots (i.e., control grave and filled graves) did not differ from each other ($p = 0.618$).

Species Succession

The undisturbed seasonal trend, observed on the control plot (Fig. 3), implies the strong decrease in plant cover during summer because of the disappearance of the annual species, many of which (*A. caryophyllea, V. myuros*) constituted a prominent part of the community. The original species composition and vegetation cover was reconstructed starting from autumn, with a pause during the coldest months (January), and was fully achieved from March onward (Fig. 3).

After the burial, *B. sterilis, C. caryophyllea, Euphorbia cyparissias, Tecurium chamaedrys, V. myuros,* and the moss *Schistidium apocarpum* were significantly more abundant in the undisturbed control plot than on the graves. Only the moss *Hypnum jutlandicum* was significantly more frequent on the control grave (Table 1). No species was significantly linked to the graves filled with the carcasses. Considering only the visits after vegetation recovery, the result did not change, except for a slight preference of *Allium carinatum* for the control grave (Tukey's *post hoc* test, $p = 0.02$).

The general overview of species abundance (Table 2) showed many species exclusive or preferential of the undisturbed control plot, two (*H. jutlandicum* and *Cerastium ligusticum*) of the control grave and only one (*Myosotis ramosissima)* of the filled graves,

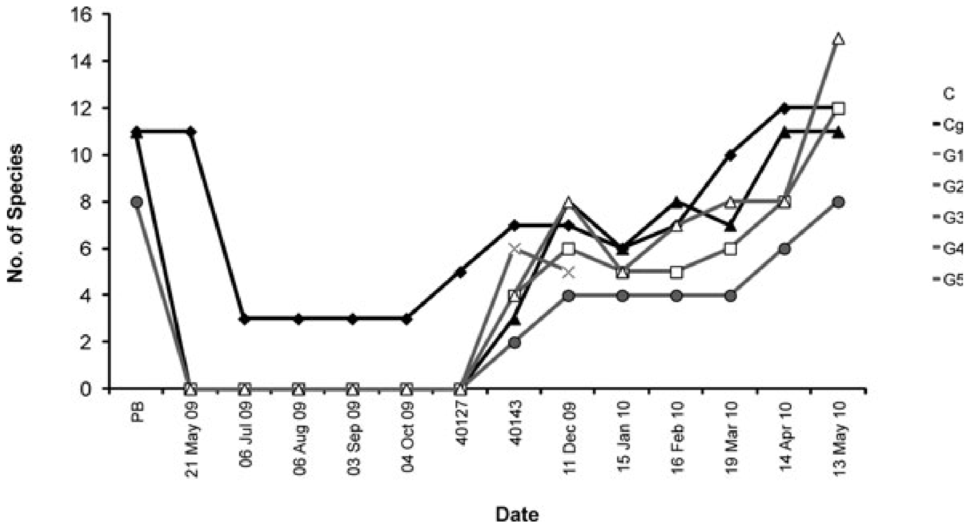

FIG. 1—*Trend of species number for each sampling plot. C: control; Cg: control grave; G1–G5: graves; PB: pre-burial.*

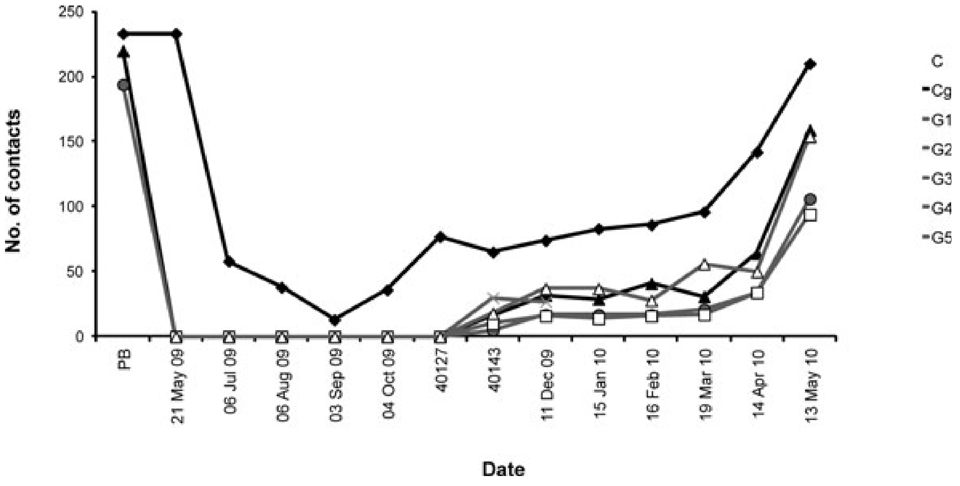

FIG. 2—*Trend of number of contacts for each sampling plot. C: control; Cg: control grave; G1–G5: graves; PB: pre-burial.*

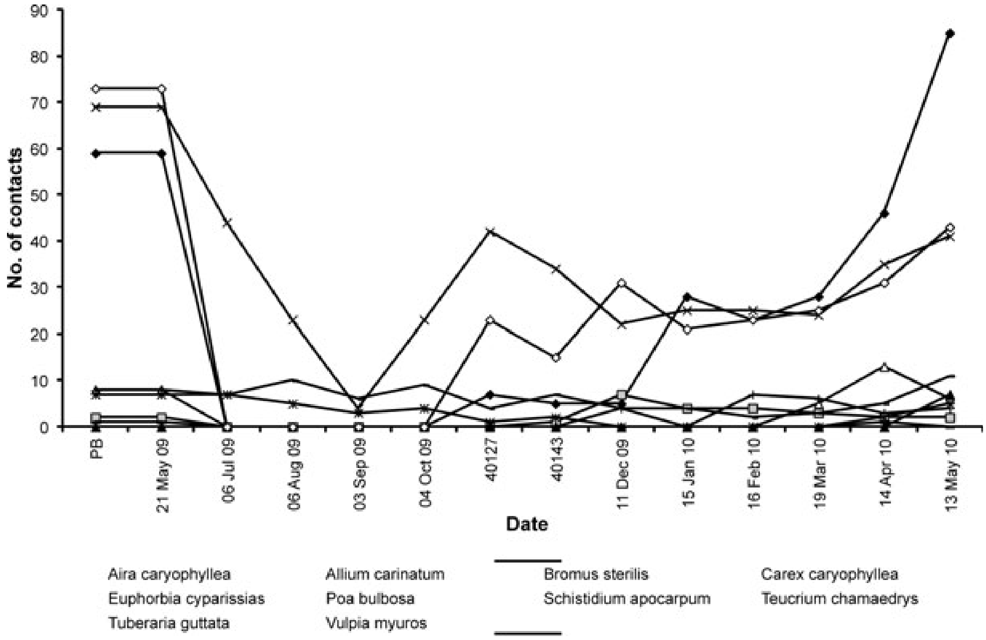

FIG. 3—*Trend of the number of contacts for the 10 most abundant species in the undisturbed control plot. PB, pre-burial.*

TABLE 1—*Tukey's HSD test of the difference among the number of contacts recorded on the control plot (1), the control grave (2), and the graves (3).*

Species	(*I*) Location	(*J*) Location	Mean Difference (*I–J*)	SE	Sig.
Bromus sterilis	1	2	2.46154	0.68430	**
		3	2.42072	0.54429	***
	2	1	−2.46154	0.68430	**
		3	−0.04082	0.54429	n.s.
	3	1	−2.42072	0.54429	***
		2	0.04082	0.54429	n.s.
Carex caryophyllea	1	2	31.07692	2.50225	***
		3	31.41130	1.99028	***
	2	1	−31.07692	2.50225	***
		3	0.33438	1.99028	n.s.
	3	1	−31.41130	1.99028	***
		2	−0.33438	1.99028	n.s.
Cerastium ligusticum	1	2	−2.69231	1.28255	n.s.
		3	0.06279	1.02013	n.s.
	2	1	2.69231	1.28255	n.s.
		3	2.75510	1.02013	*.
	3	1	−0.06279	1.02013	n.s.
		2	−2.75510	1.02013	*
Euphorbia cyparissias	1	2	2.61538	0.42847	***
		3	2.72841	0.34080	***
	2	1	−2.61538	0.42847	***
		3	0.11303	0.34080	n.s.
	3	1	−2.72841	0.34080	***
		2	−0.11303	0.34080	n.s.
Hypnum jutlandicum	1	2	−1.46154	0.44375	**
		3	0.07692	0.35295	n.s.
	2	1	1.46154	0.44375	**
		3	1.53846	0.35295	***
	3	1	−0.07692	0.35295	n.s.
		2	−1.53846	0.35295	***
Schistidium apocarpum	1	2	1.84615	0.45803	***
		3	1.74411	0.36431	***
	2	1	−1.84615	0.45803	***
		3	−0.10204	0.36431	n.s.
	3	1	−1.74411	0.36431	***
		2	0.10204	0.36431	n.s.
,	1	2	6.15385	0.44743	***
		3	6.15385	0.35589	***
	2	1	−6.15385	0.44743	***
		3	0.00000	0.35589	n.s.
	3	1	−6.15385	0.35589	
		2	0.00000	0.35589	n.s.
Vulpia myuros	1	2	20.38462	3.57659	***
		3	20.02512	2.84480	***
	2	1	−20.38462	3.57659	***
		3	−0.35950	2.84480	n.s.
	3	1	−20.02512	2.84480	***
		2	0.35950	2.84480	n.s.

Only the species showing at least a significant difference are reported. ***$p < 0.001$, **$p < 0.001$, *$p < 0.05$.

although not significantly given its low frequency. This trend did not change considering only the samplings after vegetation recovery. *Teesdalia nudicaulis, Rumex acetosella,* and *Tuberaria guttata* formed a group of species linked to all the disturbed sites (graves), which became more apparent after vegetation recovery (Table 2).

Ecological and Functional Profile

Concerning nutrient requirement, the highest frequency was reached by species belonging to class 2 of Landolt index N, followed by those of class 1 (Fig. 4) indicating an overall occurrence of oligotrophic species. Nutrient-requiring species (class 4) did not occur on the control grave, and their frequency was always lower than 10%; species belonging to class 5 did not appear at all.

TABLE 2—*Average number of contacts of each species in the undisturbed control plot, the control grave, and the graves considering the whole year (left) and the period after vegetation recovery (i.e., from November onward; right).*

	Whole Experiment			After Vegetation Recovery		
	Control	Control Grave	Grave	Control	Control Grave	Grave
Total Species	7.38	4.08	2.92	8.83	8.33	6.68
Total Contacts	116.13	28.62	17.08	115.17	59.33	40.74
Teucrium chamaedrys	6.38	0.00	0.00	4.83	0.00	0.00
Carex caryophyllea	33.75	0.54	0.20	28.67	1.17	0.53
Vulpia myuros	24.81	1.54	1.90	29.00	3.33	4.47
Bromus sterilis	5.69	0.00	0.04	4.00	0.00	0.11
Schistidium apocarpum	1.50	0.00	0.10	4.00	0.00	0.26
Euphorbia cyparissias	3.50	0.15	0.04	1.17	0.33	0.11
Poa bulbosa	0.44	0.00	0.00	1.17	0.00	0.00
Festuca ticinensis	2.25	0.00	0.00	0.00	0.00	0.00
Koeleria pyramidata	4.56	0.00	0.00	0.00	0.00	0.00
Festuca rubra	0.13	0.00	0.00	0.00	0.00	0.00
Brachypodium pinnatum	0.13	0.00	0.00	0.00	0.00	0.00
Tamus communis	0.13	0.00	0.00	0.00	0.00	0.00
Hypnum jutlandicum	0.06	1.54	0.00	0.17	3.33	0.00
Cerastium ligusticum	0.25	3.00	0.24	0.67	6.50	0.63
Myosotis ramosissima	0.00	0.00	0.04	0.00	0.00	0.11
Teesdalia nudicaulis	0.00	1.92	0.76	0.00	4.17	1.95
Tuberaria guttata	0.63	1.00	0.80	0.17	1.83	1.47
Rumex acetosella	2.06	1.62	1.02	0.00	3.50	2.37
Aira caryophyllea	26.50	12.85	9.78	35.83	26.33	23.74
Allium carinatum	1.69	3.15	1.45	3.67	6.00	3.26
Erigeron annuus	0.19	0.00	0.14	0.50	0.00	0.37
Helianthemum nummularium	0.44	0.38	0.10	0.17	0.83	0.26
Helianthemum sp.	0.31	0.54	0.14	0.83	1.17	0.37
Hypericum perforatum	0.06	0.00	0.06	0.00	0.00	0.16
Hypochoeris glabra	0.06	0.23	0.08	0.00	0.50	0.11
Luzula campestris	0.56	0.15	0.16	0.17	0.33	0.42
Potentilla tabernaemontani	0.06	0.00	0.02	0.17	0.00	0.05

Species showing significant differences between sites (Table 1) are in bold type. Shading represent the abundance classes: light gray: <1 contacts; medium gray: >1 and <10 contacts; dark gray: >10 contacts.

Landolt's index R showed the overall dominance of acidophilic species (class 2); the high occurrence of species belonging to class 4 indicated a slightly higher pH on the undisturbed control plot, at least at the rhizosphere level.

The undisturbed community (control) showed the occurrence of a wide range of plant strategies, but the overall dominance was of species exhibiting a stress-tolerant strategy (*C. caryophyllea, A. caryophyllea, K. pyramidata,* and *Teucrium chamaedrys*); among the dominant species, only *V. myuros* showed a substantial R strategy (Fig. 5). The control grave showed an increasing weight of species placed near or at the R corner, except for *A. caryophyllea;* the graves exhibited a very similar plot, with the increasing role of ruderal species. No change was observed between the different locations concerning the competitive (C) component of the community.

Discussion

Our data indicated that the observed successional patterns were mainly determined by disturbance, but under the control of an overall unproductive and climatically (summer drought) limited environment. The role of disturbance is apparent when considering the specific arrangement and the functional profile of the investigated

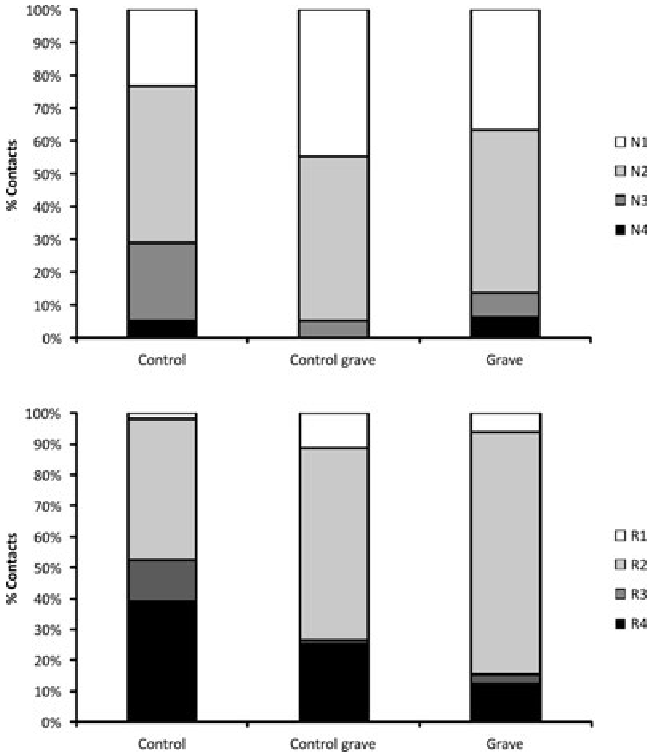

FIG. 4—*Frequency (expressed as the % of contacts during the whole year of sampling) of species belonging to classes 1–4 of Landolt's index N (upper panel) and R (lower panel). Class 5 was not represented.*

communities: disturbed plots were marked by the onset (or the persistence) of annual species, like *T. nudicaulis, T. guttata*, and *A. caryophyllea*, exhibiting R strategies, while perennial, mainly stress-tolerant species like *C. caryophyllea, T. chamaedys,* and *K. pyramidata* disappeared or strongly decreased. The important role of the summer drought is marked by the slow recovery of the disturbed plot until November and the parallel decrease in the number of species and in the cover value of the undisturbed control plot.

The role of decomposition, that is, of the actual presence of the carcasses, seemed much less critical. No significant difference was found between the control grave and those filled with a carcass; no species occurred exclusively on the filled graves (except the sporadic *M. ramosissima*), and no important ecological or functional difference was detected between the species assemblages occurring on the different kind of graves. Given the established increase in nitrogen and phosphorous compounds released with decomposition (3,18,26,27), this observation contrasts with the expected increase in nutrient-demanding species, even if a similar pattern has already been observed (5). Large mammal carcasses located on soil surface were reported to have a strong influence on vegetation turnover, because of the increased fertility and the disturbance consequent to ground covering by the carcass itself (26). The influence of swine carcasses on the underlying soil in terms of nutrient enrichment was found to be detectable up to 100 days for phosphorous and to 72 days for nitrogen (18,27). The observed lack of response to nutrient increase could be due to the limiting drought during summer, when plants could not take advantage of the increased nutrient availability. Thus, the expected and observed increased role of fast-growing species is mostly because of R and SR species (like *A. caryophyllea, V. myuros, and T. nudicaulis*), and such species occur almost indifferently on filled and empty graves. The high permeability of the soils of the study area may have an important role, as the coarse soil showed a less pronounced increase in nitrogen compounds in experimental graves and a general slower decomposition rate (2,28), thus further limiting nutrient availability, besides having a strong influence on water balance in addition to climatic drought.

Burial depth also should be taken into account. Even if the soil layer above the decomposing carcasses could not be considered too thick to be reached by roots, the mainly annual species occurring above the graves were probably not able to access the "cadaver decomposition island" (CDI [27]), which was mainly driven by the gravitative leaching of fluids from the decomposing carcass (27).

Forensic Applications of Vegetation Analysis

The present work aimed at evaluating the reliability of relatively fast and simple observation that could be performed on the field without expensive, sophisticated, or destructive analyses. Community ecology suggests that plant species assemblages consistently indicate environmental constraints *via* predictable adaptive responses (12,13,29); such responses are constant for each species and coherent throughout different regions (24,29). This means that information associated with floristic composition alone, if available, can tell us a lot about the underlying ecological conditions. Although restricted in time and space, our trial served as a test for realistic scenarios of restricted budgets, time, and availability of control situations.

At least in some conditions, the nutrient enrichment caused by a buried body does not significantly affect herbaceous vegetation, in contrast to the observations made on bodies left at soil surface (26,27). Burial depth could be one of the main factors affecting the degree of influence on surface vegetation; our experiment seems to indicate that an "average" burial (40 cm of soil on the top of the carcass) could have negligible effects on the nutrient balance of the plant community. This observation agrees with data reported by Van Belle et al. (3), indicating no significant increase in ninhydrin reactive

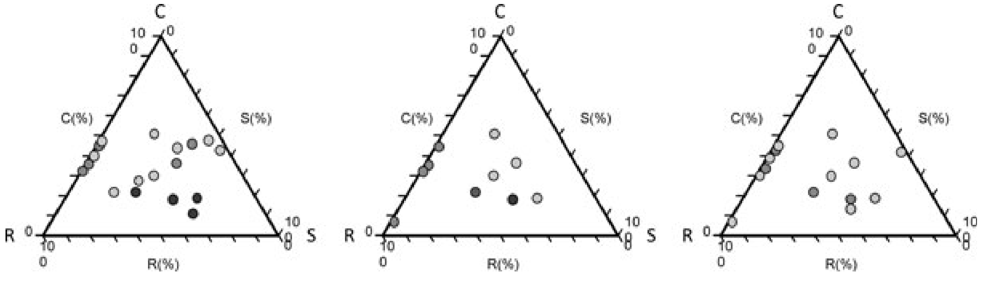

FIG. 5—*CSR classification of the species found at the undisturbed control plot (left), the control grave (center), and the graves (right) during the whole year of sampling. Each circle represents a species; gray scales represent species abundance as in Table 2.*

nitrogen in samples taken from the surface to 20 cm depth above a burial experiment with swine carcasses placed at 40 cm depth. Also, the experiment by France et al. (5) indicated "that the presence of a decaying pig has not significantly affected plant growth" (p. 1451).

On the other hand, the effects of disturbance are apparent both from the species assemblage and from the functional point of view. Species exhibiting ruderal strategies replace stress tolerators that dominate undisturbed communities. This observation agrees with data obtained in different biogeographic context, for example, in Southern Ontario (6), *Digitaria sanguinalis* and *Panicum capillare* were found to be "grave indicators": Both species are linked to disturbed habitats, and the first one is known to exhibit a CR strategy (24), while for the second, data are lacking even if congeneric species show CR strategies as well (24). Furthermore, disturbance reduces the degree of dominance by one or few species, similarly to what was observed by Pierce et al. (16).

In case of "stressed," unproductive ecosystems, that is, those affected by drought or other limiting factors, the effects of nutrient increase could be masked by the overall low resilience of the community, but the effect of disturbance may last longer, and the low recovery rate, together with the onset of annual ruderal species, could be an even more visible burial marker than the arrival of nutrient-requiring species. This could be of great interest given the probable low nutrient influx from a buried body toward the surface. Even if our results agree with the statement by France et al. (5), indicating that the disadvantages of vegetation analysis derive from "similar succession pattern for any disturbance within the ecosystem: not limited to burial" (p. 1455), a thoughtful evaluation of the functional profile of the plant species involved could give important information about the processes underpinning the observed vegetation patterns. The increasing availability of ecological data sets (24) now makes this task easier, even for research or investigative teams lacking the necessary equipment for the assessment of plant strategies.

Further studies should include productive environment and different burial depths to detect the degree of influence of the nutrient released by decomposition on the involved plant communities.

The present study, although not conclusive, begins to shed some light on the issue of botanical markers of clandestine graves. More research needs to be performed within different environments and at different depths; nonetheless, initial data do seem to suggest that regardless of the presence of putrefactive liquids, the mere disturbance of soil may produce important botanical signals for the search of buried bodies.

Acknowledgments

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