



Flow network indices signal a directional change in ecosystems: Evidence from a small mountain lake (Lake Santo, northern Italy)

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ABSTRACT

Empirical evidence of the theoretically expected trends of ecosystem development is scarce so far. In this research, we used long-term empirical data about the plankton community of a small mountain lake (Lake Santo, northern Apennines, Italy) to reconstruct its developmental trajectory during a period comprised between early 1970 s and 2010 s. We exploited these data to build yearly ecological networks and from their configuration of energy flows we computed network information indices. The trends of these indices enlighten about the developmental trajectory of this ecosystem during the period covered by the data set. In particular, they indicate that Lake Santo evolved in the direction of increasing stability at the expense of efficiency in energy transfer. We compared these results with current hypotheses about the directionality of ecosystem development, which are rooted in ecosystem theory, and discussed the possibility that, counter to some theoretical models of ecosystem development, Lake Santo followed an unimpeded direction of development rather than a trajectory typical of an ecosystem under stress. Finally, the long-term trends of flow network indices provided insights about the health status of the ecosystem.

1. Introduction

Ecosystems vary over time and their patterns of change led scholars to conceive the model of ecological succession, an orderly sequence of steps that form a directional pathway and whose endpoint is the climax community (Clements, 1936; Margalef, 1963; Odum, 1969). Defined trends for a series of indices would signal the progress along this evolutionary path (Odum, 1969; Ulanowicz, 1980), although stress and chance variability may reverse it or render it uneven, if not erratic (Holling, 1986; Archer and Stokes, 2000; Zhou et al., 2014). Identifying ecosystem successional stages is problematic because the community assembly may take centuries to complete. However, trajectories of ecosystem change may show up over shorter periods. Capturing these trajectories offers the opportunity to grasp developmental tendencies of ecosystems, whether they result from an unimpeded natural evolution or

are the product of major disturbances (Ulanowicz, 1980; Odum, 1985; Zhou et al., 2014).

MacArthur (1955), following Lindeman's (1942) earlier narrative, described the ecosystem as a configuration of flows, showing how ecosystem status over time could be characterized using information theory. Ulanowicz (1980, 2004) applied the information theory to the topological arrangement that one obtains from mapping out the energy exchanges between ecosystem components and formulated seven fundamental indices that can be used to quantitatively track ecosystem development: (1) flow diversity (H); (2) average mutual information (AMI); (3) residual diversity (H_C); (4) total system throughput (TST); (5) development capacity (DC); (6) ascendency (A); and (7) overhead (O). These indices have been since applied to understand ecosystem development (Ulanowicz, 1980; Christensen, 1995; Boit and Gaedke, 2014), assess ecosystem health (Mageau et al., 1998; Christian et al., 2010), and

Abbreviations: A , ascendency; AMI , average mutual information; $BPOC$, benthic particulate organic matter; DC , development capacity; DOC , dissolved organic carbon; ENA , ecological network analysis; F , fitness for evolution; H , flow diversity; H_C , residual diversity; IA , internal ascendency; I_C , internal connectance; IDC , internal development capacity; I_R , flow redundancy; O , overhead; O_D , dissipative overhead; O_E , overhead on exports; O_I , overhead on imports; O_R , internal redundancy; PP , phytoplankton primary production; S_B , Shannon's index of diversity based on compartments' biomass; TOM , total organic matter; TST , total system throughput; $WDOC$, water dissolved organic carbon; $WPOC$, water particulate organic carbon.

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signal the effect of stress on ecosystems (Ulanowicz, 1996; Bondavalli et al., 2006). In this research, we employed the same information indices, which we computed from yearly ecological flow networks constructed exploiting a data set of the plankton community structure of a small mountain lake ecosystem (Lake Santo, northern Italy). Such data set was collected within a long-term research project (Rossetti et al., 2004) and describe the community of Lake Santo as it was in the years 1972–1974, 1991, 2001, 2003, 2007–2010, and 2012. The results of earlier limnological campaigns at Lake Santo (from 1952 to 1954; see Moroni, 1962; Moroni et al., 1973), which also included the study of hydrochemistry and zooplankton, are not considered here because the methods were different from those used in more recent investigations.

In the literature, ecosystem development and ecological succession are often used interchangeably (Mageau et al., 1998; Latham and Scully, 2002). On the other hand, ecological succession is nothing else than the tangible, long-term expression of ecosystem development. Considering that our data set covers a limited time frame and that when data collection started the lake was at some unknown state in its successional dynamics, we stress that the trends of indices reconstructed in this research do not describe a successional pathway; neither can they be used to identify any specific successional stage(s) that characterized Lake Santo in the period of investigation. Nevertheless, they indicate a tendency to change that is expression of the lake developmental dynamics. Accordingly, we discuss the trends obtained for network information indices in the light of the ecosystem development framework.

This research aims to address the following questions: (i) do information indices in Lake Santo show trends that highlight a directional tendency of change? (ii) Is this tendency coherent with the theoretical expectations about the relative behavior of the indices during ecosystem development? (iii) Can these trends inform about some form of stress that affected Lake Santo, possibly to enable drawing conclusions on its health status?

2. Materials and methods

2.1. Study area

Lake Santo (10°00'38" E, 44°24'06" N) is a dimictic lake of glacial origin, located in the northern Apennines (1,507 m above sea level, upper Parma Valley in the Province of Parma, Italy; Fig. 1). The lake surface extends over about 8 ha, its average depth is 11.3 m, and the maximum depth is 22.5 m. Due to its altitude, the Lake Santo is relatively non-impacted by anthropogenic activities (Viaroli et al., 1994), which have remained confined to local tourism.

The lake was naturally fishless but underwent regular fish stocking since the beginning of the last century (Brian, 1924; Maldini et al., 2004). Fish introduction seems not to have significantly affected the trophic state of the lake, which has since remained oligo-mesotrophic (Mazzola, 2013). During last decades, species composition of the zooplankton community has remained fairly constant, with the seasonal succession that exhibited definite patterns and levels of abundance remained relatively stable (Rossetti et al., 2006; Mazzola, 2013).

2.2. Network construction

In what follows, we offer a sketch of the general procedure adopted for assembling the networks. This procedure is detailed in SM1, Appendix 1. For the years 1972–1974, 1991, 2001, 2003, 2007–2010, and 2012 the data set at our disposal was the most complete and was compiled in the framework of a long-term ecological research project coordinated by one of the authors (GR). Sampling was conducted monthly (from ice-melting date to end of June, and from October to ice formation in winter) and biweekly (in the summer season, from July to the end of September) in the period of the open waters. The free ice-period lengthened probably due to climate change, and the beginning and ending of the sampling campaign was set up accordingly. Thus, the

data set reflects the community structure as it was shaped by this type of variability.

Given the reduced size of the lake, sampling was conducted at a single station that coincided with the point of maximum depth. DEIMS-SDR (Dynamic Ecological Information Management System - Site and dataset registry¹), an online information management system, provides additional information regarding the dataset at disposal. For this study, we assembled average annual networks describing carbon exchanges among trophic groups during the ice-free period. The sampling effort was concentrated in the open water period, for logistical reasons related to sampling activities in the winter period, but also on the basis of the results obtained during the occasional winter campaigns conducted in the lake. The presence of ice cover, combined with low water column temperatures, limits primary production for several months of the year. Primary production is further drastically reduced in the case of snow cover, due to the very poor penetration of solar radiation. In general, biological activity under the ice cover is extremely low. There are few overwintering zooplankton species, and these have a reduced metabolism. For example, in species of the genus *Eudiaptomus* it has been reported that they can survive the whole winter with no food. They rely upon the lipids accumulated for reproduction during the open-water period (Rautio et al., 2011). It is highly plausible that the same applies to *E. intermedius*, the dominant species in the zooplankton community in terms of biomass. At the benthic level, decomposition and mineralization of organic matter may continue under the ice cover at rates not dissimilar to the open water phase, as temperatures in the hypolimnion layer are subject to slight variations throughout the year. But the nutrients made available by these processes are used by the primary producers in the short spring overturn, immediately after the melting of the ice cover. Allochthonous carbon inputs to the lake can be considered negligible in winter, due to the presence of the ice cover and the frozen soil in the lake catchment. Again, a conspicuous allochthonous carbon input can be associated with the spring snowmelt. It can therefore be assumed that carbon fluxes during the ice cover are extremely low and do not significantly affect the overall lake metabolism.

To build the networks we captured as much detail in trophic structure as possible from the data collected during the sampling campaigns. This allowed resolving the networks down to the species level for the zooplankton community. Photoautotroph primary producers (phytoplankton taxa) were instead aggregated in a single compartment. A fine partitioning of energy flows from phytoplankton species to zooplankton species would have increased unnecessarily the complexity of the network given that no selective grazing by some zooplankters over specific phytoplankton taxa was documented in the ecological studies conducted on the lake (Rossetti et al., 2006) and other waterbodies nearby (Paris et al., 1993). Standing stocks of species or trophic groups and carbon fluxes were different quantitatively from one year to the next in the time series.

One major question involved how to treat functional groups for which data at the level of individual species were not available. We grouped the heterotrophic microorganisms (i.e. bacterioplankton, flagellates and protozoa) in a single compartment representing the “living particulate organic matter” (living POC; we called it MLOO, microbial loop, in the network models) and did the same for fish species (FISH). Although benthic fauna comprised several species, their contribution to the benthic carbon was negligible in comparison with the amount of organic material that appeared as detritus (Ferrari and Villani, 1978). Accordingly, we decided to have only one benthic component called “benthic particulate organic matter” (BPOC). Finally, “water dissolved organic carbon” (WDOC) and “water particulate organic carbon” (WPOC) were added as non-living nodes to complete the ecosystem representation. Considering that trophic aggregation has been widely

¹ <https://deims.org/21d8695a-c932-4534-9819-e267e5befefc>.

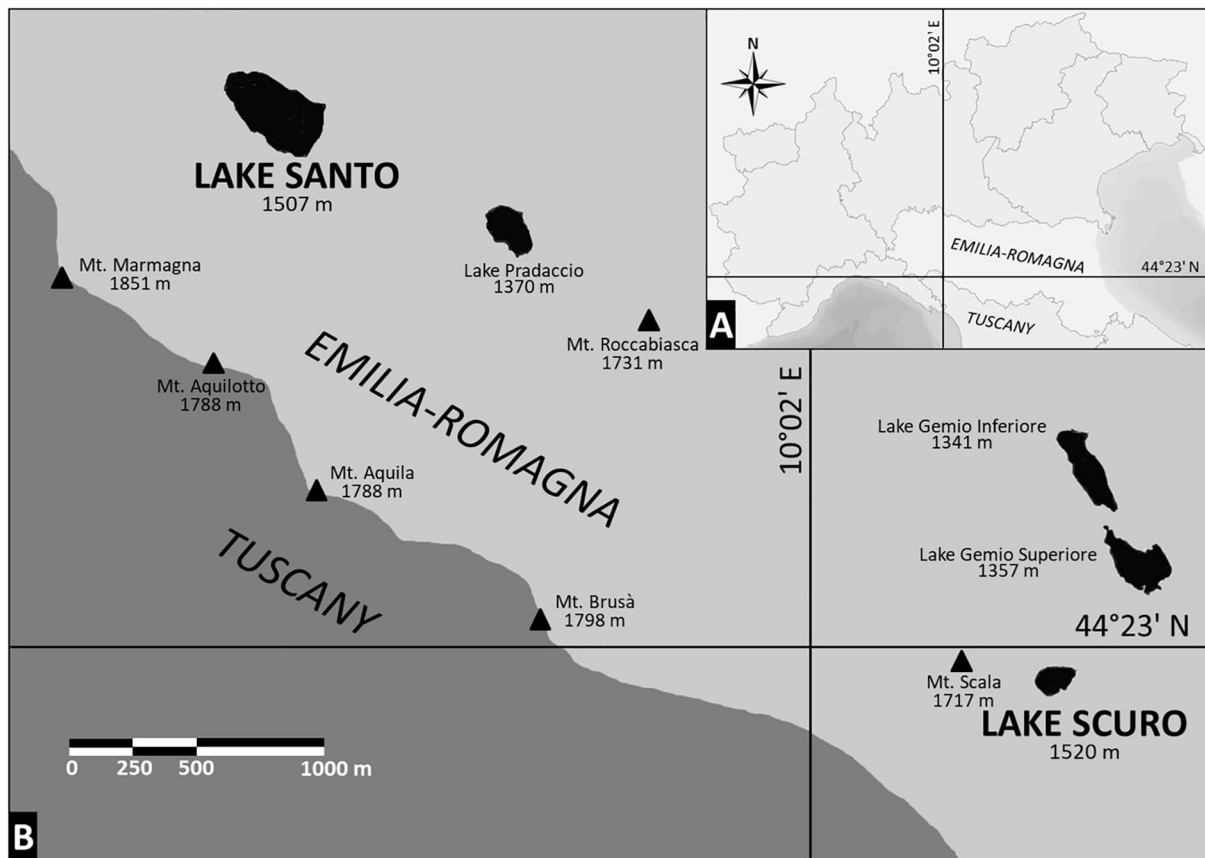


Fig. 1. The geographical location of Lake Santo. The lake is in the Tuscan-Emilian Apennines National Park (northern Apennines, Italy) and its altitude is 1,507 m above sea level.

applied in ecosystem ecology (Hart et al., 2000; Luczkovich et al., 2002; Johnson et al., 2009), parsing the network in this way seemed appropriate to grasp essential features of ecosystem organization while keeping model complexity within the limits of methodological tractability.

Sampling activity provided data of chlorophyll-a concentration ($\mu\text{g chl-a l}^{-1}$) that we used as proxy for phytoplankton biomass. Zooplankton species were sampled as number of individuals per unit volume (ind. l^{-1}). Information concerning fish abundance in Lake Santo was gathered from local organizations that rule recreational fishing. We used all these data to calculate standing stocks and quantify carbon flows as usually done in network analysis (Wilson and Parkes, 1998; Hart et al., 2000; Johnson et al., 2009). Details about estimation techniques are in SM1, Appendix 1. In what follows, we provide a quick thumbnail sketch of the basic criteria behind the procedure.

To obtain phytoplankton standing stock, we multiplied chlorophyll-a concentration ($\mu\text{g chl-a l}^{-1}$) by the carbon/chl-a ratio derived from technical literature (Jørgensen et al., 1991; SM1, Appendix 1). Phytoplankton primary production (PP) per unit of biomass was determined through in situ experiments using the light-dark bottle oxygen productivity method; such measures were integrated with the method of the ^{14}C (Ferrari and Villani, 1978).

To estimate the standing stock of the living POC, which comprises bacteria, ciliates, and flagellates, first we applied the logarithmic equations that relate bacterial biomass with field data about chl-a (Jørgensen et al., 1991; SM1, Appendix 1). We transformed the values we obtained into grams of carbon using the carbon content per cell (Ulanowicz et al., 1998; Bondavalli et al., 2006; SM1, Appendix 1). To estimate the biomasses of flagellates and ciliates, we used the biomass ratios bacteria/flagellates and bacteria/ciliates (Jørgensen et al., 1991; Ulanowicz et al., 1998; Bondavalli et al., 2006). From the number of

individuals, we calculated the biomass of zooplankton species. Because the standard units adopted for our networks are grams of carbon per cubic meter (g C m^{-3}), we transformed the data to obtain dimensional consistency. To this end, we gathered data on the average dry weight (g ind.^{-1}) of animals and the percentage of carbon per gram of dry weight (% DW) from the literature and technical manuals (Jørgensen et al., 1991; Ulanowicz et al., 1998; Bondavalli et al., 2006; SM1, Appendix 1). Such parameters allowed converting the number of individuals to g C m^{-3} of biomass. Data about fish species were treated in a similar way. Laboratory analysis of samples yielded the POC and DOC standing stocks (Bondavalli et al., 2006; Ulanowicz et al., 1998; SM1, Appendix 1). We subtracted the values of living POC, phytoplankton and zooplankton standing stocks from the POC to obtain that of WPOC. Measures of organic matter performed on sediment samples (ash-free dry weight, AFDW, with ignition at $550\text{ }^{\circ}\text{C}$ in muffle furnace) served to quantify the carbon content in sediment, which was used as an estimate of BPOC standing stock. The biomasses of water particulate organic carbon (WPOC), water dissolved organic carbon (WDOC) and benthic particulate organic carbon (BPOC) were summed to quantify the total organic matter (TOM, g C m^{-3}).

After quantifying the standing stocks for each compartment, we characterized their carbon budget according to the general equation apportioning consumption or total intake (C) between production (P), respiration (R) and egestion (E). This latter is the portion of non-assimilated material released back into the environment; for primary producers, it is commonly referred to as excretion (Pujo-Pay et al., 1997; Aota and Nakajima, 2001). For each species/trophic group (i.e. compartment), we estimated the consumption rate per unit biomass per year from the literature (Jørgensen et al., 1991; SM1, Appendix 1); then, we multiplied this factor by the biomass to attain the total intake. Next, we apportioned each compartment's total intake among the various

resource items using dietary proportions described in the literature (Jørgensen et al., 1991; SM1, Appendix 1). For some compartments, only a list of prey species was available, while details on feeding preferences were lacking. In this case, to set the magnitude of nutritional flows we had no other option than apportioning the total input to the consumer in proportion of the standing stocks of its resources. In this procedure, we did not take into account differences that may characterize the life-stages of certain species, but considered the main dietary habits documented in various pieces of the available literature. We then apportioned the total compartmental intake among the output processes. However, in the case of cyclopoids copepods we considered different life stages as explicit compartments and their specific diet was taken into account (SM1, Appendix 1). Respiration and excretion (i.e. egestion) rates per unit of biomass were available from the literature for most of the species, so that these outputs could be immediately quantified (Jørgensen et al., 1991; Ulanowicz et al., 1998; Bondavalli et al., 2006; SM1, Appendix 1). Most of the losses to predation were estimated from the predator (i.e. input) side, as described above. Finally, the assumption that the networks must be at steady state (in each compartment inputs must equal the outputs) eased the estimation of several flows

(Ulanowicz et al., 1998; Bondavalli et al., 2006; SM1, Appendix 1). However, uncertainties characterized some estimations and prevented many of the compartments from balancing exactly inputs and outputs. The degree of imbalance was investigated entering flow estimates in a spreadsheet format. We balanced manually all compartments to within a 10% difference between inputs and outputs using field data and literature values (SM1, Appendix 1). We then achieved the final balance using the program NET BALANCE, which assumes linear donor control (Allesina and Bondavalli, 2003, 2004). All flows (exogenous inputs, internal exchanges, respiration/dissipations, and exports of usable medium) in the 11 networks of the time series are reported in the Supplementary Materials 2 (SM2), Appendix 1.

2.3. Network models

Following Scharler and Borrett (2021) we assembled the budget of the carbon exchanges for Lake Santo during the open water season (from May through November) in the years 1972–1974, 1991, 2001, 2003, 2007–2010, and 2012, for a total of 11 networks. The basic structure of the network did not change over the years as the community remained

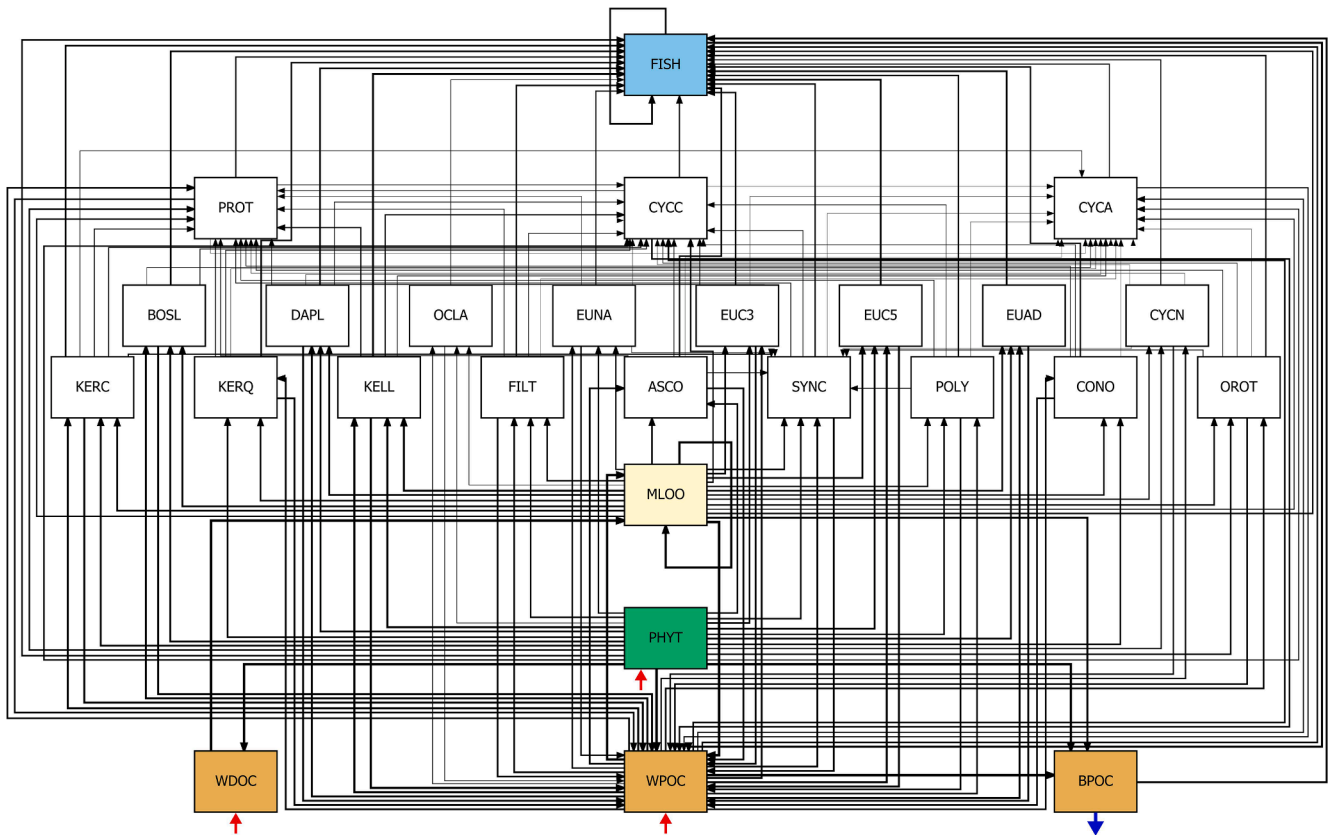


Fig. 2. Network that illustrates carbon flows ($\text{g C m}^{-3} \text{y}^{-1}$) in Lake Santo ecosystem in 2009. The network is composed of 26 compartments. Three non-living compartments (brown colored) are at the bottom and the position of the other compartments along the vertical axis reflects their trophic position (Scotti et al., 2006). The box of the primary producer (PHYT) is green, microbial loop (MLOO) is yellow, all zooplankton groups are white and the top-predator of the system (FISH, which includes *Salmo trutta fario* and *Oncorhynchus mykiss*) is light blue. Inter-compartmental exchanges among the groups composing the ecosystem are black and the direction of carbon circulation is illustrated by arrow-headed links (from prey/resources to predators/consumers). Imports from outside (i.e. primary production of PHYT and organic material contributing to WDOC and WPOC) are visualized by red links pointing to the compartments, while export from BPOC (i.e. carbon burial into the sediment) is depicted with a blue link leaving the compartment. The strength of each interaction (i.e. amount of carbon flowing) is proportional to the thickness of the links. Respiration flows are associated to each compartment but not visualized here for the sake of clarity (usually they are represented as ground symbols). Keys for node labels: WDOC – water dissolved organic carbon; WPOC – water particulate organic carbon; BPOC – benthic particulate organic carbon; PHYT – phytoplankton; MLOO – microbial loop (bacteria, ciliates and flagellates); KERC – *Keratella cochlearis*; KERQ – *Keratella quadrata*; KELL – *Kellicottia longispina*; FILT – *Filinia longiseta-terminalis*; ASCO – *Ascomorpha* spp.; SYNC – *Synchaeta* sp.; POLY – *Polyarthra* sp.; CONO – *Conochilus unicornis-hippocrepis*; OROT – Other rotifers; BOSL – *Bosmina longirostris*; DAPL – *Daphnia longispina*; OCLA – Other cladocerans; EUNA – *Eudiaptomus intermedius* nauplii; EUC3 – *Eudiaptomus intermedius* copepodites (CI-CII-CIII); EUC5 – *Eudiaptomus intermedius* copepodites (CIV-CV); EUAD – *Eudiaptomus intermedius* adults; CYCN – Cyclopoids nauplii; PROT – Predatory rotifers; CYCC – Cyclopoids copepodites; CYCA – Cyclopoids adults; FISH – fish species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fairly constant and only biomasses and flow weights differed from one network to the next (Fig. 2 depicts the network of 2009 as reference model).

The creation of the trophic networks began with the identification of the key components of the ecosystem. Resolution at the level of single species was possible for zooplankton, but data availability did not allow such fine modelling for the other components of the ecosystem because intensive campaigns of data collection along the whole period were directed mainly to study the zooplankton community. Phytoplankton (PHYT) was the only compartment considered for primary production. All heterotrophic organisms, such as bacterioplankton and protozoa, were included in the microbial community (MLOO). Only one fish compartment (FISH) grouped the two species that were present in the lake (*Salmo trutta fario* and *Oncorhynchus mykiss*), which share identical feeding habits. A compartment called BPOC (benthic particulate organic carbon) stored the benthic carbon: phyto-benthos and detritus are its major contributors. Water dissolved organic carbon (WDOC) and water particulate organic carbon (WPOC) completed the list of the compartments. Standing stocks were quantified as grams of carbon per cubic meter (g C m^{-3}) and flows in grams of carbon per cubic meter per year ($\text{g C m}^{-3} \text{y}^{-1}$), as usually required in network analysis applications.

Estimation techniques for standing stocks and flows are in the SM1, Appendix 1. The intensity of flows in all networks is reported in the SM2, Appendix 1. Annual models were constructed consistently and taking into account guidelines and instructions given in Fath et al. (2007). They were obtained by averaging over the year some markedly seasonal parameters (e.g. P/B ratios). In fact, the open water season counts of three periods, with two complete mixing events that take place at the beginning (April-May) and toward the end (September). When complete mixing occurs, the production peaks and affects the entire biological community (Paris, 1993; Paris et al., 1993; Rossetti, 1994).

2.4. Information theory indices

Information indices based on communication theory have been devised to quantify ecosystem development (Rutledge et al., 1976; Ulanowicz, 1980, 1997). Flow diversity (H) represents the total flow activity (e.g. diversity of flows, as number and intensity of the flows) in a system, given a certain amount of currency (e.g. total matter or energy, TST , see below). It expresses the amount of choice the energy has in following its way up through the ecosystem; as such, it is a measure of system uncertainty (joint entropy or joint uncertainty; Ulanowicz and Norden, 1990; Allesina and Bodini, 2008). Thus, H reflects the total complexity of a system and can be regarded as a metric of the total capacity of the ecosystem to undergo change (Ulanowicz et al., 2009). In a system with N compartments, it takes the form:

$$H = -k \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \frac{T_{ij}}{T_{..}} \log_2 \left(\frac{T_{ij}}{T_{..}} \right) \quad [1]$$

where T_{ij} stands for the flow of medium from compartment i to compartment j . Labels 1, 2 ... N identify the compartments that form the network. Summations include the outside system as: (i) source of input (compartment 0) to the system (e.g. phytoplankton primary production); (ii) receiver of usable medium (compartment $N + 1$) from the system (i.e. export); (iii) sink of medium (compartment $N + 2$) dissipated by the system (i.e. respiration). Because the algorithms of network analysis manipulate flow values in a matrix format (matrix of flows), it is assumed that $T_{..}$ stands for summation across all rows (first dot) and columns (second dot) and it corresponds to TST . In the same way, T_i is the sum over the i^{th} row and T_j the sum over the j^{th} column. Rutledge et al. (1976) used the notion of conditional probability to decompose H into two complementary terms, amending the measure of total flow diversity as follows:

$$H = AMI + H_C \quad [2]$$

The average mutual information (AMI) quantifies the portion of flow diversity encumbered by structural constraints. It expresses the degree to which the flow structure reduces the amount of choice that energy has at disposal to flow in the ecosystem. AMI depends on flows as follows:

$$AMI = k \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \left(\frac{T_{ij}}{T_{..}} \right) \log_2 \left(\frac{T_{ij} T_{..}}{T_i T_j} \right) \quad [3]$$

The chief advantage of using information theory to describe organization is that it allows one to quantify the opposite (or the complement) to information in similar fashion. Whence, a non-negative variable called residual diversity (H_C) captures everything that is disordered, incoherent and redundant in the network. It represents the residual redundancy of connections, which informs about the amount of "choice" (i.e. residual freedom) that remains for energy to flow in the ecosystem:

$$H_C = -k \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \left(\frac{T_{ij}}{T_{..}} \right) \log_2 \left(\frac{T_{ij}^2}{T_i T_j} \right) \quad [4]$$

Thus, the overall complexity of the flow structure, as measured by flow diversity (H), can be resolved into a component that gauges how orderly and coherently the flows are connected (AMI), and a residual that measures the disorder and freedom that remains (H_C). This latter index includes those aspects that detract from system's organization and performance: disorganized, stochastic, inefficient and incoherent aspects of a system's activity all contribute to the residual diversity (H_C). Rutledge et al. (1976) proposed that these aspects concur to the stability of the system. Under favorable (i.e. undisturbed) conditions, these inefficiencies tend to encumber system's performance. During times of novel or stochastic stress, however, the same processes act as a reservoir from which the system can draw to reconfigure itself (i.e. adapt) following perturbations.

Summing all flows that make an ecological network, one obtains a measure of total ecosystem activity, the total system throughput (TST). This metric imparts physical dimension to indices derived from information theory, as it replaces the coefficient k in their expression (Ulanowicz, 1986). Therefore, substituting TST in [3] yields an index called ascendancy (A):

$$A = TST \times AMI = T_{..} \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \left(\frac{T_{ij}}{T_{..}} \right) \log_2 \left(\frac{T_{ij} T_{..}}{T_i T_j} \right) \\ = \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} T_{ij} \log_2 \left(\frac{T_{ij} T_{..}}{T_i T_j} \right) \quad [5]$$

It measures the fraction of matter or energy that an ecosystem distributes in an efficient way (i.e. along efficient routes); it combines ecosystem activity and organization, thus providing a single measure of ecosystem growth and development, as TST quantifies system's size and AMI informs about the level of organization in flow structure. Scaling H in [1] by TST yields the development capacity (DC) (Ulanowicz, 1986; Scotti, 2008):

$$DC = TST \times H = -T_{..} \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \frac{T_{ij}}{T_{..}} \log_2 \left(\frac{T_{ij}}{T_{..}} \right) \\ = - \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} T_{ij} \log_2 \left(\frac{T_{ij}}{T_{..}} \right) \quad [6]$$

DC quantifies how much currency benefits from the potential choices offered by the flow diversity. It combines the realized efficiency level at which the system processes matter/energy with the fraction of matter/energy that the system processes inefficiently (i.e. the potential for further development). Together, these fractions represent the maximum level of development a given system can reach (its value depends on number of components and TST ; Ulanowicz, 1986; Scotti, 2008). DC can also be seen as the scope for network development because it considers the currency available for establishing connections (TST) and the overall level of structural organization portrayed by H , which combines realized

organization and potential for further organization (*AMI* and *H_C*, respectively). The higher the *DC*, the greater the potential for the ecosystem to become an organized whole is; *DC* represents the upper limit for ascendancy. When scaling the residual diversity of flows (*H_C*) by the *TST* [4], one obtains the overhead (*O*), which pertains to redundant flows:

$$O = TST \times H_c = -T \cdot \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} \left(\frac{T_{ij}}{T_{..}} \right) \log_2 \left(\frac{T_{ij}^2}{T_i T_j} \right) \\ = - \sum_{i=0}^{N+2} \sum_{j=0}^{N+2} T_{ij} \log_2 \left(\frac{T_{ij}^2}{T_i T_j} \right) \quad [7]$$

The overhead is made of different contributions, each related to a certain type of flow: overhead on imports (*O_I*), overhead on exports (*O_E*), dissipative overhead (*O_D*), and redundancy of internal exchanges (*O_R*).

$$O = O_I + O_R + O_E + O_D \quad [8]$$

The mathematical expression of all the components of the overhead can be found in the literature (Ulanowicz, 1986). While dissipative overhead is related to what system's internal processes dissipate through respiration (*O_D*), the other terms are strongly tied to the effective multiplicity of parallel flows by which energy is imported (*O_I*), exported as usable medium (*O_E*), or exchanged between compartments (*O_R*). Dividing every term that composes the overhead by the *TST* one obtains the expression of the intensive overhead fractions, i.e. the parts pertaining the flow structure (as much as *AMI* is the intensive component of *A*):

$$H_c = \frac{O_I}{TST} + \frac{O_R}{TST} + \frac{O_E}{TST} + \frac{O_D}{TST} \quad [9]$$

Ulanowicz (1980, 1986, 1997) combined the information indices in a coherent framework that helps quantify ecosystem development. Within this framework he derived expectations about indices trends along ecosystem developmental trajectories, which he then substantiated through a simulation approach (Mageau et al., 1995). In what follows we provide a summary of such expectations. System size is measured by the *TST* and is expected to increase during the early stages of development, to gradually level off as the development proceeds. Abundant resources characterize in fact early developmental phases and when the exogenous inputs tend to be bounded the *TST* may increase further via recycling. This latter, however, attenuates due to thermodynamics constraints so that the *TST* may converge to a finite quantity in the later stages of development. Flow diversity (*H*) as well is expected to increase during development. In parallel with resource availability (i.e. *TST*), the potential exists for a greater number of exchange pathways to appear. However, *H* does not increase without limits as connections are lost whenever they are too weak and cannot withstand perturbations. This occurs as the *TST* is partitioned among an ever greater number of pathways associated with an increase in diversity. Such phenomenon provides an upper bound for *H*, which is also expected to level off. *AMI* also increases with the development as the flow network would be streamlined to favor the most efficient material transfers. This would occur at the expenses of the inefficient, redundant flows, thus leading the residual diversity (*H_C*) to decrease. The fractions composing this latter index (see equation [9]) are expected to decrease. Dissipative overhead (*O_D/TST*) would be reduced as the scenario of increased *AMI* would imply increasing efficiency and then reducing dissipative losses. Pathway redundancy (*O_R/TST*) would diminish as less efficient energy transfers would be progressively pruned away during development. Also the overhead on import flows (*O_I/TST*) would be decreasing: sources from which medium is most available and less costly to import would be privileged and the multiplicity of import channels would decrease. Finally, greater internalization of medium leads to a reduction of export flows and also this last component of the overhead (*O_E/TST*) is expected to diminish. Ascendancy (*A*) augments throughout development as both

its component, i.e. *AMI* and *TST*, are predicted to increase. The development capacity (*DC*) would increase in earlier stages of development as both its components, i.e. *H* and *TST*, are expected to do so. However, this index is constrained by the limits on *TST* and *H*. Therefore, it levels off in later stages of development. Overhead (*O*) is calculated as the difference between development capacity (*DC*) and ascendancy (*A*). As the ecosystem develops, *A* would continue to increase thus approaching *DC*, its upper bound. At first, both *A* and *DC* would increase, but ultimately *DC* would be limited and *A* would continue to increase at the expense of *O*. Table 1 provides a synthesis of these trends.

Synthetic indices of ecosystem maturity have been introduced to test these expectations. Some confirmed them (Pérez-España and Arreguín-Sánchez, 2001) but in other cases contrasting results emerged (Christensen, 1995). In this paper we use trends expected as a benchmark because they form the most complete and coherent view of ecosystem development.

These expected trends led Ulanowicz (1986) to introduce the principle of optimal ascendancy. Such principle allows interpreting ecosystem development using a single goal function (Christensen, 1995) that unifies seemingly unconnected (and sometimes disparate) observations and hypotheses. In fact, the principle of optimal ascendancy synthesizes most of the attributes of developing ecosystems that were described by Odum (1969; see Ulanowicz, 1980). Several goal functions to describe ecosystem development were derived also from the thermodynamic framework (Fath et al., 2001). Jørgensen and Mejer (1979, 1981) applied the thermodynamic concept of exergy to ecological systems. It is a measure of the thermodynamic distance of a system from the equilibrium with the surrounding environment, and quantifies the (free) energy incorporated into a system. These authors posited that ecosystems would develop towards states of maximal exergy; this principle is not counter to that of optimal ascendancy (Ulanowicz, 1986). Scholars (Christensen, 1995; Vassallo et al., 2006) found a positive correlation between exergy and ascendancy, thus confirming that no fundamental contradiction exists between maximal exergy and optimal ascendancy. Exergy can be split in two forms (Fath et al., 2004): exergy degradation and exergy storage. It has been shown that exergy storage would continuously increase during all stages of ecosystem development whereas exergy degradation increases initially and then levels off.

Table 1
Expected trends for network information indices during ecosystem development. Ecosystem development may take centuries to complete and, for sake of simplicity, we condensed complex trajectories in two main phases, i.e. early and late developmental stages.

Key	Index	Tendency during development	
		Early	Late (Toward Maturity)
<i>H</i>	Flow diversity	Increases	Levels off
<i>AMI</i>	Average mutual information	Increases	Increases relative to <i>H</i>
<i>H_C</i>	Residual diversity	Decreases	Decreases relative to <i>H</i>
<i>O_R/TST</i>	Internal flow redundancy	Decreases	Decreases relative to <i>H</i>
<i>O_D/TST</i>	Dissipation flow redundancy	Decreases	Decreases relative to <i>H</i>
<i>O_I/TST</i>	Import flow redundancy	Decreases	Decreases relative to <i>H</i>
<i>O_E/TST</i>	Export flow redundancy	Decreases	Decreases relative to <i>H</i>
<i>DC</i>	Development capacity	Increases	Levels off
<i>A</i>	Ascendancy	Increases	Increases relative to <i>DC</i>
<i>O</i>	Overhead	Decreases	Decreases relative to <i>DC</i>
<i>O_R</i>	Overhead on internal connections	Decreases	Decreases relative to <i>DC</i>
<i>O_D</i>	Dissipative overhead	Decreases	Decreases relative to <i>DC</i>
<i>O_I</i>	Overhead on import	Decreases	Decreases relative to <i>DC</i>
<i>O_E</i>	Overhead on export	Decreases	Decreases relative to <i>DC</i>
<i>TST</i>	Total system throughput	Increases	Levels off

An issue faced when analyzing DC , A , and O is that major TST changes may mask the trends otherwise dictated by the structural components of the indices (H , AMI and H_C , respectively). The computation of relative ascendancy and overhead, obtained dividing these indices by their upper bound, helps overcoming the problem (A/DC and O/DC , respectively). Trends shown by these two relative indices are complementary and Ulanowicz (2009) introduced a new measure, i.e. the fitness of ecosystems for evolution (F), to quantify the system's potential for a change:

$$F = -k \log(a) \quad [10]$$

When $a = A/DC$ decreases (and, consequently, O/DC increases) F rises because the fraction of unorganized flows is higher (Ulanowicz et al., 2009). This condition indicates larger potential of the system to organize/structure the architecture of energy/matter circulation. Other indices inspired by the same logic of scaling degrees of organization and pathway multiplicity with the upper bound (i.e. DC) can be calculated. These indices may quantify either the relative amount of disorder that pertains to internal flows, as a proxy for stability (i.e. internal redundancy ratio, O_R/DC ; Rutledge et al., 1976), or the relevance of efficient routes with respect to internal flows only (i.e. internal ascendancy/internal development capacity, IA/IDC ; Scotti, 2008).

To characterize structural changes specific to the compartments we used flow redundancy (I_R) and internal connectance (I_C). They provide details on the fraction of residual diversity due to the redundancy of flows linking the compartments. Flow redundancy, calculated dividing the redundancy of internal exchanges by the total system throughput ($I_R = O_R/TST$), quantifies the degrees of freedom to energy/matter circulation due to multiplicity of connections between the compartments:

$$I_R = - \sum_{i=1}^N \sum_{j=1}^N \frac{T_{ij}}{T_{i..}} \log_2 \left(\frac{T_{ij}^2}{T_{i..} T_{.j.}} \right) \quad [11]$$

High levels of I_R can be indicative of poorly efficient ecosystems but such degrees of freedom represent an advantage in case of stress (i.e. they confer the system the potential to withstand disturbance). The internal connectance is the fraction of realized connections, weighted by their strength. It considers inter-compartmental connections only, without taking into account flows that cross the system's boundaries (i.e. excluding imports, exports and respirations; Ulanowicz, 1997):

$$I_C = e \left[-0.5 \sum_{i=1}^N \sum_{j=1}^N \frac{T_{ij}}{T_{i..}} \log_2 \left(\frac{T_{ij}^2}{T_{i..} T_{.j.}} \right) \right] \quad [12]$$

where e is the base of the natural logarithm. The elements $T_{i..}$ and $T_{.j.}$ in equations [11] and [12] indicate the sums of all flows from node i and to node j , respectively, calculated by excluding transfers that do not involve system's compartments (i.e. they do not consider the indices: 0 = inputs, $N + 1$ = exports, and $N + 2$ = dissipations/respirations). Finally, since during the phase of network construction the flow strength is deduced from compartments' biomass, the Shannon's index of diversity was calculated using compartments' standing stocks (S_B).

All network indices were computed in the R statistical environment (R Core Team, 2017) using either ad hoc scripting or functions from the *enaR* (version 3.0.0) package (Borrett and Lau, 2014).

2.5. Statistical and uncertainty analysis

Statistical analysis and uncertainty analysis were performed in the R statistical environment (R Core Team, 2017). Linear models were fitted using generalized least squares and applied to model temporal trends of the indices (function *gls*, R package *nlme*, version 3.1.149; Pinheiro et al., 2021). To correct for the effect of correlated values along the time series, a *corAR1* (i.e. "lag-1") auto-correlation structure was used. Models were fitted by maximizing restricted log-likelihood (i.e. method = *REML*).

To test the robustness of the findings obtained with networks that include high-resolution zooplankton compartments (i.e. that represent single species and/or ontogenetic development stages), we repeated the analysis using aggregated networks. These networks were assembled grouping only zooplankton nodes that represent functionally similar species with comparable diet. For rotifers, we clustered all nodes including herbivorous species (KERC, KERQ, KELL, FILT, ASCO, POLY, CONO, and OROT; see the caption of Fig. 2 for correspondence between species and keys) and grouped together the two compartments related to predatory rotifers (SYNC, PROT). The same criteria was adopted for copepods; herbivorous (EUNA, EUC3, EUC5, EUAD, and CYCN) and predatory (CYCC, CYCA) components were clumped in two separate nodes. Cladocerans become one single, generic compartment (BOSL, DAPL, and OCLA) as all its species show a similar diet. All other nodes remained unchanged.

Uncertainty analysis was conducted following two strategies. First, the construction of random networks not bound by the condition of being at steady state. This option could be implemented because information theory indices do not require networks to be balanced for their calculation (Ulanowicz, 2004). Second, the assembly of plausible, steady-state networks using the function *enaUncertainty* of the package *enaR* (Borrett and Lau, 2014, Hines et al., 2018). In both cases, intervals of varying breadths were explored to reflect the reliability of input data, using an approach analogous to Corrales et al. (2017). Carbon flows of empirical networks served as a reference to identify the center of sampled intervals. Flows were classified as with low, medium and high uncertainty (SM2, Appendix 1, 3), which translated into randomly sampling symmetric intervals that were $\pm 5\%$, $\pm 10\%$, and $\pm 20\%$ compared to reference empirical values, respectively. Low uncertainty was assigned to zooplankton compartments for which site-specific data on body size and diet were available. Medium uncertainty was adopted for flows related to phytoplankton, because they were estimated combining field chl-a data and experiments with data from the literature. High uncertainty was associated to flows related to fish and detritus compartments for which few site-specific data were available, and also assigned to a number of flows that have been estimated by assuming mass balance. In the case of aggregated networks, both randomization approaches were applied. Due to difficulties in attaining convergence to build balanced networks, only the approach not constrained by steady-state conditions was adopted for disaggregated networks. All uncertainty analyses were performed assembling 999 networks for each of the 11 years in the time series. Pairwise comparisons between all possible networks combinations were carried quantifying the overlap between the tails of frequency distributions generated for each index (see Hines et al., 2015).

3. Results

3.1. Index trends and statistical analysis

Linear models that consider *corAR1* auto-correlation revealed that most of the information indices computed with the four types of network flows (i.e. imports, internal exchanges between compartments, exports and respirations) show significant linear trends (Tables 2-3).

The size of the system (TST) did not change significantly along the time series (ANOVA: $F_{1,9} = 2.308$, $p = 0.163$). Fig. 3 illustrates the trends for flow diversity (H), average mutual information (AMI), residual diversity (H_C) and their scaled versions – development capacity (DC), ascendancy (A) and overhead (O) while the scaling factor total system throughput (TST) is in Fig. 5h.

The indices H , H_C and their TST -scaled versions, DC and O , increased significantly over the period covered by the data set. The trend shown by ascendancy (A), the scaled version of AMI , is not significant (ANOVA: $F_{1,9} = 2.210$, $p = 0.171$) although that of AMI is (ANOVA: $F_{1,9} = 6.765$, $p = 0.029$). Total system throughput (TST) does not hide the behavior of H and H_C , whose trends reflect in that of DC and O , respectively. Total

Table 2

Results of linear models with *corAR1* auto-correlation structure. Models were fitted using generalized least squares and express changes of the indices as a function of time.

Index	Term	Value	Standard error	t-value	p-value	
<i>H</i>	intercept	-9.391	4.980	-1.886	0.092	.
	year	0.007	0.002	2.682	0.025	*
<i>AMI</i>	intercept	2.494	0.260	9.595	<0.001	***
	year	-3.387E-04	1.302E-04	-2.601	0.029	*
<i>H_C</i>	intercept	-11.916	5.185	-2.298	0.047	*
	year	0.007	0.003	2.712	0.024	*
<i>DC</i>	intercept	-9619.181	5184.599	-1.855	0.097	.
	year	5.133	2.597	1.976	0.080	.
<i>A</i>	intercept	-3477.836	2533.192	-1.373	0.203	.
	year	1.886	1.269	1.487	0.171	.
<i>O</i>	intercept	-6178.619	2766.819	-2.233	0.052	.
	year	3.265	1.386	2.355	0.043	*
<i>O_R</i>	intercept	-4257.284	1744.824	-2.440	0.037	*
	year	2.244	0.874	2.567	0.030	*
<i>O_D</i>	intercept	-890.077	314.536	-2.830	0.020	*
	year	0.462	0.158	2.931	0.017	*
<i>O_I</i>	intercept	-1020.633	837.565	-1.219	0.254	.
	year	0.553	0.420	1.319	0.220	.
<i>O_E</i>	intercept	-4.006	0.945	-4.242	0.002	**
	year	0.002	4.730E-04	4.541	0.001	**
<i>O_R/TST</i>	intercept	-9.587	4.034	-2.377	0.041	*
	year	0.006	0.002	2.724	0.023	*
<i>O_D/TST</i>	intercept	-3.122	1.650	-1.892	0.091	.
	year	0.002	0.001	2.020	0.074	.
<i>O_I/TST</i>	intercept	0.790	0.668	1.183	0.267	.
	year	-1.299E-04	3.345E-04	-0.388	0.707	.
<i>O_E/TST</i>	intercept	-0.003	0.013	-0.239	0.816	.
	year	2.457E-06	6.542E-06	0.376	0.716	.
<i>A/DC</i>	intercept	220.064	60.597	3.632	0.005	**
	year	-0.087	0.030	-2.874	0.018	*
<i>O/DC</i>	intercept	-120.064	60.597	-1.981	0.079	.
	year	0.087	0.030	2.874	0.018	*
<i>O_R/DC</i>	intercept	-124.481	54.968	-2.265	0.050	*
	year	0.080	0.028	2.907	0.017	*
<i>IA/IDC</i>	intercept	240.973	70.529	3.417	0.008	**
	year	-0.103	0.035	-2.906	0.017	*
<i>I_C</i>	intercept	-16.294	6.341	-2.570	0.030	*
	year	0.009	0.003	2.933	0.017	*
<i>PP</i>	intercept	-99.341	218.333	-0.455	0.660	.
	year	0.056	0.109	0.513	0.620	.
<i>TOM</i>	intercept	-24.008	51.988	-0.462	0.655	.
	year	0.044	0.026	1.677	0.128	.
<i>TST</i>	intercept	-1973.481	1403.223	-1.406	0.193	.
	year	1.068	0.703	1.519	0.163	.
<i>F</i>	intercept	-0.038	0.123	-0.312	0.762	.
	year	1.981E-04	6.160E-05	3.215	0.011	*
<i>S_B</i>	intercept	-22.644	9.606	-2.357	0.043	*
	year	0.012	0.005	2.518	0.033	*

system throughput (*TST*) prevails over *AMI* in determining the behavior of *A*. The degrees of flow redundancy, which are expressed by the overhead (*O*), can be dissected in the contribution of four sub-indices that account for the multiplicity of pathways at the level of internal exchanges among compartments (redundancy, *O_R*), imports (*O_I*), exports (*O_E*) and respirations (dissipative overhead, *O_D*). The trends of these four indices and their structural components, obtained by dividing them by the *TST*, are in Fig. 4.

Internal redundancy (*O_R*) and dissipative overhead (*O_D*) exhibit significant increasing trends (ANOVA: $F_{1,9} = 6.589, p = 0.030$; and $F_{1,9} = 8.592, p = 0.017$). The model fitted for the overhead on exports (*O_E*) is significant (ANOVA: $F_{1,9} = 20.619, p = 0.001$) whereas that of the overhead on imports (*O_I*) is not (ANOVA: $F_{1,9} = 1.740, p = 0.220$). Among their intensive (i.e. structural) counterparts, *O_R/TST* and *O_D/TST* remain (at least marginally) significant (ANOVA: $F_{1,9} = 7.420, p = 0.023$; and $F_{1,9} = 4.080, p = 0.074$) while the scaled versions of overhead on imports (*O_I/TST*) and exports (*O_E/TST*) do not show significant trends (ANOVA: $F_{1,9} = 0.151, p = 0.707$; and $F_{1,9} = 0.141, p = 0.716$).

Trends of relative ascendancy (*A/DC*) and relative overhead (*O/DC*) were studied to elucidate the relative status of flow organization and the

relative degree of disorder in the ecosystem, respectively (Fig. 5a,b). The tendencies exhibited by these two indices are complementary. They indicate that the system developed reducing the organization of flows compared to the total flow diversity. The level of flow organization specific to internal exchanges was modelled with $I_R = O_R/TST$ (Fig. 4e) and considering the internal connectance, *I_C* (Fig. 5e). Fig. 5 also includes the trends of classical parameters used in ecological research such as phytoplankton primary production (*PP*) and total organic matter (*TOM*), as well as the sum of all network flows (i.e. *TST*). All indices reporting relative changes in the levels of organization or redundancy as well as those focusing on the ordered arrangement of internal flows are significant (ANOVA: $A/DC, F_{1,9} = 8.258, p = 0.018$; $O/DC, F_{1,9} = 8.258, p = 0.018$; $I_R, F_{1,9} = 6.589, p = 0.030$; and $I_C, F_{1,9} = 8.602, p = 0.017$) while traditional ecological indicators such as phytoplankton primary production and total organic matter do not show any significant tendency along the time series (ANOVA: $PP, F_{1,9} = 0.263, p = 0.620$; and $TOM, F_{1,9} = 2.811, p = 0.128$).

The fitness for evolution (*F*) displays a positive trend (ANOVA: $F_{1,9} = 10.338, p = 0.011$; Fig. 6a), which indicates that, during the period 1972–2012, Lake Santo increased its potential to evolve or self-organize.

Table 3

ANOVA results for system-level indices. All indices are expressed as a function of time (i.e. years) using linear trends with *corAR1* auto-correlation structure.

index	F-value	p-value	
<i>H</i>	7.191	0.025	*
<i>AMI</i>	6.765	0.029	*
<i>H_C</i>	7.354	0.024	*
<i>DC</i>	3.906	0.080	.
<i>A</i>	2.210	0.171	.
<i>O</i>	5.548	0.043	*
<i>O_R</i>	6.589	0.030	*
<i>O_D</i>	8.592	0.017	*
<i>O_I</i>	1.740	0.220	.
<i>O_E</i>	20.619	0.001	**
<i>O_R/TST</i>	7.420	0.023	*
<i>O_D/TST</i>	4.080	0.074	.
<i>O_I/TST</i>	0.151	0.707	.
<i>O_E/TST</i>	0.141	0.716	.
<i>A/DC</i>	8.258	0.018	*
<i>O/DC</i>	8.258	0.018	*
<i>O_R/DC</i>	8.451	0.017	*
<i>IA/IDC</i>	8.447	0.017	*
<i>I_C</i>	8.602	0.017	*
<i>PP</i>	0.263	0.620	.
<i>TOM</i>	2.811	0.128	.
<i>TST</i>	2.308	0.163	.
<i>F</i>	10.338	0.011	*
<i>S_B</i>	6.342	0.033	*

This tendency is a consequence of the change observed for *A/DC* (and *O/DC*). The Shannon's index of diversity, calculated using the biomass of each compartment, exhibits a positive and statistically significant trend (ANOVA: S_B , $F_{1,9} = 6.342$, $p = 0.033$; Fig. 6b).

Results presented here were obtained by modelling indices time series derived from empirical networks (indices values are stored in SM2, Appendix 2). Significant findings persist even after scrutiny with

uncertainty analysis, which enabled assembling time series of plausible networks with flow strengths modulated in the vicinity of those in the empirical, reference models (SM1, Appendix 2). Pairwise comparisons revealed striking differences between earlier (1970s) and later networks (2007–2008, and 2012). Robustness of findings obtained with the disaggregated networks including 26 compartments is supported by comparisons with trends generated using aggregated networks composed of 11 compartments (SM2, Appendix 3). Strengths of all flows in the aggregated networks are in SM2, Appendix 3; indices computed using these aggregated networks are in SM2, Appendix 4. With the aggregated networks, (1) the direction (either positive or negative) of linear relationships was in fact preserved and significance levels almost remained unaltered; and (2) pairwise comparisons between networks assembled according to two randomization algorithms (i.e. by either preserving or ignoring the steady-state conditions) returned consistent results when compared with uncertainty analysis conducted for the disaggregated networks. In general, both the linear models constructed using aggregated networks and all uncertainty analysis scenarios confirmed (i) a significant increase of stability along the time series (e.g. see *H_C*, and *O*) and (ii) a key role of internal connections in driving such an increase (e.g. see *O_R* and *I_C*).

This outcome demonstrates that although the indices change as a function of network aggregation, their mutual relationships are not altered by it (i.e. at a certain extent, they are simply scaled). Hence, the use of aggregated networks highlights the robustness of the trends found for the information theory indices in Lake Santo, irrespective of the number of compartments considered.

4. Discussion

4.1. Directional tendency of change and ecosystem development

Summarizing the trends observed in the indices, Lake Santo is a

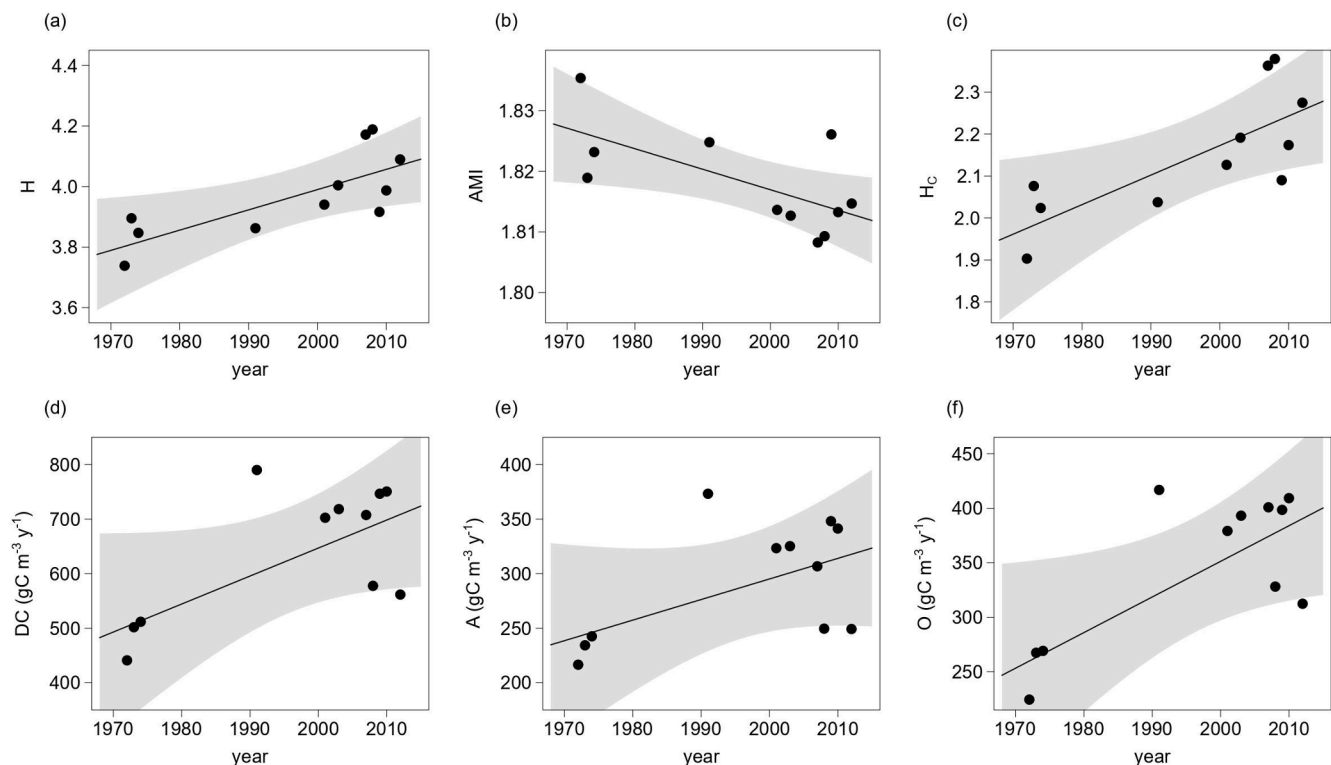


Fig. 3. Trends of (a) flow diversity (H ; $F_{1,9} = 7.191$, $p = 0.025$), (b) average mutual information (AMI ; $F_{1,9} = 6.765$, $p = 0.029$), (c) residual diversity (H_C ; $F_{1,9} = 7.354$, $p = 0.024$) and their scaled counterparts – (d) development capacity (DC ; $F_{1,9} = 3.906$, $p = 0.080$), (e) ascendancy (A ; $F_{1,9} = 2.210$, $p = 0.171$), and (f) overhead (O ; $F_{1,9} = 5.548$, $p = 0.043$). Statistics ($F_{1,9}$) and probabilities (p) in parenthesis refer to ANOVA, and grey shaded areas delimit 95% confidence interval of the fitted linear trends.

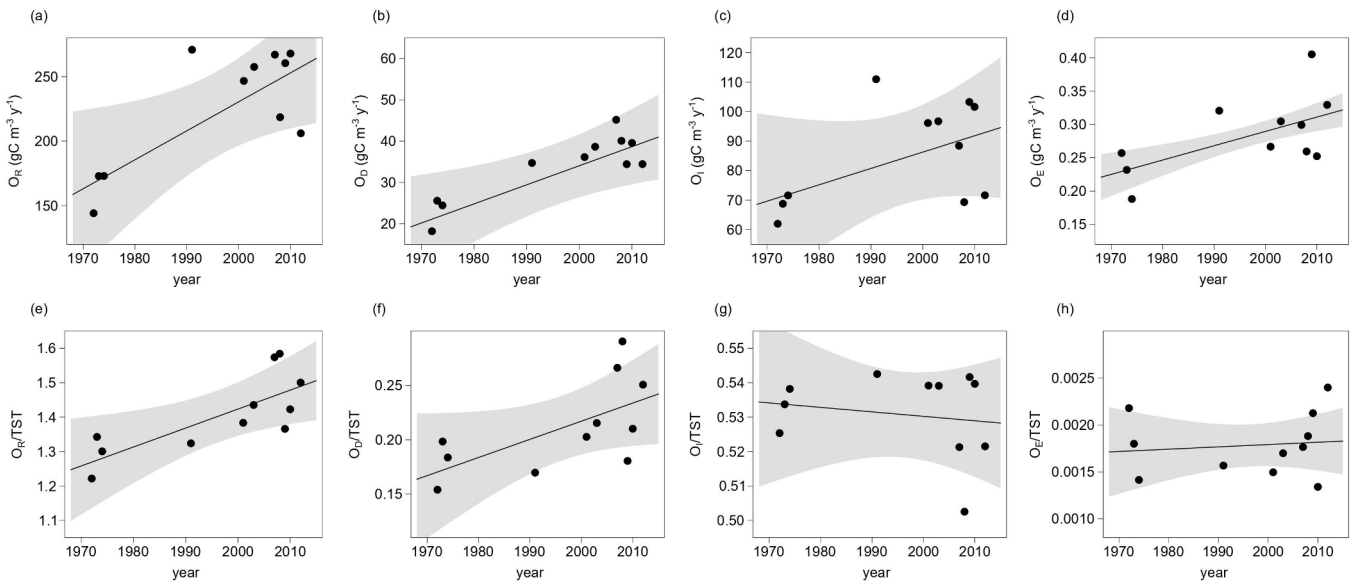


Fig. 4. Trends of (a) internal redundancy (O_R ; $F_{1,9} = 6.589$, $p = 0.030$), (b) dissipative overhead (O_D ; $F_{1,9} = 8.592$, $p = 0.017$), (c) overhead on imports (O_I ; $F_{1,9} = 1.740$, $p = 0.220$), (d) overhead on exports (O_E ; $F_{1,9} = 20.619$, $p = 0.001$), and their unscaled counterparts (i.e. obtained dividing each index by the TST), (e) O_R/TST ($F_{1,9} = 7.420$, $p = 0.023$), (f) O_D/TST ($F_{1,9} = 4.080$, $p = 0.074$), (g) O_I/TST ($F_{1,9} = 0.151$, $p = 0.707$), and (h) O_E/TST ($F_{1,9} = 0.141$, $p = 0.716$). Statistics ($F_{1,9}$) and probabilities (p) in parenthesis refer to ANOVA, and grey shaded areas delimit 95% confidence interval of the fitted linear trends.

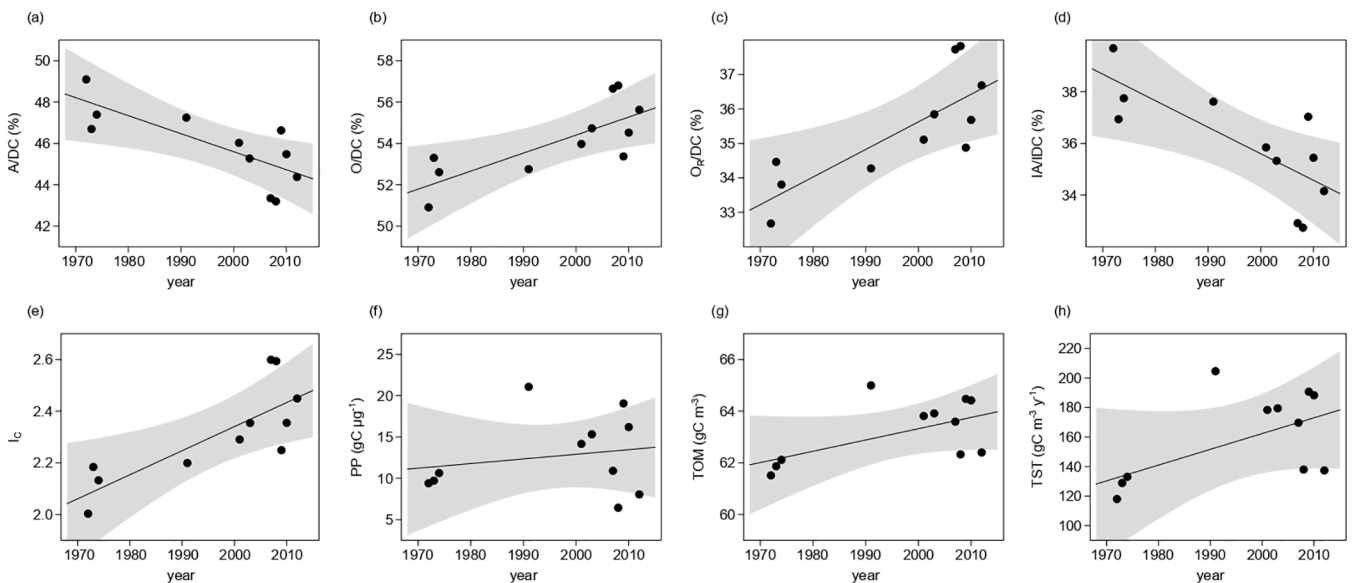


Fig. 5. Trends of (a) relative ascendency (A/DC ; $F_{1,9} = 8.258$, $p = 0.018$), (b) relative overhead (O/DC ; $F_{1,9} = 8.258$, $p = 0.018$), (c) relative redundancy (O_R/DC ; $F_{1,9} = 8.451$, $p = 0.017$), (d) relative internal ascendency (IA/DC ; $F_{1,9} = 8.447$, $p = 0.017$), (e) internal connectance (I_C ; $F_{1,9} = 8.602$, $p = 0.017$), (f) primary production (PP ; $F_{1,9} = 0.263$, $p = 0.620$), (g) total organic matter (TOM ; $F_{1,9} = 2.811$, $p = 0.128$), and (h) total system throughput (TST ; $F_{1,9} = 2.308$, $p = 0.163$). Statistics ($F_{1,9}$) and probabilities (p) in parenthesis refer to ANOVA, and grey shaded areas delimit 95% confidence interval of the fitted trend.

system in which flow diversity (H) increased, information (AMI) decreased significantly while residual diversity (H_C) paralleled H . The scaled versions (multiplied by the TST) of these indices, respectively DC , A and O , maintained the trends of their intensive component except for A . TST , whose trend is not significant, predominates over AMI in shaping A . Among the components of O , significant trends are those of internal redundancy (O_R), dissipative overhead (O_D) and overhead on exports (O_E): all are increasing. Only the overhead on imports (O_I) does not show a significant trend. Among their unscaled counterparts (obtained dividing each index by the TST), O_R/TST and O_D/TST increased significantly, whereas trends of O_I/TST and O_E/TST were not significant. The relative ascendency (A/DC) significantly diminished whereas relative

overhead (O/DC), relative redundancy (O_R/DC), and relative internal ascendency (IA/DC) all increased.

With reference to the first objective of this research, trends of flow network indices indicate a defined trajectory of change that Lake Santo followed during the period 1972–2012. Trends of these indices, in particular those of H , AMI , H_C , DC , and O , seem to form a coherent pattern. We incorporated in the analysis other indices (A/DC , O/DC , I_C and O_R/DC), whose trends all confirm the directionality of the Lake Santo development. This trajectory is, however, partially confounded by the non-significant trend of TST when the focus is on A . The rich conceptual framework that has flourished around the study of ecosystems as flow networks (Rutledge et al., 1976; Christensen, 1995; Ulanowicz,

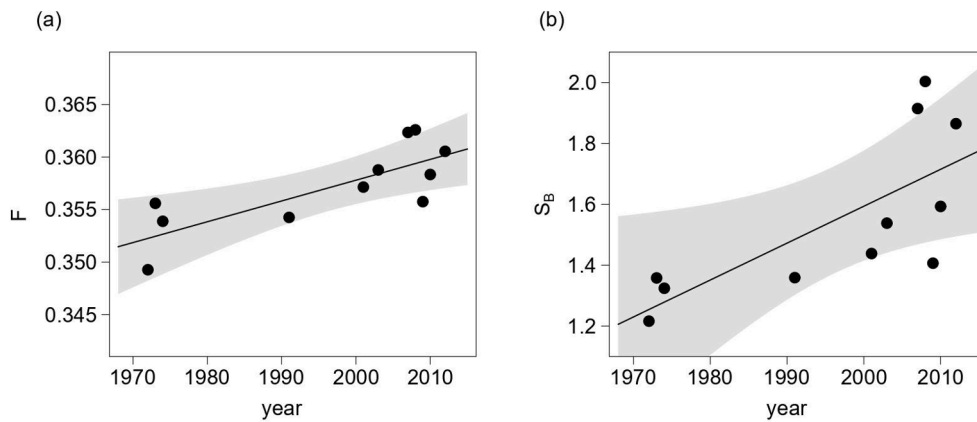


Fig. 6. Trends of (a) the fitness for evolution (F ; $F_{1,9} = 10.338$, $p = 0.011$) and (b) the Shannon's index of diversity calculated for each network using the biomass of the 26 compartments (S_B ; $F_{1,9} = 6.342$, $p = 0.033$). Statistics ($F_{1,9}$) and probabilities (p) in parenthesis refer to ANOVA, and grey shaded areas delimit 95% confidence interval of the fitted trend.

1997; Latham and Scully, 2002; Ludovisi et al., 2005; Fath et al., 2019) offers a benchmark for interpretation of these trends.

Rutledge et al. (1976) posited that H would increase during initial stages of development, to level off as succession proceeds toward more mature stages. In a simulation that generated outputs characteristic of an ecosystem advancing through various successional steps, Mageau et al. (1995) observed that H initially increased until it reached a maximum and then declined to level off, a result coherent with Rutledge's statement. While theoretical studies converge toward the above-described pattern for H (Saint-Béat et al., 2015), it is difficult to find long-term experimental works that highlight trends of network information indices and discuss them in the light of ecosystem development. One such work regards the below-ground terrestrial ecosystem on the island of Schiermonnikoog in The Netherlands (Holtkamp and Tobor-Kapton, 2007), which confirmed the expected trend for H . However, this study covered a period of 100 years with only four points representative of the system status. This limited data set precludes reconstructing a trend that helps grasping the detail of the developmental trajectory for that ecosystem.

According to the trend expected for H (see Table 1) the period covered by our data set may coincide with an early phase of ecosystem development for Lake Santo (e.g. early phase of a secondary succession). In the early stages of ecosystem development H would be increasing because the TST would be partitioned among a greater number of exchange pathways associated with an increase in diversity (Mageau et al., 1995). We tested this hypothesis by computing the Shannon's index of diversity, based on compartments' biomass. This index showed a significant positive trend (Fig. 6b) and this evidence confirms that increasing species diversity may have driven flow diversity, according to the theory. Because community structure (number and type of species) did not change during the whole period of analysis, species diversity must have increased because of a more even apportionment of the total biomass among the different components of the network. Such more even distribution of the biomass among the compartments must have driven the diversity of flows because flow values are apportioned proportionally to the standing stocks of resources and consumers (Bondavalli and Ulanowicz, 1999; Fath et al., 2007).

DC augmented significantly over time, coherently with what expected at early stages of development (see Table 1). DC is the product of TST and H and its significant positive trend reveals that the index is mostly driven by its intensive component (H) rather than ecosystem size (TST), whose trend shows a slight, non-significant increase. Trends of DC , H and TST suggest further hypotheses. At very early stages of development, DC and H would augment, ignited by the increasing TST associated with the pulse of growth provided by abundant resources (Mageau et al., 1995). At later stages of development, TST stops

increasing due to thermodynamic constraints and limits to external inputs (Ulanowicz, 1980). The non-significant trend observed in the TST could signal that Lake Santo might have completed the initial phase of pure growth, that in which in which DC , H and TST all increase (Mageau et al., 1995; Ulanowicz, 1997), and reached later stages of development, in which H and DC may continue to increase meanwhile TST levelled off (Ulanowicz, 1997).

The theoretical framework indicates that H should dominate over the TST during later stages of succession, as the TST would augment less than at the earliest stages (Mageau et al., 1995; Ulanowicz, 1997). This is another indication that Lake Santo could have been caught at early stages of some developmental path but not the earliest. The dominance of the intensive component in the trend for DC should be paralleled by the dominance of AMI over TST in the trend for A (Mageau et al., 1995), which however does not occur. Because A tracks DC but lags behind it (Ulanowicz, 1997), it may be that trends of index values may overlap along the evolutionary gradient confounding the various temporal phases, so that ecosystem developmental trajectories would be less clear than conceptual model would predict. The relative behavior of the indices offers the opportunity to contrast the directional change exhibited by Lake Santo with what predicted by the theory. Ulanowicz (1980, 1986, 1997) posited that the unimpeded natural development of an ecosystem would be in the direction of increasing AMI and decreasing H_C . The former index would augment because autocatalytic competition would prune away less efficient links at the advantage of mutual constraints and efficient connections for energy transfer. The network would become more streamlined and less redundant, so the flowing of energy results more constrained. AMI would increase at the expenses of H_C . Because this latter index quantifies the redundancy of the network, it must decrease when AMI grows (Mageau et al., 1995). Rutledge et al. (1976) held an opposite view, and posited that as ecosystems develop AMI should fall, resulting in a gradient towards greater food web redundancy, signaled by an increasing H_C , the proxy index for stability (Rutledge et al., 1976). A larger choice of paths in fact would compensate for the loss of some connections eventually disrupted by perturbations (Rutledge et al., 1976). The trends of AMI and H_C (Fig. 3b,c) suggest that Lake Santo moved in the direction of increasing stability at the expense of efficiency and organization, thus confirming Rutledge's view that ecosystem development would be accompanied by a falling level of information coupled with increasing stability and entropy (i.e. complexity).

Scaling H_C by the TST one obtains O (total overhead, Fig. 3f). It quantifies the amount of total matter that the system handles through inefficient connections. Such inefficiency is apportioned among an increased redundancy (O_R , Fig. 4a), a greater dissipation (O_D , Fig. 4b) and an increased overhead on exports (O_E , Fig. 4d), while the

contribution of overhead on imports (O_i , Fig. 4c) is negligible as its trend is not significant. These indices indicate that Lake Santo developed in the direction of increasing redundancy (uncertainty associated to the presence of parallel pathways amongst the network components), dissipation (i.e. respiration), and of reducing internalization of medium (i.e. higher exports). These results are not in agreement with Ulanowicz's view, according to which all the components of O would be decreasing during ecosystem development². Our results, on the contrary, may seem in agreement with other several pieces of literature dealing with dissipation tendency during ecosystem growth and development (Jørgensen et al., 1991; Fath et al., 2004; Maes et al., 2011) although this overhead quota is not simply an index of increasing dissipation but pertains also to the structure of the dissipation flows.

Further confirmation of the direction of development comes from the relative ascendancy (A/DC , Fig. 5a) and relative overhead (O/DC , Fig. 5b). Lake Santo reduced the fraction of DC devoted to efficiency in exchanging matter and energy, incrementing the portion encumbered by disorganization, which quantifies the potential for adapting to novel perturbations. More specifically, the relative redundancy (O_R/DC), the fraction of development pertaining to internal redundant connections (Fig. 5c), has been indicated as an index of system resilience to disruption (de la Vega et al., 2018). In Lake Santo this index increased significantly over time supporting the conclusion that Lake Santo evolved toward greater protection against perturbations and thus greater stability. Two other information indices are coherent with this picture: I_C (internal connectance, Fig. 5e), and O_R/TST (the intensive component of redundancy, Fig. 4e).

In relation to the second objective of this analysis, that is whether observed trends are coherent with the theoretical expectations about ecosystem development, our results contradicts the hypothesis that in the absence of major perturbations ecosystems would develop in the direction of increasing AMI and A at the expenses of H_C and O (Ulanowicz, 1986, 1997). Interpreting the observed trends according to this view, Lake Santo would be classified as an ecosystem under stress, an issue that we cover in section 4.2.

Finally, we explored the ratio of internal ascendancy to internal capacity (IA/IDC), which was indicated as a possible metric of ecosystem maturity (Baird et al., 1991; Christensen, 1995; Latham and Scully, 2002). Ulanowicz (1986, 1997) would expect a positive trend for this index, according to the idea that ascendancy and organization would augment during development. On the contrary, the trend we found (Fig. 5d) is negative and highly significant, confirming the negative relationship found between this index and the maturity index set up by Christensen (1995). However, our result does not match with the trend of another maturity index proposed by Pérez-España and Arreguín-Sánchez (2001).

4.2. Signs of unimpeded development or ecosystem under stress?

Predictions of the original theory (Ulanowicz, 1980, 1986, 1997) would classify Lake Santo as an ecosystem under stress. The concomitant increase of H_C and decrease of AMI indicate that efficiency is reduced in favor of protection that the multiplicity of connections would provide against perturbation, i.e. a signal of ecosystem's response to disturbance. Moreover, A displays a non-significant trend (Fig. 3e), a further indication that Lake Santo may not have followed a normal course of development given that, as said, an ecosystem would develop along a gradient of increasing ascendancy in the absence of major perturbations.

² Dissipation would be minimized at later stages of succession. As long as resources remain abundant (growth phase; Ulanowicz, 1997), it is unlikely that dissipation is minimized because ascendancy would more easily increase by a growing TST . Later toward maturity, i.e. when limitations on resources become more stringent, minimizing dissipative overhead would be an appropriate route for increasing ascendancy.

Aoki (1995) posited that the evolution of lake ecosystems towards eutrophication would be characterized by higher diversity-complexity of flows/pathways (H), reduced information (AMI), and an increased residual diversity (H_C). He extended his conjectures to ecosystem maturation in general, and hypothesized that the persistent input of nutrients from the outside would lead unavoidably to eutrophication while ecosystems mature (Aoki, 1997). Trends of network flow indices in Lake Santo mirror those hypothesized by this author. Hence, one possibility is that Lake Santo had been progressively moving towards a eutrophic state. To verify this hypothesis, we analyzed the levels of chl-*a* during the long-term campaigns conducted on the lake (Rossetti et al., 2004; Mazzola, 2013). Such data revealed persistent oligo-mesotrophic conditions: from this point of view, thus, the assumption that Lake Santo had been facing a progressive eutrophication loses its strength. Furthermore, the expectation is that eutrophication would bring about greater production (PP) and total organic matter (TOM), but the trends of these quantities are not significant (Fig. 5f,g). Phytoplankton biomass in Lake Santo was in general low ($<500 \text{ mg m}^{-3}$; see Mazzola, 2013) and the only exception is a seasonal peak occurring in late summer, a feature shared by many mountain lakes (Rott, 1988). Data thus allow classifying the Lake Santo as oligo-mesotrophic (Rott, 1984) as it was since the 1970 s. This would confirm that this water body was not undergoing eutrophication in the period covered by our data set. Further confirmation comes from the information indices. Eutrophication would be characterized by the rise of TST that more than compensate for a concomitant fall in the level of AMI , so that A would increase (Ulanowicz, 1986, 1997). The non-significant trends of both TST (Fig. 5h) and A (Fig. 3e) contradict the hypothesis that Lake Santo progressively moved towards eutrophication. In a previous work, Bondavalli et al. (2006) posited that Lake Santo exhibited early signs of eutrophication. However, the analysis was based on a restricted set of data, which allowed to compare the networks for two years only, i.e. 1973 and 1991. The more extended data set used in this research suggests to rectify that position: the trends displayed by the indices do not indicate the lake as affected by eutrophication.

The picture offered by network flow indices in Lake Santo seems to contradict Ulanowicz's (1986, 1997) hypothesis in favor of Rutledge's model (Rutledge et al., 1976). Latham and Scully (2002) pointed out that also in Rutledge's view under severe environmental conditions the energy flow in ecosystems would become more diffuse, leading to a drop in AMI and an increase in H_C (or stability; Rutledge et al., 1976). Rutledge et al. (1976) came to this conclusion that apparently contradicts their model of ecosystem development simulating the evolution of a shortgrass prairie ecosystem over a 20-year period for both normal and perturbed conditions (reduced moisture). Under normal conditions (normal moisture), H_C showed an increasing trend and the ecosystem moved along a gradient of increasing stability (H_C). This trend disappeared under stress (reduced moisture), although the values of H_C were higher than under normal moisture. Thus, stress induced greater stability but disrupted a regular trend for H_C that the ecosystem would exhibit under normal conditions. Lake Santo showed a gradient of increasing H_C and of decreasing AMI . As such, it resembles Rutledge et al. (1976) prairie ecosystem under normal conditions.

Only limited human interference was observed in Lake Santo (Viaroli et al., 1994; Mazzola, 2013). The remote position it occupies in northern Apennines prevents the occurrence of most anthropogenic impacts typical of rural and urban areas. A possible interference is that produced by local tourism, which may reach conspicuous levels in summer, but it is made essentially of daily visitors and cannot be classified as mass tourism. Despite these possible disturbing factors, the trophic conditions and the composition of the zooplankton community of Lake Santo have remained substantially the same during the years and comparable to those expected in similar natural lakes located in the same area (Viaroli et al., 1994; Rossetti et al., 2006; Mazzola, 2013). Toward the end of the period covered by our data set observational data led to hypothesize only an increase in the density of small-bodied zooplankton, in

particular of some species of rotifers and of *Bosmina longirostris* (a micro-filter feeder cladoceran), and a reduction in the abundance of the cladoceran *Daphnia longispina* (an efficient macro-filter feeder). To test a possible variation in the abundance of these two groups we analyzed their biomass trends and using ANOVA we found that both increased significantly in the period of investigation (small-bodied zooplankton: $F_{1,9} = 4.634$, $p = 0.060$; large bodied zooplankton: $F_{1,9} = 6.783$, $p = 0.029$). Details regarding the zooplankton and phytoplankton community of the Lake Santo are in the [Supplementary Materials 1 \(SM1\)](#), [Appendix 1](#).

Climate change has impacted most places during last decades. In Lake Santo, signs potentially traceable to climate alterations have been identified. A more delayed formation of the ice cover in autumn and an earlier melting in spring is one such signature (Rogora et al., 2018). It has been hypothesized that a shorter persistence of the ice cover leads to a longer growing season and higher water temperatures (Rogora et al., 2018). However, no significant differences were detected for Lake Santo in water temperature, trophic conditions, chl-a, and biomass of meso-zooplankton (Morabito et al., 2018). Rogora et al. (2018) found signs that large-scale climate variations could be linked to changes in plankton phenology. Nevertheless, the response of Lake Santo to large-scale climatic events seems largely dependent on its thermal structure and mixing regime (Rogora et al., 2018).

Given the lack of documented evidence concerning drastic changes attributable to perturbations, we think that the trends shown by the indices suggest that Lake Santo followed a normal course of development. Variations attributable to climate did not modify significantly parameters and features of the lake (Morabito et al., 2018; Rogora et al., 2018). As such, effects of climate change are confounded within the range of the normal inter-annual variability (e.g. temporal fluctuations, stochastic events), which is an inescapable ingredient of natural dynamics. Any characterization of the normal course of development should incorporate variability, which exerts a constant pressure on ecosystems. While environmental variability is always at work, the unimpeded development remains a theoretical concept. Thus, Ulanowicz's model could be an ideal representation of ecosystem development that does not take into account environmental variability, like in physics the ideal motion does not consider friction as a real entity that counteracts it.

An alternative explanation could be considered as well. Although signs of deterioration are not yet visible, climate change may have acted like a press perturbation, driving the system slowly away from its natural trajectory. This condition may have reflected in the indices, whose trends could anticipate deeper, macroscopic consequences (Bertani et al., 2016). If it would be so, trends of flow information indices could provide early warning signs of stress, a fascinating hypothesis that needs to be confirmed by solid evidence to be gathered, i.e. contrasting pristine and perturbed ecosystems. In relation with the third objective of this work, that is whether index trends may inform about some form of stress that affected Lake Santo, the more likely hypothesis is that this ecosystem followed an unperturbed trajectory of development.

4.3. A healthy ecosystem?

A theoretical definition of ecosystem health has been proposed as a combination of the three indices *TST*, *AMI* and *O* as proxies for vigor, organization and resilience, respectively (Mageau et al., 1995; Costanza and Mageau, 1999). A system lacking ascendancy (*vigor* \times *organization*) has neither sufficient activity nor internal organization needed to thrive. By contrast, systems that are too tightly constrained (i.e. no resilience) appear prone to collapse in the face of novel disturbances. Systems that endure – that is, are sustainable or healthy – lie somewhere between these extremes. Recognizing the importance of a trade-off between these features, Ulanowicz et al. (2009) revisited the hypothesis of a single directional evolution that progresses toward increasing organization. They proposed the existence of a dynamic tension between efficiency

and robustness, and suggested a metric combining organizational constraints and redundancy instead of using ascendancy as a goal function (Fath, 2015). They introduced the ratio *AMI/H*, equivalent to *A/DC*, as a potential index of health or sustainability. From this index descends what Ulanowicz et al. (2009) defined as fitness of ecosystems for evolution (*F*):

$$F = -k\alpha^\beta \log(\alpha^\beta) \quad [13]$$

The authors showed that this function can be normalized by setting $k = e/\log(e)$, so that $F_{max} = 1$ at $\alpha = e^{1/\beta}$, where β can be any positive real number; *F* is dimensionless and varies between 0 and 1. It describes the fraction of activity effective in creating a sustainable balance between *A* and *O* (*AMI* and *H_C*), i.e. between organization and redundancy. For an optimal configuration of flows, that is a healthy ecosystem, parameters α and β should derive from ecosystem networks that lie in the so-called window of vitality (Zorach and Ulanowicz, 2003), a restricted area in the plot of link density ($c = 2^{O/2}$, with *O* = overhead) vs. roles ($n = 2^A$, with *A* = ascendancy). Systems that more likely stay healthy would be those closer to the center of the window of vitality, which corresponds to $\alpha_{opt} = 0.4596$. Systems can risk unsustainability in relation to this optimum on two accounts. When $\alpha < 0.4596$, the system likely requires more coherence and cohesion. There may be insufficient or under-developed autocatalytic pathways to confer supplementary degrees of robustness to the system. Conversely, when $\alpha > 0.4596$, the system may be over-developed or too tightly constrained and thus more fragile. Computing α as the ratio between *AMI* and *H*, which corresponds to *A/DC* (Fig. 5a), Lake Santo exhibited a significant decreasing trend of this index. At the beginning of the 1970s, it showed the highest value ($\alpha_{1972} = 0.4910$), revealing a preponderance of organization, and a tendency to be fragile. It approached the optimum in the early 2000 s ($\alpha_{2001} = 0.4603$ and $\alpha_{2003} = 0.4527$) and decreased further in the following years. Ulanowicz et al. (2009) admitted the possibility that if $\beta \approx 1$ the optimum value (i.e. $\alpha_{opt} = 0.4596$) may be an overestimate. Morris et al. (2005) showed that in a large collection of randomly assembled food webs α approached an asymptote very close to $1/e$ (i.e. for most healthy systems this value should be close to 0.37). Accordingly, the decreasing trend observed in Lake Santo for α would reflect a tendency to progress toward its optimum value, which would guarantee healthier conditions (Morris et al., 2005; Ulanowicz et al., 2009). Assuming $\beta = 1$ in equation [13], we calculated the trend for *F* (Fig. 6a), which increased significantly. Lake Santo, developed in the direction of reducing α towards its optimum, thus increasing its fitness for evolution. This occurred through reducing part of the quota of organization in favor of redundancy and disorder. In relation to the third objective of this work, more specifically whether index trends allow drawing conclusions on the health status of Lake Santo, our results suggest that the ecosystem, while proceeding along its unimpeded trajectory of development, was moving toward healthier conditions.

5. Concluding remarks

Network analysis has been given credit as a powerful tool to study ecosystem development. However, to build up a convincing, rigorous and coherent framework, empirical studies are required as validating benchmarks for theories and/or hypotheses. Empirical studies must necessarily be long-term, however, and this constraint has led scholars to rely on simulations (Rutledge et al., 1976; Ulanowicz, 1986; Field et al., 1989; Herendeen, 1989; Mageau et al., 1995; Pérez-España and Arreguín-Sánchez, 1999; Coll et al., 2008; Ludovisi and Scharler, 2017) eventually calibrated using field data (Heymans et al., 2007). Empirical confirmation of the theoretically expected trends of ecosystem development are scarce so far. We deliberately built upon temporally highly resolved, long-term empirical data from Lake Santo to avoid artifacts from simulations or pooling inevitably coarser cross-system data. Accordingly, this study recalls what Woodward et al. (2010) call it a

“natural experiment”, a tool essential for benchmarking ecosystem dynamics.

In respect to the objectives of the research, trends for network information indices reveal a directional tendency of change for Lake Santo, which may not be fully in agreement with theoretical expectations. The tendency shown towards greater stability and less organization contradicts Ulanowicz’s model of ecosystem development in favor of Rutledge’s view. The lake in fact seems to have followed an “unimpeded” trajectory of change, given the lack of evidence about major perturbations. However, the increase of stability (i.e. residual diversity, H_C) at the expenses of efficiency and organization (i.e. average mutual information, AMI) could be the response of the ecosystem to the continuous pressure of environmental variability, whose role has not found place yet in the theoretical framework of ecosystem development.

By converting a quota of organization and constraints in favor of redundancy and freedom, Lake Santo seemed to move in the direction of healthier conditions, which require a balance between organization and plasticity. Since A and O quantify the fractions of medium that the system handles through efficient and redundant connections, respectively, their trends signal that in Lake Santo an increasing portion of the energy available for development was devoted to system stability rather than being employed for improving efficiency and organization. This trajectory of change however does not allow identifying specific stages of the successional development that characterized Lake Santo in the period covered by our data set.

Recent works underlined the role that the ecological network analysis may play in the context of management (Fath et al., 2019) and in response to the pressing needs of developing, testing, and validating reliable ecosystem state indicators (Safi et al., 2019). Network information indices are promising in this respect and have a sound theoretical basis. However, their practical application is hampered by the lack of a thorough understanding of their behavior in real ecosystems. Contrasting results about ecosystem developmental trajectories, response to perturbations and health conditions may be found, and such discrepancies must be settled to obtain a reliable framework. This study wants to contribute to this direction and acts as stimulus for refinement and further analysis.

CRediT authorship contribution statement

Marco Scotti: Conceptualization, Methodology. **Cristina Bondavalli:** Conceptualization, Methodology. **Giampaolo Rossetti:** Data curation. **Antonio Bodini:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.108896>.

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