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Abstract Southern Portugal is experiencing a rapid change in land use due to the spread of intensive farming systems, namely olive production systems, which can cause strong negative environmental impacts and affect the ecological integrity of aquatic ecosystems. This study aimed to identify the main environmental disturbances related with olive grove intensification on Mediterranean-climate streams in southern Portugal, and to evaluate their effects on fish assemblage structure and integrity. Twenty-six stream sites within the direct influence of traditional, intensive, and hyper-intensive olive groves were sampled. Human-induced disturbances were analyzed along the olive grove intensity gradient. The integrity of fish assemblages was evaluated by comparison with an independent set of least disturbed reference sites, considering metrics and guilds, based on multivariate analyses. Along the gradient of olive grove intensification, the study observed overall increases in human disturbance variables and physicochemical parameters, especially organic/nutrient enrichment, sediment load, and riparian degradation. Animal load measured the impact of livestock production. This variable showed an opposite pattern, since traditional olive groves are often combined with high livestock production and are used as grazing pasture by the cattle, unlike more intensive olive groves. Stream sites influenced by olive groves were dominated by non-native and tolerant fish species, while reference sites presented higher fish richness, density and were mainly occupied by native and intolerant species. Fish assemblage structure in olive grove sites was significantly different from the reference set, although significant differences between olive grove types were not observed. Bray–Curtis similarities between olive grove sites and references showed a decreasing trend in fish assemblage integrity along the olive grove intensification gradient. Olive production, even in traditional groves, led to multiple in-stream disturbances, whose cumulative effects promoted the loss of biota integrity. The impacts of low intensity traditional olive groves on aquatic ecosystems can be much greater when they are coupled with livestock production. This paper recommends best practices to reduce negative impacts of olive production on streams, contributing to guide policy decision-makers in agricultural and water management.

Keywords Land use intensification · Olive grove · River degradation · Water quality · Fish assemblages · Portugal

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Introduction

The integrity of inland waters is an issue central to water policies and nature conservation. Within the European Union, the Water Framework Directive (WFD) (European Commission 2000) requires that all member states assess, monitor, and improve, if necessary, the ecological quality of water bodies. The identification of human-induced disturbances and the evaluation of their effects on aquatic ecosystems are therefore crucial to the implementation of effective environmental policies and the minimization of impacts through appropriate recommendations to farmers, technicians, and agriculture/forestry organizations.

Land use and land cover change play an important role in global change phenomena (e.g., Foley et al. 2005; Lambin and Geist 2006), and threaten the ecological integrity of fluvial ecosystems (e.g., Allan 2004; Roth et al. 1996). Diffuse pollution from agriculture is one of the main factors impacting the ecological quality of southern Portuguese rivers (Matono et al. 2012), and is highly related to the intensification of agrosystems. These have strong negative environmental impacts, particularly on soil erosion, runoff to water bodies, habitats and quality of scarce water resources (Beaufoy and Pienkowski 2000). Excessive fertilization and irrigation in intensive and hyper-intensive agriculture may result in degradation of aquatic ecosystems and depletion of superficial and groundwater resources (e.g., Zalidis et al. 2002). Intensive agriculture and forestry can result in several types of stress, which individually or together affect the structure and functioning of inland waters. For instance, the decrease of river flows due to water over-abstraction (both underground and surface) may change river hydrology, increasing siltation, and reducing habitat heterogeneity, with negative effects on aquatic biota, namely on fish bio-integrity (e.g., Bernardo et al. 2003; Meador and Carlisle 2011). Runoff from irrigated cultures washes topsoil, fertilizer, and herbicides into water bodies, causing contamination and loss of biodiversity (Beaufoy 2001). Thus, the expansion of hyper-intensive olive groves is likely to be an important driving force affecting aquatic ecosystem integrity.

The Mediterranean region has been shaped by human activity and maintained by traditional practices of land use for centuries. Southern Portugal has long been characterized by the maintenance of evergreen oak agroforestry systems combined with extensive non-irrigated farming (Pinto-Correia 1993). However, in recent decades the region has experienced a more accelerated change in farming systems, due to the expansion of more profitable irrigated agriculture (Pinto-Correia and Vos 2004). Increased demand for products such as olive oil and wine has resulted in a significant conversion of open arable land to permanent and intensive uses such as vineyards and irrigated olive groves (Diogo and Koomen 2010; Ramos and Santos 2009).

Currently, Portugal is one of the five top olive producers in the European Union, with approximately 160,000 ha of olive groves in the south of the country, the largest cultivated area (INE 2011; DRAPAL 2012). About 25 % of this area is occupied by intensive systems in large cultivated landscapes. The area per land parcel tends to increase with intensification of the culture, reaching up to 100 ha (unpublished data of the authors). Intensive olive cultivation systems are characterized by a high density of trees, systematic irrigation, and mechanized harvesting. The use of hyper-intensive olive groves has recently been increasing, incorporating new production techniques with strong input of energy and water at the expense of natural resources.

Fish fauna is recognized as an indicator of aquatic ecosystem health because: (i) it is sensitive to various kinds of pressures; (ii) it responds over longer timescales than other biological quality indicators, because some fish species are relatively long lived; (iii) some fish species represent the upper trophic levels and thus provide an integrated view of the ecosystem, possibly revealing problems at lower levels (Barbour et al. 1999). Fish functional characteristics provide information about several aspects of a community, and enable testing of hypothetical changes in species traits along environmental gradients. In this context, the assessment of water quality coupled with fish guilds allows evaluation of aquatic biota integrity and its relationship with human activities.

The objective of this study was to identify the main environmental disturbances to Mediterranean-climate streams in southern Portugal related with olive grove intensification, namely those disturbances related to water quality and river morphology, and to evaluate their effects on fish assemblage structure and integrity.

Methods

Study Area

The study sites were located in the south of Portugal, distributed throughout the Tagus (19 % of sites), Sado (27 %), and Guadiana (54 %) river basins (Fig. 1). This is a lowland region with a few low-altitude mountains. The climate is typically Mediterranean, with high intra- and interannual precipitation and discharge variation, severe and unpredictable floods between autumn and spring, and persistent summer droughts (Miranda et al. 2002). Mean annual air temperature is high (16 °C), and mean annual precipitation ranges from 350 to 1200 mm (APA (2011), available from http://sniamb.apambiente.pt/webatlas). Although it is not an overpopulated area, the rural landscape has been deeply transformed by agricultural activity during the last century. As a consequence of growing water demands, numerous reservoirs have been built in this region, one of them being the largest in Europe (Alqueva). The irrigated area has increased considerably in the last decade, and is currently about 30 % of the cultivated land (INE 2011).

Within the region, olive groves and pastures are now the most widespread culture in terms of area (>70 %). Pastures are mainly used as grazing area for livestock production

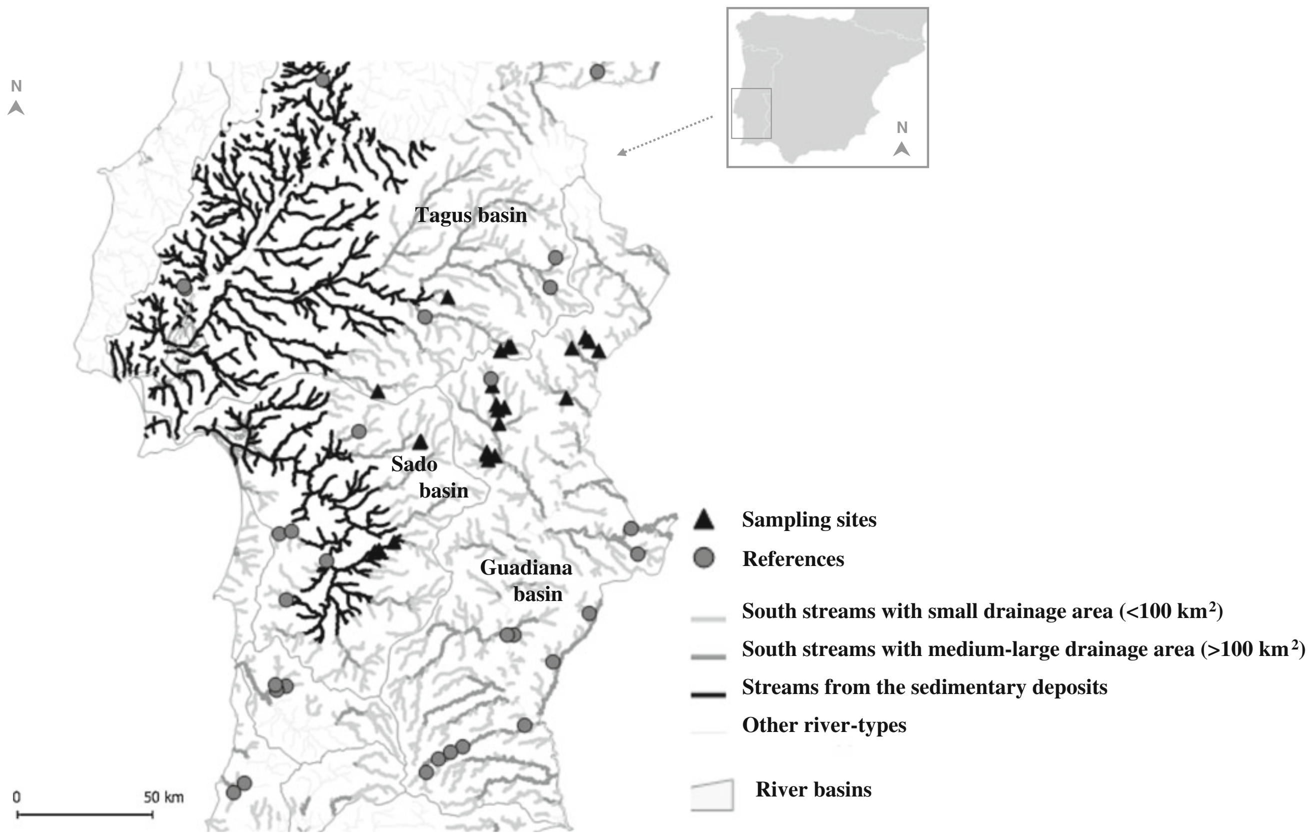

Fig. 1 Location of the sampling sites and the reference sites in the Tagus, Sado, and Guadiana river basins, considering three south river-types previously defined under the implementation of the EU Water Framework Directive in Portugal (Ilhéu et al. 2009)

(beef cattle), and this use has increased by about 42 % in the last decade. Although livestock are mostly produced in extensive systems, there are often a high number of animals per farm (often >300) (INE 2011). The management of livestock is based on the rotational grazing of cattle on partitioned pasture areas, so that at each moment a high stock rating may be observed. During the summer, when pastures become dry, livestock are often found in paddocks with free access to streams, which are used as refuges from the heat (e.g., providing shade) and drinking-water areas.

The area used for olive groves has also increased in the last decade (by about 20 %), mostly due to the expansion of intensive and hyper-intensive production systems (INE 2011). Many traditional olive groves have been converted to intensive and hyper-intensive systems, and many others are increasingly used simultaneously as fertilized pasture for grazing by livestock, mainly beef cattle. Besides diffuse pollution caused by agriculture and livestock production impacts on water quality and water abstraction, other common human-induced disturbances include river channelization and destruction of riparian vegetation. All these factors have been responsible for major changes in aquatic ecosystems, threatening native fish fauna. Native freshwater fish fauna of Portuguese rivers present relatively low species richness, being dominated by cyprinids. Most of the fish species are endemic with high conservation status and many of them are threatened with extinction (Cabral et al. 2005).

Site Selection and Sampling

Sampling took place during the spring of 2010 at 26 stream sites within the direct influence of traditional ($N = 9$), intensive ($N = 11$), and hyper-intensive ($N = 6$) olive groves. Olive grove areas ranged between 2 and 10 ha and were within a maximum lateral distance of 100 m from the sampled streams. All the sites were located and evenly distributed within a relatively homogeneous environmental area, comprising three south river-types (see INAG 2008a) defined under the implementation of the WFD in Portugal (Fig. 1).

The classification of olive grove types was based on tree density: (i) less than 200 trees/ha—traditional olive grove; (ii) between 200 and 1000 trees/ha—intensive olive grove; and (iii) more than 1000 trees/ha—hyper-intensive olive grove (Fontanazza et al. 1998). In addition, a Kruskal–Wallis test supported this classification, confirming the existence of significant differences in tree density between the olive grove types ($H_{2, 26} = 21.8$; $P < 0.001$), therefore preventing a possible bias resulting from the dispersion of values within each type.

Unlike traditional olive groves, all the intensive and hyper-intensive systems were irrigated. Pre-selection of sites followed a GIS screen, based on digital cartography with information on land cover (Caetano et al. 2009). Sites were then selected based on field surveys because the available cartography was not up to date, and many areas are undergoing changes in land use. All the selected olive groves included mature and productive trees, and were being actively exploited.

Sampling was undertaken according to the WFD-compliant sampling protocol (INAG 2008b), which follows recommendations by the European Committee for Standardization (CEN 2003). In order to ensure the highest habitat diversity in the streams, surveys were carried out during spring in flowing water conditions, immediately after floods and prior to the strong reduction of flow during summer. At each site, a stream reach was sampled, encompassing all the existing physically homogeneous units (mesohabitats)—pool, run, and riffle. The length of each sampled reach was defined as 20 times the mean width of the stream, up to a maximum of 150 m. Fish were collected using backpack battery-powered electrofishing equipment (IG 200/2B, PDC Hans-Grassl GmbH, Schönau am Königssee, Germany), wading in shallow reaches (<1.2 m) or from a boat in deeper streams. All captured fish were identified to the species level, measured (total length, mm) and returned alive to the stream. Captures were quantified as density (number of individuals per 100 m^2).

Landscape and regional variables were obtained from the national online database of water resources (Sistema Nacional de Informação de Recursos Hídricos (2011), available from http://snirh.pt) and freely available digital cartography, including distance from source (km), altitude (m), mean annual runoff (mm), and mean annual air temperature (°C). Local variables were measured during the field sampling procedure: water temperature (°C), conductivity (μS/cm), pH, dissolved oxygen (mg/L), water transparency (Secchi disk depth, m), mean stream wetted width (m), maximum and mean water depth (m), mean current velocity (m/s), riparian vegetation (%), shadow (%), and proportion of different mesohabitat types. The dominant substrate class was attributed according to 6 classes adapted from the Wentworth scale (Giller and Malmqvist 1998): 1-mud and sand; 2-gravel; 3-pebbles; 4-cobbles; 5-boulders; 6-boulders larger than 50 cm. To evaluate the local variables, repeated measurements were taken of each mesohabitat present within the stream reach along transverse transects at regular distances of 20 and 50 cm, depending on the mesohabitat area. The final weighted mean value was calculated considering the proportional area covered by each mesohabitat type.

Human disturbance level was evaluated using ten semi-quantitative variables assessed at each site (previously developed within the EU project FAME (2004), available from http://fame.boku.ac.at): land use, urban area, riparian vegetation, longitudinal connectivity of the river segment, sediment load, hydrological regime, morphological condition, presence of artificial lentic water bodies, toxicological and acidification levels, and nutrient/organic load. Each variable was scored from 1 (minimum disturbance) to 5 (maximum disturbance) (Table 1), and the sum of these scores represented the total human pressure in each site (ranging from 10 to 50). Land use and hydrological regime were assessed at two different scales (Table 1), though not separately as independent variables. Both approaches were used to quantify just in one value (from 1 to 5) the degradation of those two variables, integrating different levels of information.

Several physicochemical parameters were assessed to complement and support the evaluation of human-induced disturbance at each site (mainly organic/nutrient enrichment): phosphate—P_2O_5 (mg/L); total dissolved phosphorous—P (mg/L); nitrite—NO_2^- (mg/L); nitrate—NO_3^- (mg/L); ammonium—NH_4^+ (mg/L); and total dissolved nitrogen—N (mg/L). Water samples were collected at each site immediately before fish sampling. Laboratory analyses were then carried out according to the Standard Methods for the Examination of Water and Wastewater (Clesceri et al. 1998).

As mentioned above, livestock production is an important activity in the study area with potentially strong impacts on stream health, since pastures are often located near water bodies. Moreover, streams are rarely bounded by fences, allowing cattle to enter the water. Many traditional olive groves are currently used as grazing pasture by the cattle. In fact, it was not possible to select traditional olive groves exclusively without the pressure of livestock. In contrast, all the intensive and hyper-intensive olive groves studied were devoted exclusively to this land use. As such, the olive grove intensity gradient expresses intensification in the production practices but a decrease in the number of anthropogenic pressures. Livestock production (mainly beef cattle) was then considered separately from agricultural land use, designated as animal load and scored following criteria similar to those used for the other human disturbance variables, considering the stocking rate within the area surrounding each sampling site (Table 1).

Data Set

In addition to taxonomic fish data, community attributes or fish metrics were used preferentially in the analyses: total fish density; species richness and diversity (Shannon-Wiener Index); total number of native and non-native species, potamodromous and long lived species; and functional guilds related to habitat (relative abundance of rheophilic,

Table 1 Description, assessment scale, methods, and scoring criteria of the variables used to evaluate the level of anthropogenic disturbance at each sampled site

Variables	Description	Assessment scale	Score	Criteria	Methods
Land use	Impact of farming/forestry practices	River segment	5	>40 % Agricultural use (intensive agriculture), very severe impact (rice field)	Local expert assessment complemented with Corine Land Cover (2000, 2006)[a]
			4	>40 % Strong impact (area with strong forestry, including clearcuts)	
			3	<40 % Moderate impact (subsistence gardens, pastures)	
			2	<40 % Small impact (cork and holm oaks, high-growth forest)	
			1	<10 % No significant impacts (natural forest and bush)	
	Land use characterization	Local	5	Irrigated crops and/or high stocking	
			4	Horticultural crops, semi-intensive grazing	
			3	Extensive cultures (e.g., pastures, cereal crops, pine, eucalyptus), extensive grazing	
			2	Cork and holm oaks	
			1	Natural	
Urban area	Impact of urban areas	River segment	5	Very severe (location near a city without wastewater treatment)	Local expert assessment complemented with Corine Land Cover (2000, 2006)[a]
			4	Town	
			3	Village	
			2	Hamlet	
			1	Negligible (isolated dwellings)	
Riparian vegetation	Deviation from the natural state of the riparian zone	River segment	5	Lack of riparian shrubs and trees (presence of only annual plants)	Local expert assessment
			4	Fragmented vegetation with the presence of bushes or reeds	
			3	Second replacement step (dominance of dense brushwood)	
			2	First replacement step (presence of shrub or tree strata with some level of preservation)	
			1	Potential vegetation (presence of shrub and tree strata according to the geo-series)	

Table 1 continued

Variables	Description	Assessment scale	Score	Criteria	Methods
Morphological condition	Deviation from the natural state of the stream bed and banks	Local	5	Transverse and longitudinal profile of the channel completely changed, with very few habitats	Local expert assessment
			4	Channelized sector, missing most of the natural habitats	
			3	Channelized sector, missing some types of natural habitats, but maintaining much of the shape of the natural channel	
			2	Poorly changed sector, close to the natural mosaic of habitats	
			1	Morphological changes absent or negligible	
Sediment load	Deviation from the natural sediment load (both carried in the water column and deposited on the riverbed)	River segment and local	5	>75 % of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	Local expert assessment
			4	50–75 % of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
			3	25–50 % of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
			2	5–25 % of coarse particles of the bed are covered with fine sediments (sand, silt, clay)	
			1	<5 % of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
Hydrological regime	Deviation from the natural hydrological regime (flow pattern and/or quantity). Includes all sources of hydrologic alteration, such as significant water abstraction	Local	5	<50 % and strong deviation from the natural variability of the flow regime	Local expert assessment complemented with information from gauging stations (SNIRH)[b]
			4	<50 % and moderate deviation from the natural variability of the flow regime	
			3	>50 % and duration of flood periods close to the natural	
			2	>75 % and duration of flood periods close to the natural	
			1	>90 % and normal duration of natural flood periods	
		Local	5	<10 % of mean annual discharge	
			4	<15 % of mean annual discharge	
			3	>15 % of mean annual discharge	
			2	>30 % of mean annual discharge	
			1	>90 % of mean annual discharge	

Table 1 continued

Variables	Description	Assessment scale	Score	Criteria	Methods
Toxic and acidification levels	Deviation from the natural state of toxicity conditions, including acidification and oxygen levels	Local	5	Constant for long periods (months) or frequent occurrence of strong deviations from natural conditions (e.g., pH <5.0, DO <30 %)	Local expert assessment complemented with information from gauging stations (SNIRH)[b]
			4	Constant for long periods (months) or frequent occurrence of strong deviations from natural conditions (e.g., pH <5.5, DO <30–50 %)	
			3	Occasional deviations (single measurements or episodic) in relation to natural conditions (e.g., pH <5.5, DO <30–50 %)	
			2	Occasional deviations (single measurements or episodic) in relation to natural conditions (e.g., pH <6.0)	
			1	Conditions within the normal range of variation	
Organic and nutrient loads	Deviation from the normal values of BOD, COD, ammonium, nitrate, and phosphate concentrations	Local	5	>20 % of values in classes D or E	SNIRH (classification of water quality for multiple uses, according to the guidelines from INAG—National Water Institute[c]), complemented with local expert assessment
			4	>10 % of values in classes D or E	
			3	>10 % of values in class C	
			2	No obvious or too small signs of eutrophication and organic loading	
			1	No signs of eutrophication and organic loading	
Artificial lentic water bodies	Impact related to the presence of artificial lentic water bodies upstream and/or downstream of the site (upstream change in thermal and flow regimes; downstream invasion by exotic species of lentic character)	Local	5	Site immediately downstream of a large reservoir or within the influence area of its backwaters	SNIRH[b] and available cartography
			4	Site immediately downstream of a mini-hydroelectric installation or within the influence area of its backwaters	
			3	Site downstream of a large standing water body or within the influence area of the reservoir	
			2	Site downstream of a mini-hydroelectric installation or within the influence area of its backwaters	
			1	No influence of reservoirs	
Connectivity	Impact of artificial barriers to fish migration	River basin and segment	5	Permanent artificial barrier	SNIRH[b], available cartography, documental data and local expert assessment
			4	Occasional passage of some species	
			3	Passage of certain species or only in certain years	
			2	Passage of most species in most years	
			1	No barriers or existence of an effective pass-through device	

Table 1 continued

Variables	Description	Assessment scale	Score	Criteria	Methods
Animal load	Impact of livestock production considering the stocking rate in the sampling area based on the presence of beef cattle and/or fecal material and trampling in the riverbed or within a 5 m wide strip along the riverside	River segment and local	5	>60 animals and/or >50 % of the sampling area covered with fecal material and trampling	Information from INE—National Statistics Institute (2011) complemented with local expert assessment
			4	<60 animals and/or 30–50 % of the sampling area covered with fecal material and trampling	
			3	<40 animals and/or 10–30 % of the sampling area covered with fecal material and trampling	
			2	<10 animals and/or <10 % of the sampling area covered with fecal material and trampling	
			1	Absence of cattle, fecal material and animal trampling	

[a] Caetano et al. 2009

[b] Available from http://snirh.pt

[c] Available from http://snirh.pt/snirh/ dadossintese/qualidadeanuario/boletim/tabela classes.php

limnophilic, eurytopic, benthic, and water column species), breeding (relative abundance of lithophilic and phytophilic species), feeding (relative abundance of omnivorous and insectivorous species), and tolerance do degradation (total number of intolerant and tolerant species). Captured species were assigned to functional guilds according to published literature (FAME 2004; Holzer 2008; Magalhães et al. 2008) and expert judgment based on the available knowledge (Table 2). The following are brief definitions of some fish metrics included in each functional guild:

Rheophilic prefer to live in a habitat with high flow conditions and clear water using this habitat both for breeding and feeding purposes; *Eurytopic* fish exhibit a wide tolerance to flow conditions, although generally not considered to be rheophilic; *Limnophilic* prefer to live, feed, and reproduce in a habitat with slow flowing to stagnant conditions; *Benthic* prefer to live on or near to the bottom, from where they take food, and usually do not go to the surface for feeding; *Water-column* species prefer to live and feed in the water column and usually do not go the bottom to search for food; *Insectivorous* fish whose adult diet consists mostly of insects, having a terminal or supraterminal mouth, taking aerial, drifting, or swimming insects and invertebrates; *Omnivorous* adult diet consists of plant and animal material, taking food from a wide range of flora and fauna; *Lithophilic* fish spawning exclusively on gravel, rocks, stones, rubble or pebbles, whose spawning success depends on the availability of suitable sized and clean gravel, and whose larvae are photophobic; *Phytophilic* fish spawning especially on plants, leaves, and roots of live or dead vegetation, whose larvae are not photophobic.

Since several sites presented very low catches, the use of an index of biotic integrity would reduce the overall accuracy of the ecological quality assessment. Therefore, the integrity of fish assemblages was evaluated by comparison with an independent set of reference sites (i.e., in the absence of significant anthropogenic disturbances), considering metrics and guilds. References included a set of least disturbed sites ($N = 29$) representative of the sampled river-types and basins (Fig. 1). These references were previously selected under the implementation of the WFD (Ilhéu et al. 2009) based on the aforementioned ten human disturbance variables, considering only sites with scores 1 and/or 2 and only one variable scored with a 3 (following CIS-WFD 2003). The suitability of the reference sites for comparison with the sampling sites is demonstrated in Table 3, which presents the mean general environmental characteristics of the reference set and the sampling sites.

Data Analysis

Bivariate analyses were conducted to analyze the pattern of human-induced disturbance variables, physicochemical parameters, and local habitat variables along the olive grove

Table 2 Ecological classification of fish species captured from sampling sites: B (benthic), WC (water column), EUR (eurytopic), RF (rheophilic), LIM (limnophilic), PHY (phythophilic), LIT (lithophilic), OMNI (omnivorous), INSV (insectivorous), POTAD (potamodromous), NAT (native), NN (non-native), TOL (tolerant), INT (intolerant)

Species	Common name	Classification
Barbus bocagei Steindachner, 1864	Common Barbel	B; LIM; LIT; OMNI; POTAD; NAT; TOL
Barbus steindachneri Almaça, 1967	Iberian Barbel	WC; LIM; LIT; OMNI; POTAD; NAT; TOL
Barbus microcephalus Almaça, 1967	Small-head Barbel	B; LIM; LIT; OMNI; POTAD; NAT
Cobitis paludica (de Buen, 1930)	South Stone Loach	B; LIM; INSV; NAT; TOL
Iberochondrostoma lemmingii (Steindachner, 1866)	Arched-mouth Nase	WC; LIM; LIT; OMNI; NAT
Pseudochondrostoma willkommii Steindachner, 1866	Guadiana Nase	B; RF; LIT; OMNI; POTAD; NAT
Squalius alburnoides Steindachner, 1866	Roach	WC; EUR; LIT; INSV; NAT
Squalius pyrenaicus (Günther, 1868)	Iberian Chub	WC; EUR; LIT; INSV; NAT
Alburnus alburnus (Linnaeus)	Bleak	WC; EUR; OMNI; NN; TOL
Carassius auratus (Linnaeus)	Goldfish	B; LIM; PHY; OMNI; NN; TOL
Cyprinus carpio Linnaeus	Common Carp	B; LIM; PHY; OMNI; NN; TOL
Gambusia holbrooki Girard, 1859	Mosquitofish	WC; LIM; INSV; NN; TOL
Lepomis gibbosus (Linnaeus)	Pumpkinseed	WC; LIM; INSV; NN; TOL
Micropterus salmoides (Lacépède, 1802)	Largemouth Bass	WC; LIM; PHY; NN; TOL

Table 3 General environmental characterization of the reference set and sampling sites (mean ± SD) of each olive grove type, considering the regional variables altitude (m), mean annual temperature (°C), mean annual precipitation (mm), distance from source (km), and mean annual runoff (m)

Variable	Reference set	Traditional	Intensive	Hyper-intensive
Altitude (m)	148.29 ± 82.64	196.11 ± 19.17	169.55 ± 61.66	150.83 ± 62.81
Mean annual temperature (°C)	15.52 ± 1.01	16.00 ± 0.75	15.91 ± 0.70	16.25 ± 0.82
Mean annual precipitation (mm)	637.71 ± 142.24	638.89 ± 33.33	613.64 ± 102.69	516.67 ± 51.64
Distance from source (km)	24.25 ± 20.90	12.92 ± 6.31	15.64 ± 7.77	20.62 ± 10.41
Mean annual runoff (mm)	160.00 ± 62.78	166.67 ± 39.53	147.73 ± 63.69	92.08 ± 40.01

intensification gradient. Habitat diversity at each reference and sampling site was calculated using the Shannon-Wiener Index (Shannon and Weaver 1949). Significant differences were detected with Kruskal–Wallis test. A Friedman test was used to search for significant differences in habitat proportion between the reference set and each olive grove type, and along the olive grove intensity gradient.

Correspondence Analysis (CA) of reference and sampled sites based on metrics and guilds allowed identification of the main patterns of variation in fish assemblage structure, independently of the possible influence of anthropogenic disturbance and environmental gradients.

To take into account the effect of co-variation along natural gradients when interpreting biotic responses to human disturbances, a Principal Components Analysis (PCA) was carried out on environmental and human pressure variables of reference and olive grove sites, to extract independent and synthetic environmental and perturbation gradients. The environmental axis was then correlated with CA axes in order to account for natural variability. This environmental gradient was incorporated as a co-variable when analyzing responses to olive grove intensification practices. No significant correlation would be expected if community structure patterns were completely independent of environmental gradient effects.

Permutational Multivariate Analysis of Variance (Permanova) evaluated the differences in fish community structure among olive grove types and the reference set (Anderson 2001). Olive grove type was considered as a fixed factor, and PC axis expressing the environmental gradient as a co-variable. The Model was tested using the Monte Carlo test under 9999 permutations.

Bray–Curtis similarities were used to quantify the distances between each site and the reference set. A Kruskal–Wallis test was used to find significant differences in Bray–Curtis similarities along the olive grove intensification gradient.

Prior to analyses, data were transformed to improve normality: percentages using arcsin [sqrt(x)] and linear measurements using log (x + 1). Bivariate analyses and non-parametric statistical tests were undertaken with

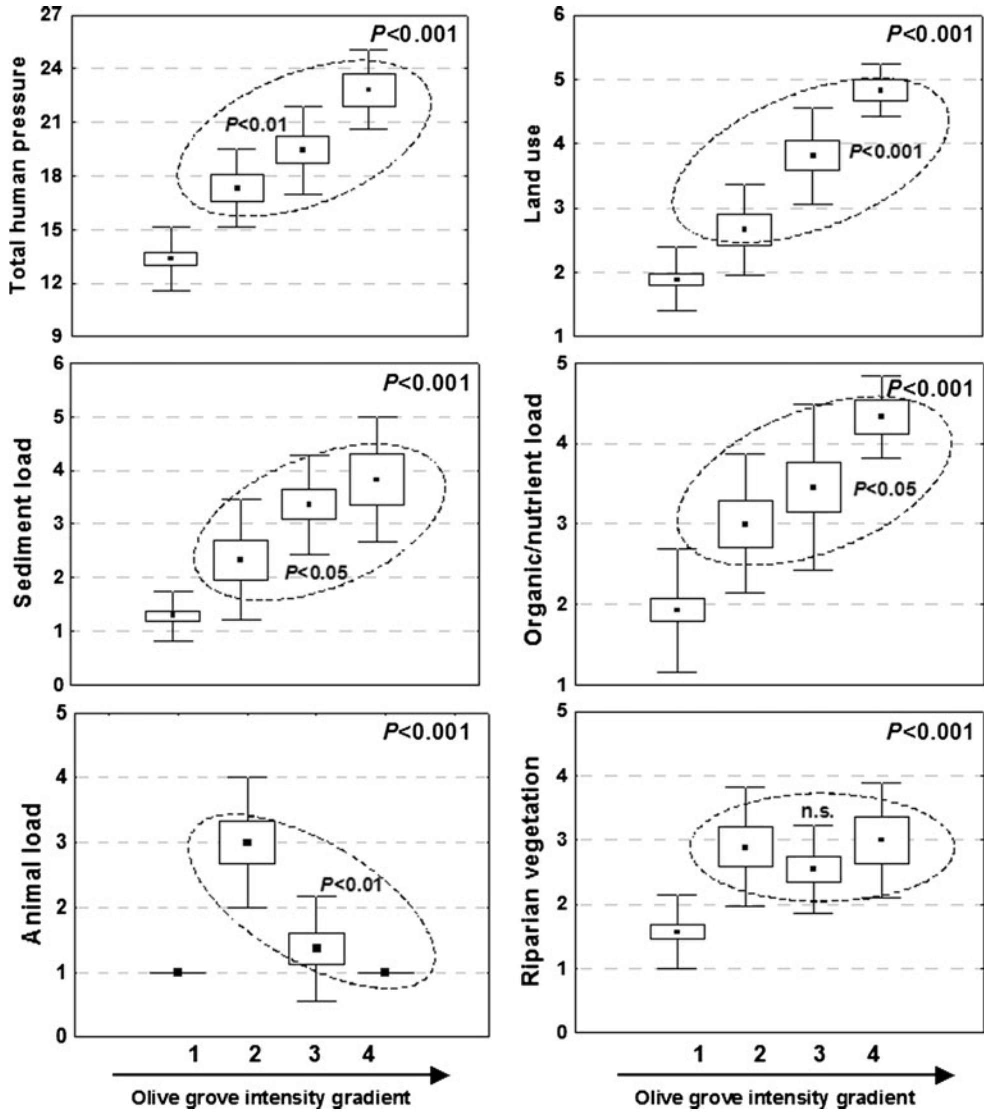

Fig. 2 *Box plots* of the most significant results for human-induced disturbances along the olive grove intensity gradient. Results from Kruskal–Wallis tests are shown for the entire gradient (*upper corner*) and considering only the three olive grove types (*inside the dotted line*). The olive grove intensity gradient follows a graphical sequence: (1) reference, (2) traditional olive groves, (3) intensive olive groves, and (4) hyper-intensive olive groves. (*Filled square*): Mean; box: ± SE; whisker: ± SD

Statistica 6.0, CA and PCA were performed with Canoco 4.5, and Bray–Curtis similarities were calculated with Primer 6, as well as Permanova.

Results

Anthropogenic Disturbance, Water Quality and Habitat Changes

Olive grove intensification was followed by a significant increase in the scores of several human disturbance variables (Fig. 2), thus reflecting a general increment in the total human pressure ($P < 0.001$). However, the total overall scores are not extreme, considering that the maximum value can reach 50. This is because the degradation of the sampling sites was specifically related with some particular variables. Indeed, land use, organic/nutrient enrichment, and sediment load showed a linear and gradual increase along the intensification gradient ($P < 0.001$), whereas degradation of riparian vegetation was significantly higher in olive groves than in the reference set ($P < 0.001$) but not significantly different among olive grove types ($P > 0.05$). Animal load showed a different pattern, decreasing along the olive grove intensity gradient ($P < 0.01$). All these variables revealed higher significant differences considering the entire gradient than among the olive grove types.

Fifty five percent of the sampled traditional olive groves were associated with cattle production and pasture. Active traditional olive groves used exclusively for olive production (i.e., without pasture) are increasingly difficult to find in the Alentejo region.

Except for water transparency, physicochemical parameters did not differ significantly ($P > 0.05$) between olive grove types (Fig. 3). Conductivity ($P < 0.05$), total phosphorous ($P < 0.001$), phosphate ($P < 0.001$), nitrite

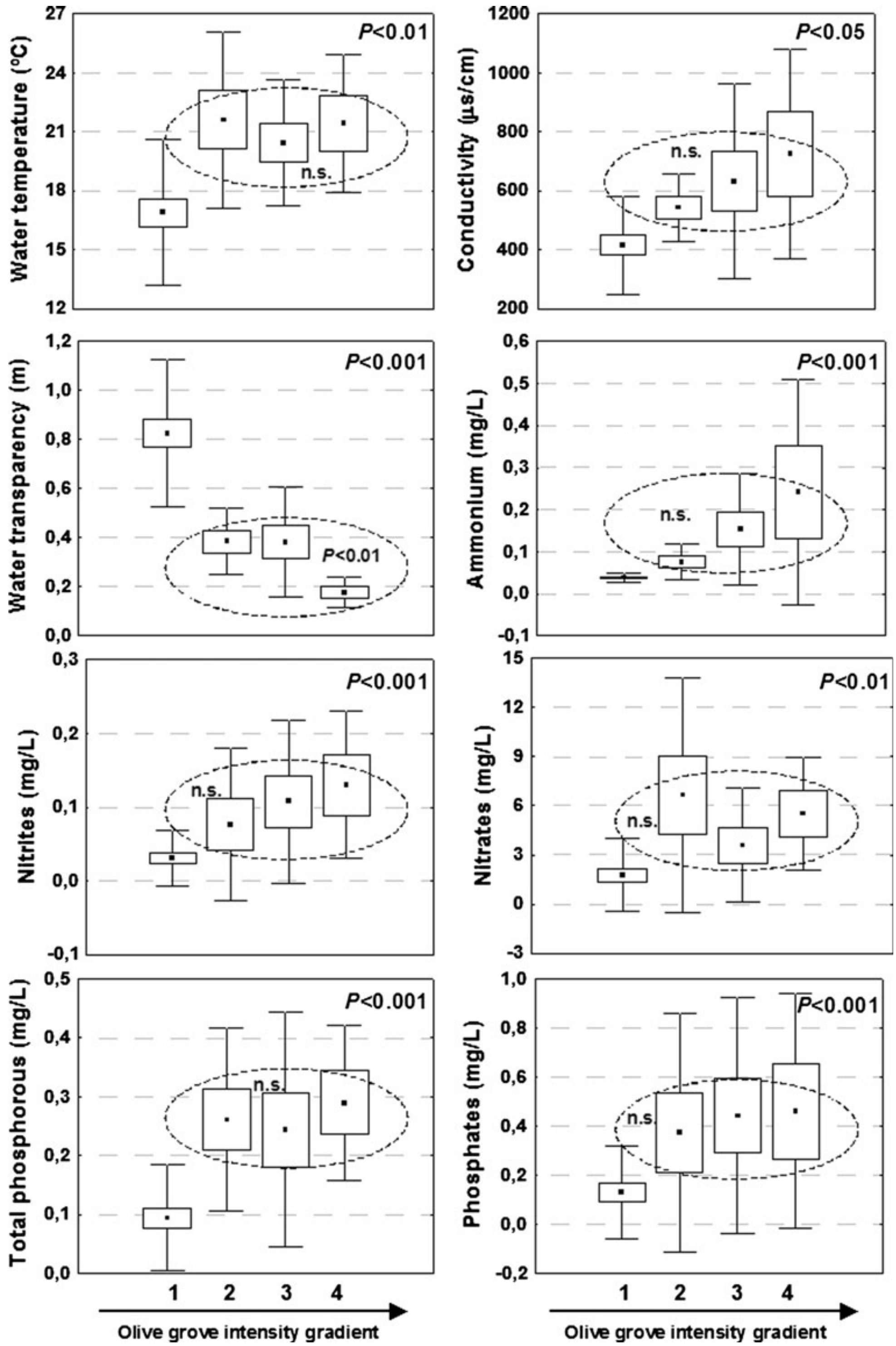

Fig. 3 *Box plots* of the most significant results for physicochemical parameters along the olive grove intensity gradient. Results from Kruskal–Wallis tests are shown for the entire gradient (*upper corner*) and considering only the three olive grove types (*inside the dotted line*). The olive grove intensity gradient follows a graphical sequence: (1) reference, (2) traditional olive groves, (3) intensive olive groves, and (4) hyper-intensive olive groves. (*Filled square*): Mean; box: ± SE; whisker: ± SD

($P < 0.001$), and ammonium ($P < 0.001$) concentrations only showed significant differences between the references and all olive grove types (Fig. 3). A large dispersion of values in each olive grove type was observed for these variables, although there seems to be an increase trend along the olive grove intensity gradient.

Substantial changes in the most relevant stream habitat variables were also observed (Fig. 4). Mean water depth gradually increased along the olive grove intensity gradient ($P < 0.01$), while current velocity showed no significant ($P > 0.05$) differences, despite the overall decreasing trend observed. The substrate showed decreasing granulometry

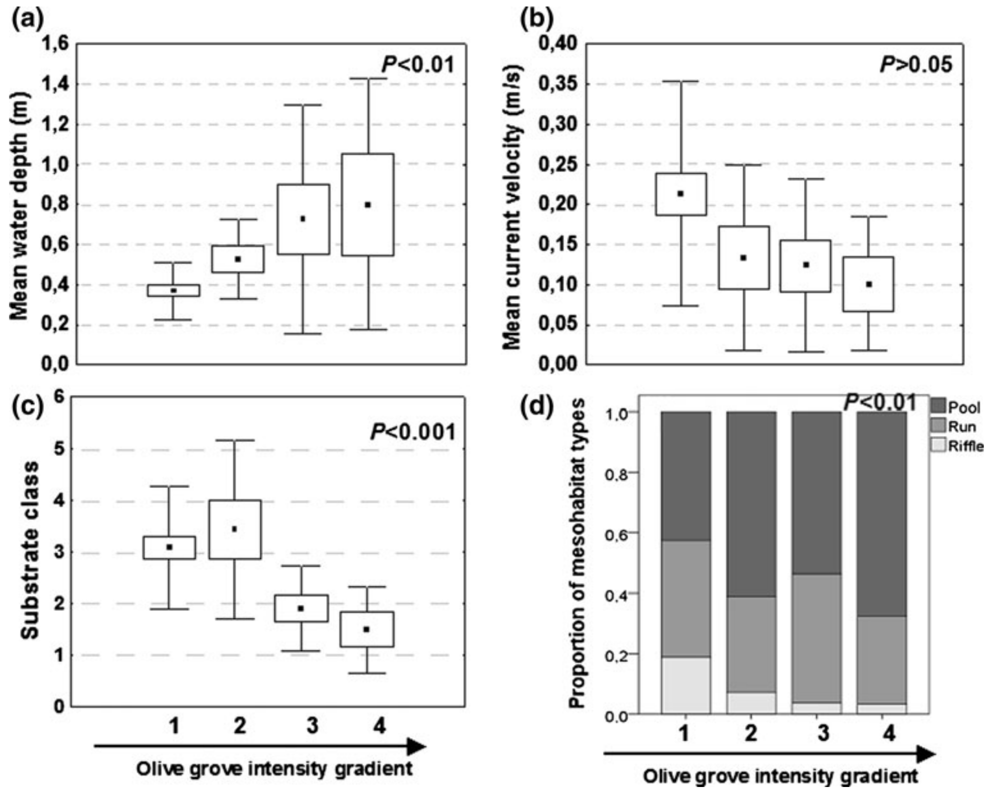

Fig. 4 **a**, **b**, **c** *Box plots* of the most significant results for local habitat variables along the olive grove intensity gradient (results from Kruskal–Wallis tests are shown). (*Filled square*): Mean; box: ± SE; whisker: ± SD. **d** Proportion of each mesohabitat type along the olive grove intensification gradient (result from Friedman test is shown). The olive grove intensity gradient follows a graphical sequence: (1) reference, (2) traditional olive groves, (3) intensive olive groves, and (4) hyper-intensive olive groves

($P < 0.001$), particularly significant between the reference set/traditional group and the intensive/hyper-intensive types, indicating that olive culture intensification lead to an increase in fine substrate (e.g., mud). Habitat patchiness also revealed changes along the intensity gradient (Fig. 4). Mesohabitat proportions differed significantly between olive grove types ($P < 0.01$), revealing a particular decrease in riffles ($P < 0.01$) and an increase in pools ($P < 0.05$) along the olive grove intensity gradient. Accordingly, habitat diversity also showed a decreasing pattern ($P < 0.05$), with a higher value in the reference set streams ($H = 0.61$) and a lower value in the streams under the influence of hyper-intensive systems ($H = 0.36$).

Changes in Fish Assemblage Structure

A total of 14 fish species were captured, including eight native and six non-native species (Table 2). In all sites under the influence of olive groves there was a high occurrence of non-native species, particularly *Gambusia holbrooki* and *Lepomis gibbosus*. On average, non-native species represented more than 50 % of the mean density and species richness per site, and were the only species captured from 17 % of the sampled sites. All the native species are endemic to the Iberian Peninsula (many are basin endemisms) and have high conservation status. Two

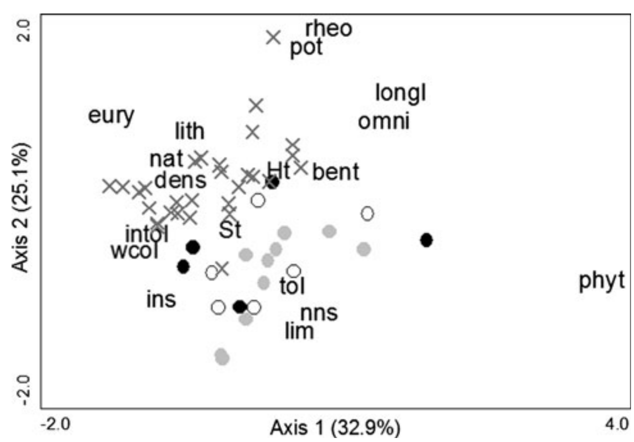

Fig. 5 Ordination diagram from Correspondence Analysis (CA) of reference and sampled sites based on fish community structure. Site symbols represent: references (*gray cross*), traditional olive groves (*white dots*), intensive olive groves (*gray dots*), and hyper-intensive olive groves (*black dots*). Metrics represent: species diversity (Ht), total number of species (St), number of native (nat), non-native (nns), intolerant (intol) and tolerant (tol) species, total density (dens), and the proportion of potamodromous (pot) and long lived (longl) individuals. Guilds abbreviations represent: habitat guilds - proportion of rheophilic (rheo), limnophilic (lim), eurytopic (eury), benthic (ben) and water column (wcol) individuals; trophic guilds—proportion of omnivorous (omni) and insectivorous (ins) individuals; and reproductive guilds—proportion of lithophilic (lith) and phytophilic (phyt) individuals

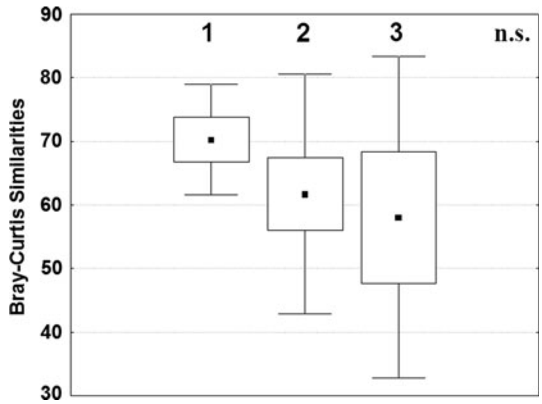

Fig. 6 *Box-plot* of Bray–Curtis similarities between references and each olive grove type: traditional (1), intensive (2), and hyper-intensive (3). Results from Kruskal–Wallis tests are shown. (*Filled square*): Mean; box: ± SE; whisker: ± SD

are endangered species: *Squalius pyrenaicus* and *Iberochondrostoma lemmingii* (Cabral et al. 2005).

The CA based on fish metrics and guilds showed a good segregation of the reference and olive grove sites, particularly along the 2nd axis, which together with the 1st axis explained 58 % of data variability (Fig. 5). The olive grove sites overlapped widely. Considering the overall fish assemblage structure, olive grove sites were particularly associated with non-native, tolerant, phytophilic, and limnophilic species. Reference sites were related to higher fish richness and density, and the species present were mostly natives. These sites were also represented by a larger diversity of functional guilds, including intolerant species.

Considering the possible co-variation of the environmental gradient in the biological analysis, results from PCA allowed definition of two main axes, which together accounted for 54 % of total variation in data. Ordination did not reveal any discrimination between olive grove types. PC1 was mainly related to human disturbance variables, e.g., organic/nutrient load ($r = 0.63$), sediment load ($r = 0.61$), riparian vegetation ($r = 0.57$), ammonium ($r = 0.66$), nitrate ($r = 0.81$), while PC2 was highly correlated with environmental variables reflecting river size, e.g., distance to source ($r = -0.60$), mean stream width ($r = -0.53$) and water depth (-0.52), habitat diversity, as proportion of pools ($r = -0.74$) and runs ($r = 0.55$), thus expressing a longitudinal environmental gradient. Spearman rank correlations between the PC2 and CA axes did not reveal any significant ($P > 0.05$) influence of the environmental gradients on the variation of fish community structure along the olive grove intensity gradient and between these sites and the reference set.

According to Permanova results significant differences were observed in community structure between the reference set and all olive grove types ($P < 0.001$). However, no differences were detected among olive grove types ($P > 0.05$). The environmental gradient expressed by PC2, and included in the analysis as co-variable, did not reveal any influence on these results. This further strengthened the previous correlations between the PC2 and CA axes, ensuring that the differences in fish assemblages between olive grove types and the reference sites are due to differences in olive production and not to underlying environmental differences.

Bray–Curtis similarities between community features of each sampled site and the reference set showed a general negative trend along the olive grove intensity gradient (Fig. 6). The results suggest a corresponding decrease in fish assemblage integrity along the stressor gradient, although no significant differences ($P > 0.05$) were observed. The increase in dispersion of similarities (reference/sampling sites) along the gradient was highest in the most intensive olive grove types.

Discussion

In this study olive grove intensification was related to an overall increase of human-induced disturbance in sampled streams. However, these values were not extreme, as olive production resulted in severe disturbances only reflected by specific variables. Indeed, major significant increases along the olive grove intensity gradient was particularly observed for organic/nutrient and sediment loads. Conductivity, ammonium, nitrite, total phosphorous, and phosphate concentrations showed increasing trends, but differences were only significant relative to the reference sites. Water transparency decreased along the gradient, in agreement with this trend towards disturbance. Stream habitat structure presented considerable changes in terms of water depth, current velocity, and substrate between the reference set and stream sites under the influence of olive groves as well as along the intensification gradient. The habitat diversity also decreased along the gradient, being the dominance of pools with fine substrate a result of natural flow modification and sedimentation. All these types of disturbance are strongly related to soil erosion, high surface runoff, and high levels of fertilization and irrigation commonly associated with intensive agriculture practices. Soil erosion is cited as one of the principal environmental problems associated with olive farming in Mediterranean regions (e.g., Graaff and Eppink 1999). In intensive olive plantations, farmers usually keep the soil bare of vegetation throughout the year; such that severe erosion occurs during heavy rains. Soil erosion and water runoff into nearby streams can be a major source of suspended sediments (and consequent turbidity), nutrients, and pesticides in watersheds dominated by agricultural land (e.g., Kuhnle et al.

2000; Vanni et al. 2001), and may change natural flow (e.g., Allan 2004; Stohlgren et al. 1998).

The main water pollutants from fertilizer use are nitrate and phosphate (e.g., Carpenter et al. 1998). Nitrate is highly mobile, leaches with water and reaches both surface and groundwater. Phosphate is less soluble in water and travels associated with sediments. Excesses of these nutrients cause eutrophication, which results in depletion of dissolved oxygen (Mallin et al. 2006). Like fertilizers, pesticides may have strong negative impacts on surface and groundwater and aquatic ecosystems (e.g., Liess and von der Ohe 2005).

All the problems mentioned can be further enhanced by the removal of riparian vegetation and channelization of streams, frequent in farmlands. Degradation/removal of the riparian vegetation was actually observed in all sampled sites and no significant differences were observed among olive grove types. Riparian zones have the ability to prevent sediment runoff and to hold excess nutrients and modify their inputs to the stream (e.g., Muenz et al. 2006), preventing negative consequences in overall water quality (Sekely et al. 2002). Due to their position at the interface between terrestrial and aquatic ecosystems, riparian zones play a crucial role in controlling the flow of nutrients from watersheds (Roth et al. 1996). Furthermore, removal of streamside vegetation can increase mean water temperature (e.g., Wohl and Carline 1996) and promote changes in stream morphology as streams typically widen and become shallower (Roth et al. 1996).

Animal load generally decreased with olive grove intensity. Traditional olive groves presented the highest animal loads as a result of livestock associated to these systems. Traditionally olive tree farming was founded on the principles of organic economy, together with the systematic use of human and animal labor. Livestock functioned not only as workforce but also as a producer of manure. More recently, production of livestock, mainly beef cattle, has undergone a huge increment in the study area associated with traditional olive groves, as well as other land uses such as pastures. This was probably the cause of the pattern observed in water temperature, nitrate concentration, and degradation of streamside riparian vegetation. These parameters presented the lowest values in the reference sites and the highest in both traditional and hyper-intensive olive groves. The literature clearly demonstrates that livestock grazing with unrestricted access to streams has negative impacts on aquatic ecosystems (e.g., Lyons et al. 2000; Magner et al. 2008). This practice increases in-stream trampling, habitat disturbance, and erosion from overgrazed stream banks, as well as reducing sediment trapping by riparian and in-stream vegetation and decreasing bank stability, leading to increased turbidity, nutrients, and suspended solids concentrations in streams as already stated (e.g., Kaufmann and Kreuger 1984; Vidon et al. 2008). Streams can be contaminated by water runoff from adjacent land during and immediately after irrigation and precipitation, and by direct excretion of fecal material into the water. In the current study, sampling was conducted during spring, immediately after heavy rains and flash floods typical of the Mediterranean climate.

Overall, results showed that for most of the disturbance variables significant differences occurred mainly between reference and olive grove sites, but not among olive grove types, even though a general increasing trend was observed along the olive grove intensity gradient. This confirms a high level of disturbance in all olive grove sites, emphasizing that despite the low intensity of agricultural practices, traditional olive groves sites are subjected to a considerable level of disturbance as a consequence of high animal loads and other land uses, which involve fertilization.

There was a strong shift in fish assemblage structure between the reference and olive grove sites. Reference sites were related to higher fish density and a richer, diverse, native community with intolerant species. In contrast, olive grove sites were greatly associated with non-native, tolerant, phytophilic, and limnophilic fish species. Land use influences aquatic organisms through interrelated impacts on water quality, hydrology, and habitat (Allan 2004; Paul and Meyer 2001). These impacts have been shown to substantially change fish assemblages (Argent and Carline 2004), decrease species richness/diversity and sensitive species, while increasing tolerant and introduced species, ultimately influencing the integrity of fish assemblages (e.g., Fischer et al. 2009; Roth et al. 1996). A loss of intolerant species accompanied by a gain in tolerant ones has been identified as a potential factor contributing to the homogenization of biotic assemblages in freshwater systems also reported in other studies (e.g., Olden and Poff 2004; Scott and Helfman 2001). On the other hand, less disturbed streams tend to support more trophic, reproductive, and habitat specialist species (Poff and Allan 1995).

No differences were observed in fish assemblage structure among olive grove types, but major differences existed between each olive grove group and the reference set. Furthermore, community similarities between references and each olive grove type showed a decreasing trend along the olive grove intensity gradient, though without significant differences. Results from the present study are in accordance with several previous studies (e.g., Heitke et al. 2006; Lammert and Allan 1999; Nerbonne and Vondracek 2001; Roth et al. 1996) documenting the deviation of biological assemblages from reference conditions with increasing land use within watersheds. Furthermore, high values of dispersion in similarities between references and most intensive practices can also be interpreted as signs of high environmental variability and degradation. One important observation of this study is that, although fish assemblages under the influence of traditional olive groves tended to show higher similarities with reference sites than the other olive grove types, they are far from being communities with high integrity, as river basins are increasingly subjected to multiple pressures.

Conclusions and Guidelines for Mediterranean Agro-Systems Management

This study illustrates the relationships between land use and the ecological integrity of southern Portuguese streams as regards to water quality, stream habitat, and fish community structure. Intensive agro-systems, such as olive culture can result in several types of stress, which individually or together affect the structure and functioning of streams. In the current study, olive production led to multiple in-stream disturbances, with emphasis on sediment, nutrient, and organic loads, as well as degradation of riparian vegetation. The cumulative effects of these multiple disturbances reduced water quality and habitat diversity, and promoted the loss of biota integrity. Even when traditional farming practices seem to inherently support the highest natural value, as is the case of traditional olive groves, the impact of these systems on the aquatic ecosystems can be dramatically different when they are coupled with other land uses such as livestock production. The impacts of agro-systems depend not only on the degree of intensity of the main culture but also on associated multi-pressures. From this perspective it is important that streams nearby farms be managed following simple guidelines as follows:

(i) Conserve/rehabilitate riparian corridors by restoring their natural vegetation, since this will protect stream channels by decreasing erosion and sedimentation, filtering nutrients, and therefore reducing eutrophication. Other suggested bank protection measures include stone or log walling, brush matting, deflectors, and rock riprap.

(ii) Ensure that small tributaries or surface waters discharge to floodways rather than directly to streams. These small floodways (natural or artificial) may be vegetated to retain soil and nutrients that would otherwise be washed into streams.

(iii) Reduce soil-intensive tillage and increase plant cover on groves, which will decrease soil erosion and consequently surface runoff flow, leaching and sedimentation, promoting enrichment of the soil in organic matter and infiltration.

(iv) Reduce direct water abstraction from streams and unstable crossing structures, since natural flow enhances stream function, habitat diversity, and consequently community integrity, while minimizing the effects of floods.

(v) Fence watercourses and floodplains to control access by livestock from pasture areas to reduce organic loads on streams, bank grazing, and trampling. Both sides of watercourses and crossing access should be fenced and additional sources of water supply should be provided to the cattle.

Making olive production practices compatible with the "good" status of surface and groundwater required by the WFD represents a major challenge in Mediterranean regions. Management plans, legislation, and restoration programs will need to consider all these aspects simultaneously to protect aquatic ecosystem health and promote sustainable agricultural development.

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