Original Articles

A new index of resilience applicable to external pulse-disturbances that considers the recovery of communities in the short term

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ABSTRACT

Resilience is a key concept in the study of the recovery of ecosystems affected by disturbances. Currently, there are numerous indices to measure resilience, but many of them do not show the accuracy of the resilience value or the behaviour of ecological parameters in the face of disturbances. New approaches and technologies enable large amounts of information to be obtained, facilitating the proposal of new resilience indices that work consistently and intuitively for a wide variety of ecological response variables under different scenarios after pulse-disturbances. In this study, we propose and verify a new resilience index, comparing its performance with others previously published. We validated the performance of the new index using real data based on field measurements of changes in soil bacterial OTUs diversity and abundance after a wildfire. The new resilience index provided an automatic and robust functional classification of the behaviour of ecosystems after disturbances, supported by a bootstrap analysis. We identified 5 scenarios of ecosystem resilience performance according to their behaviour after a pulse-disturbance: resilient, non-resilient, recovering, rebound, and continuing.

1. Introduction

The ecological impacts of disturbances on the functioning, composition and structure of ecosystems are dependent on their intensity and frequency, as well as the spatial distribution and size of the disturbed patches. Consequently, these impacts can move through a wide range from unaltered to completely altered. In general, many authors (e.g. D'Antonio and Thomsen, 2004) consider that the concept of resistance refers to the extent to which the composition of the community in the ecosystems remains unchanged. Together with the resistance concept, resilience is defined as the capacity of communities to return to their original state prior to the disturbance, both concepts forming the components of ecological stability (Allison and Martiny, 2008).

Orwin and Wardle (2004) proposed two indices to quantify the resistance (iRS) and resilience or recovery (iRL.OW, these indices were labelled as RS and RL in the Orwin and Wardle original paper) of soil biota after different types of disturbances, which have been used in numerous studies (more than 240 citations in Scopus). Although the indices were designed to analyse the response of soil microbial (frequently, bacterial) parameters, they have sometimes been used with

variables other than soil microorganism diversity, e.g. by Wyszkowska et al. (2021) to measure maize yield resistance to herbicide applications; or iRS and iRL by Borowik et al. (2019) to explore the effects of contamination by diesel oil and unleaded petroleum in *Elymus elongatus* yields.

A different number of variables have been used to estimate resistance and resilience, such as enzymatic activity (Cycoń et al., 2020), gene abundance measured with qPCR (Tao et al., 2021), richness or the Shannon diversity (Liang et al., 2020), biotic integrity index (Niu et al., 2021), or abundance of different taxa (Zhang et al., 2017).

The concept of resilience in ecology and its quantification is currently in constant debate, with some partially contradictory definitions (Angeler and Allen, 2016; Ingrisch and Bahn, 2018). Nevertheless, many authors (Nikinmaa et al., 2020) consider it necessary to find a way to measure the resilience of communities that have suffered a disturbance for a limited time (pulse-disturbance). The Orwin and Wardle resilience index, as well as others, measures community recovery capacity in time after a disturbance, to achieve the initial state at a specific moment in time, comparing with the behaviour of the control community (without disturbance). So, the identification of recovery during that

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time serves as an indicator of resilience, understood to be the ability to return to the original stable state. However, in an analysis carried out on the recovery capacity of soil bacterial communities in different forestry ecosystems two years after a large wildfire (in preparation), we found that the Orwin and Wardle resilience index did not reflect exactly the functioning of the community after this disturbance because the index values did not differentiate between not reaching the pre-disturbance state or surpassing it. In a the study carried out immediately after the fire (Sáenz de Miera et al., 2020), the abundance of diverse OTUs or taxa showed different degrees of resistance to disturbance. Diverse studies show that the abundances of different taxa are not independent variables and it is therefore essential to understand how each taxon performs under the framework of disturbances.

The objective of this work is to propose a new resilience index with a clear ecological conceptualization to estimate the resilience of bacterial communities to pulse-disturbances for one or multiple functional response variables, enabling them to be classified automatically.

2. Material and methods

2.1. Theory

The ecological resilience of a community defined as the recovery capacity can be estimated as the recovery rate of a functional variable (diversity and abundance among others) at a given time. The simplest way to measure recovery in time t_x is to consider the variation of the response variable (P) from the moment the disturbance ends (t_o) to time t_x, normalized by the variation in the variable produced by the disturbance in relation to the control, which is the proposed new index (iRL, Eq. (1)):

$$iRL = (Px - Po)/(Co - Po) \tag{1}$$

where Px and Po are the values of the response variable at times t_x and t_o respectively, and Co the value in an undisturbed equivalent community (control). If Co = Po no effect in the analysed variable is detected and it will be considered as resistant. Fig. 1, inspired by Orwin and Wardle (2004), shows the theoretical variation in the response variable over time (t_x and t_o).

Do is the difference in the variable with respect to the control community after the disturbance, and Dx is the difference at the time of estimating resilience (t_x). Rx is the difference between Do and Dx and is equivalent to the numerator of the proposed index when the control community does not change over time (Eq. (2)):

$$Rx = Do - Dx = (Co - Po) - (Cx - Px) = Px - Po \tag{2}$$

the index must be relativized to the total change produced by the disturbance (Co – Po) = Do (Fig. 1a1). The iRL could also be defined as in Eq. (3).

$$iRL = Rx/Do = (Do - Dx)/Do \tag{3}$$

In order to apply the index properly (Eq. (1) or Eq. (3)), it is necessary to verify that the response variable does not change during the period of analysis in control communities (Co = Cx). If the variation occurs due to other different causes (e.g. a new disturbance or stress situation) than the disturbance, the value of the disturbed community produces an incorrect estimation of resilience.

There are five different theoretical scenarios with regard to the behaviour of the response variable after a pulse-disturbance (Fig. 1), which enable the community to be classified in relation to its resilience. The expected value of Px for the response variable would be between Co and Po, Px ∈ (Co, Po), in this case the variable is recovering (RC, Fig. 1a1), although the change produced by the disturbance may lead to a decrease (blue in Fig. 1a1; Po < Co) or an increase (red in Fig. 1a2, Po > Co), the decrease or increase cases could be produced in the five theoretical scenarios, although Fig. 1 only shows they in the first scenario. The second scenario occurs when the response variable returns to the exact original value prior to the disturbance, Px = Co, and the community is considered to be fully resilient in t_x time (RL, Fig. 1b). The third post-disturbance scenario represents the situation in which the response variable cannot recover, maintaining the value reached after the disturbance in time, Px = Po, in which case it is considered to be non-resilient (NR, Fig. 1c). There are two other alternatives: the response variable exceeds the initial value during its recovery, |Rx| > |Do|, producing a rebound effect (RB, Fig. 1d), or, it can continue changing, |Dx| > |Do|, even though the agent that caused the disturbance disappears
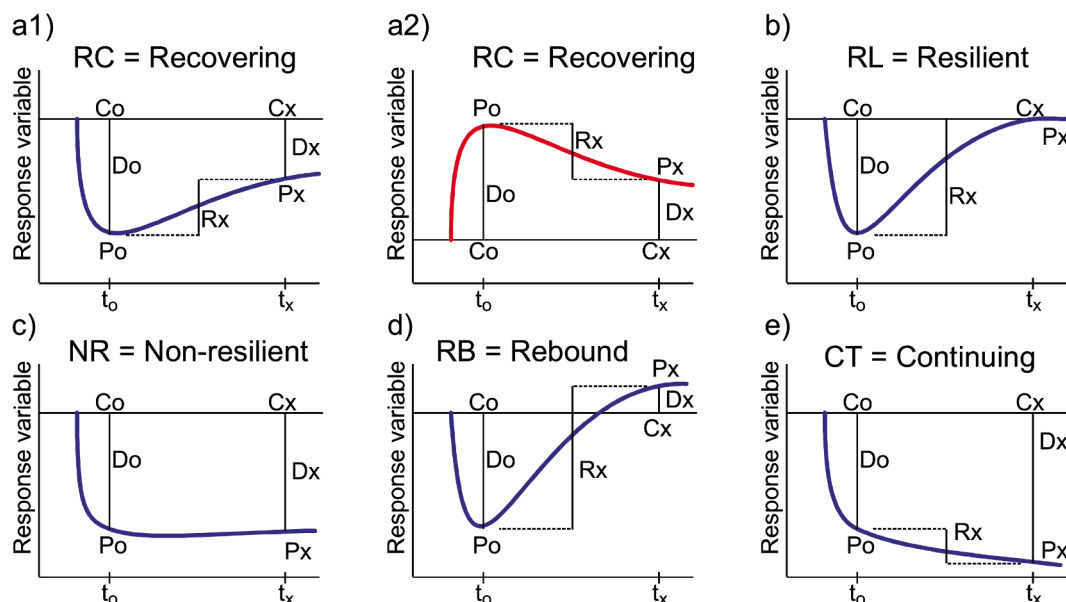
Fig. 1. Different theoretical models (scenarios a-e) of response to a pulse-disturbance. The analysed variable may suffer an initial (a1) decrease (blue lines) or (a2) increase (red line). Once the disturbance is over, the variable can: partially recover (it is recovering RC; a1 and a2); reach exactly the same value as before the disturbance at time t_x (it is resilient, RL; b); remain in the altered state (non-resilient, NR; c); exceed the original value (with a rebound effect, RB, d); or continue with the change caused by the disturbance (continuing, CT; e). Each scenario (a-e) would have a symmetrical graph (with a decrease of variable) which is only displayed in the RC scenario (a1 and a2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and the community is classified as continuing changing (CT, Fig. 1e). A good resilient index must distinguish among all these possible scenarios.

2.2. Procedure

All statistics, including graphical explorations and calculation of indices were performed with R statistical software. The first set of data consisted of simulated data used to estimate resilience with different indices and checking the values obtained in each of the scenarios shown in Fig. 1. The new proposed index has been tested with real data from a study on the recovery of soil bacterial communities carried out in three Mediterranean ecosystems two years after a large wildfire. The communities were analysed in the control situation, two months and two years after the fire (pulse-disturbance) (Sáenz de Miera et al., 2020). The response variables analysed were the Shannon diversity and the abundance of different OTUs, although other variables such as abundance of taxa or other measures of diversity can be used. Abundance was estimated from sequencing the variable regions V3-V4 of coding genes for the 16S ribosomal with Illumina, reads were clustered with *vsearch* to obtain OTUs, analysed with Vegan package of R, and normalized to 100 thousand reads per community (see Sáenz de Miera et al., 2020 for references and more details).

In the analysis of the new proposed resilience index performance with real data, 10 samples of burnt and control heathlands ecosystems (biological replicates) were taken. The mean values of the response variables from the replicates were used to calculate the new resilience index. Two different statistical approaches were used to assign the variables to the different scenarios described in Fig. 1. The first approach consists of using linear models with biological replicates, where the null hypothesis is that the response variable does not change according to the treatment: it can be analysed under three considerations (i) $Cx = Co$, (ii) the variable is resistant if $Po = Co$; and (iii) the variable is resilient if $Px = Co$. The second approach is based on producing 100 Bootstrap resampling taking 10 biological samples with repetition (100 Bootstrap

replicates). The iRS, iRL and the differences between the controls in time (Co–Cx) are estimated based on each one of these 100 replicates. Normal distributions with their means and standard deviations obtained with the 100 Bootstrap replicates can be used to measure the error of estimation. In the latter approach, the null hypothesis is that the critical values of the indices are included in the distribution (1 for iRS and iRL and 0 for the difference between the controls).

Linear models and Bootstrap are two different ways of performing a hypothesis test to determine if a variable behaves like RS or RL. Linear models are statistically simpler to interpret, while Bootstrap provides estimates of index errors and seems more reliable to avoid type II errors in contrasts. The R script is available at https://github.com/microGEAT/iRS_iRL.

3. Results and discussion

3.1. Comparison of the indices

The Y-axis in Fig. 2 indicates the variation in resilience predicted by the 6 different indices previously published plus the new proposed one (new iRL index). In all cases, it has been assumed that the response variable in the control community had the same value prior to the disturbance (Co) and over time (Cx). After the disturbance, the response variable value could either decrease (blue line) or increase (red line). In the X-axis it is represented the change of the response variable Dx in the range (-40, 40). Critical ranges corresponding to the different scenarios described in Fig. 1 are shown in Table 1 and indicated in Fig. 2.

Indices #1, #2, #3 and #4 are normalized or relativized to the value of the response variable without disturbance or before the disturbance (Co), whereas indices #5, #6 and #7 are relativized with the value of the impact (Do in #5 and 7, and $|Do| + |Dx|$ in #6). The graphs for disturbances that increase or decrease the value of the response variable of indices #1, #2, #3 and #6 are coincident (blue and red lines in Fig. 2), although only indices #1 and #6 are symmetrical because they
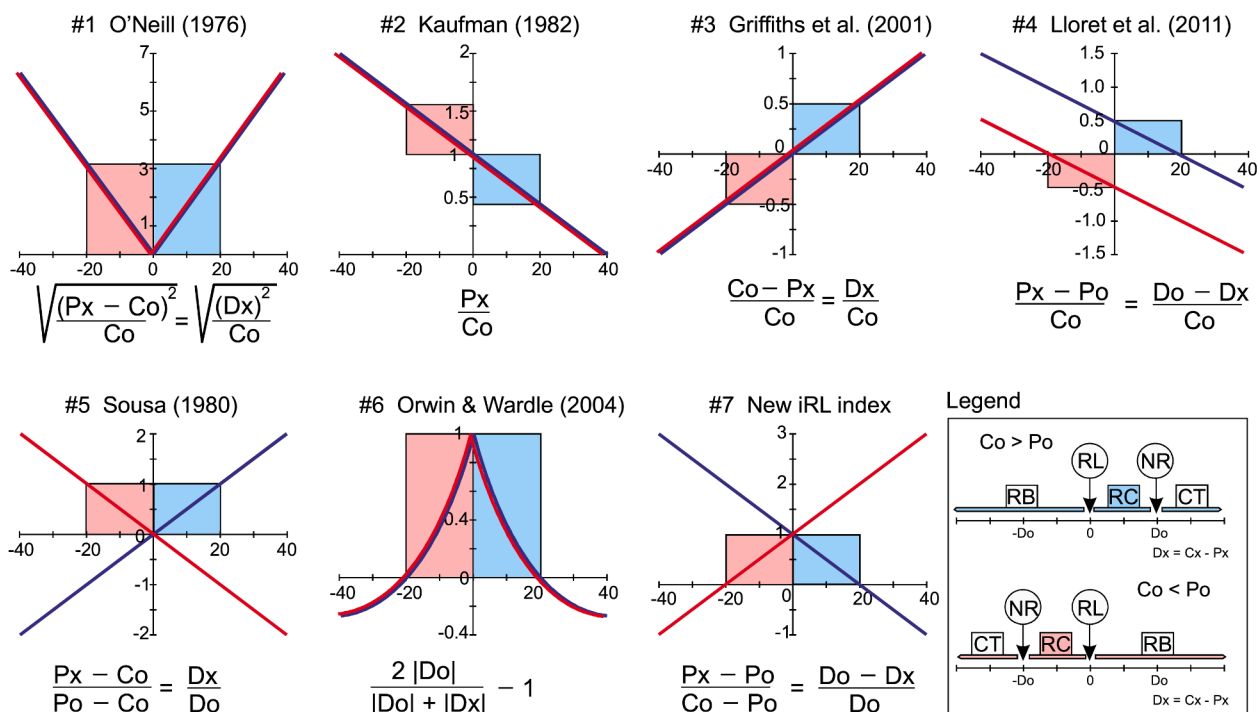
Fig. 2. Resilience indices (Y-axis) as a function of Dx (i.e. Co − Px, in X-axis) (#1 to #7). Co is fixed at 40 and Po at 20 (Po < Co, blue lines) or at 60 (Po > Co, red lines). The legend indicates the different scenarios of behaviour of the response variable described in Fig. 1 as a function of the x-axis values: RL = resilient, NR = non-resilient, RC = recovering, RB = rebound effect, and CT = continuing. The range in which the variable is found when it is recovering is shaded (cyan when Po < Co and pink when Po > Co). All equations have been simplified assuming that Cx = Co. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of the values obtained for different resilience indices for critical values of Dx (i.e. Co – Px). Classification of cases is in accordance with Fig. 1.

Pulse time	Resilience time			Resilience indices							Px position
	Class.	Px	Dx	#1	#2	#3	#4	#5	#6	#7	
Group 1 Co > Po Co = 40 Po = 20 Do = 20	RL	40	0	0	1	0	0.50	0	1	1	Px = Co
	NR	20	20	3.16	0.50	50	0	1	0	0	Px = Po
	RC	**30**	**10**	**1.58**	**0.75**	**25**	**0.25**	**0.5**	**0.33**	**0.5**	**Co > Px > Po**
	RB	50	−10	1.58	1.25	−25	0.75	−0.5	0.33	1.5	Co < Px > Po
	RB	70	−30	4.74	1.75	−75	1.25	−1.5	−0.20	2.5	Co ≪ Px > Po
	CT	10	30	4.74	0.25	75	−0.25	1.5	−0.20	−0.5	Co > Px < Po
Group 2 Co > Po Co = 80 Po = 40 Do = 40	RL	80	0	0	1	0	0.50	0	1	1	Px = Co
	NR	40	40	4.47	0.50	50	0	1.0	0.00	0	Px = Po
	RC	**60**	**20**	**2.24**	**0.75**	**25**	**0.25**	**0.5**	**0.33**	**0.5**	**Co > Px > Po**
	RB	100	−20	2.24	1.25	−25	0.75	−0.5	0.33	1.5	Co < Px > Po
	RB	140	−60	6.71	1.75	−75	1.25	−1.5	−0.20	2.5	Co ≪ Px > Po
	CT	20	60	6.71	0.25	75	−0.25	1.5	−0.20	−0.5	Co > Px < Po
Group 3 Co < Po Co = 40 Po = 60 Do = -20	RL	40	0	0	1	0	−0.50	0	1	1	Px = Co
	NR	60	−20	3.16	1.50	−50	0	1	0	0	Px = Po
	RC	**50**	**−10**	**1.58**	**1.25**	**−25**	**−0.25**	**0.5**	**0.33**	**0.5**	**Co < Px < Po**
	RB	30	10	1.58	0.75	25	−0.75	−0.5	0.33	1.5	Co > Px < Po
	RB	10	30	4.74	0.25	75	−1.25	−1.5	−0.20	2.5	Co ≫ Px < Po
	CT	70	−30	4.74	1.75	−75	0.25	1.5	−0.20	−0.5	Co < Px > Po

The index compared and the first group of cases (Group 1) are similar to that used by Orwin and Wardle (Index #6). In the second group of cases there is a variation in the values scale, and in the third (Group 3), the pulse disturbance produces an increase in the value of the response variable. The indices are: #1, (O'Neill, 1976); #2, (Kaufman, 1982); #3, (Griffiths et al., 2001); #4, (Lloret et al., 2011); #5, (Sousa, 1980); #6, (Orwin and Wardle, 2004); and #7 the proposed new index.

calculate the absolute value of Dx or Do (the square root of its square in #1). Indices #5 and #7 produce the same behaviour in resilience values when Po is greater or lower than Co, which means resilience is independent of the sign of Do. However, resilience is not independent of the Do sign in indices #2, #3, and # 4 as shown by the cyan and pink shaded areas in Fig. 2, or first and third group of cases in Table 1.

Table 1 also shows that when comparing the first and second group of cases (with Do = 20 as Fig. 2, or with Do = 40 but with a 50% reduction from Co to Po), the magnitude of the initial variation caused by the disturbance affects the absolute value of resilience only in index #1. This problem associated with index #1 could be corrected if the square root of the index only affected the numerator of its equation.

Another difference in the performance of the indices is found in the values obtained when the effect of the disturbance continues (CT), or there are rebounds (RB). Indices #2, #3 and #4 allow differentiated scenarios of CT and RB only when the sign of Do is known, whereas, indices #5 and #7 differentiated these situations for Po both with greater and lower values than Co.

Indices #1, #3 and #5 have a value of zero for fully recovered variables in t_x (RL) and a higher value when the variable is recovering and Po < Co (cyan RC), whereas indices #2, #6 and #7 showed values that were much easier to interpret: 1 for RL variables, and lower values for RC (when RC is cyan). Both #6 and #7 indices move between zero and 1 in all cases of response variables in RC (Fig. 2 and Table 1).
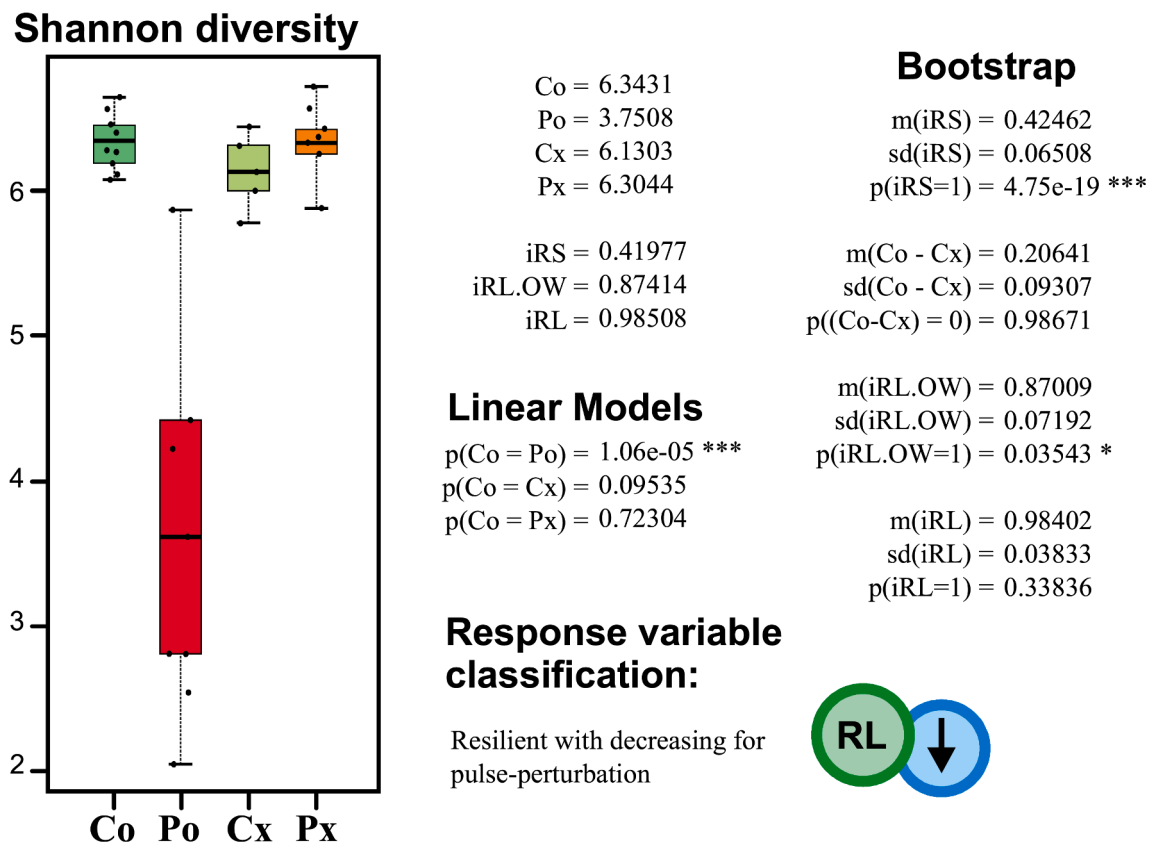
The new index proposed (#7) had performance and interpretation advantages over the others: i) it is normalized with regard to the impact value (Do). The effect of the impact just after the disturbance would be measured by resistance, the other component of stability. In this way, the response of communities to a disturbance (stability) would be characterized using a bivariate framework as proposed by (Ingrisch and Bahn, 2018), resistance and resilience, respectively, report the initial variation and recovery capacity in a specific time, ii) the values of the resilience index allow the response variables to be classified into resilient (RL) when iRL = 1; recovering (RC) when iRL belongs to the intervals (0, 1); non-resilient (NR) when iRL = 0; rebound effect (RB) when iRL greater than 1; and continuing the effect caused by the disturbance (CT) if iRL < 0. This classification is independent of the sign of the variation (Do) produced by the disturbance, and iii) the simplicity, continuity and linearity of the function that allows the new index to interpret the results easily.

3.2. Validation data and bootstrap statistics with one variable.

The Shannon diversity of the bacterial communities variable was used to estimate the Orwin and Wardle (2004) resistance index (iRS) and the resilience indices #6 described by the same authors (Orwin and Wardle, 2004), as well as the new proposed index #7 (iRL.OW and iRL respectively). The wildfire caused a significant decrease in the diversity of bacterial communities (Fig. 3). These changes showed that bacterial community diversity had relatively low resistance (iRS = 0.420), but high resilience estimated with the Orwing and Wardle index (iRL.OW = 0.874, #6), and with the new index proposed iRL = 0.985, #7) (Fig. 3). Also, with the biological replicas and applying linear models, it was found that the communities did not show resistance when Shannon's diversity variable is considered since Co and Po are significantly different to this variable ($p = 1.06 \times 10^{-5}$). However, they can be classified as totally resilient two years after the fire, Px values being similar to those for diversity in the control (p (Co = Px) = 0.723) (Fig. 3).

The analysis of 100 bootstrap replicates, taking 10 samples from biological replicates of each of the four categories of communities (control or burned in both times, Co, Po, Cx and Px), was used to obtain a distribution of the values for each of the three indices (iRS, iRL.OW # 6 and iRL #7), in addition to the differences in diversities of the control communities (Co - Cx). The distributions of the three indices were adjusted to a normal distribution (Shapiro's test), although sometimes (with different bootstrap attempts) the Orwin and Wardle indices were not. The new proposed index iRL adjusted to the normal distribution in all tests, possibly because it is a continuous and linear function.

Considering the Shannon diversity response variable (Fig. 3) an interpretation of the resistance and resilience values obtained by different indices showed that the communities are not resistant, since the iRS index differs significantly from 1 with the bootstrap analysis (p = 4.75×10^{-19}). The resilience index #6 would indicate that it is not completely resilient since it differs significantly from 1 (p(iRL.OW = 1) = 0.035). With this index (#6) the value of 0.87 would not indicate if the communities were in recovery (RC in previous figures) or whether there was a rebound effect (RB) (Fig. 3). However, the proposed index (#7) enables the bacterial communities to be classified as resilient (RL) because their value does not differ significantly from 1 (p(iRL = 1) = 0.338).

Shannon diversity

Co = 6.3431
Po = 3.7508
Cx = 6.1303
Px = 6.3044

iRS = 0.41977
iRL.OW = 0.87414
iRL = 0.98508

Linear Models

p(Co = Po) = 1.06e-05 ***
p(Co = Cx) = 0.09535
p(Co = Px) = 0.72304

Response variable classification:

Resilient with decreasing for pulse-perturbation

Bootstrap

m(iRS) = 0.42462
sd(iRS) = 0.06508
p(iRS=1) = 4.75e-19 ***

m(Co - Cx) = 0.20641
sd(Co - Cx) = 0.09307
p((Co-Cx) = 0) = 0.98671

m(iRL.OW) = 0.87009
sd(iRL.OW) = 0.07192
p(iRL.OW=1) = 0.03543 *

m(iRL) = 0.98402
sd(iRL) = 0.03833
p(iRL=1) = 0.33836

Fig. 3. Shannon diversity of validation data. After the disturbance produced by a large wildfire, t_0, (see Sáenz de Miera et al., 2020) and two years later (preliminary results). The mean values for diversity, the indices of Resistance and Resilience calculated with the averages including three checkpoints to classify the variable, and the description of the distributions obtained by 100 bootstrap resamples of the communities are shown. iRS = Resistance; iRL.OW = #6 index Resilience; and iRL = proposed resilience index. When both, linear models or bootstrap distributions were used, the three checkpoints (see Fig. 4) indicated that the response variable (Shannon diversity) behaved as resilient with a decrease caused by the pulse-disturbance.

3.3. Automatic classification of variables using validation data.

The automatic classification of the response variables follows the proposed workflow (Fig. 4). First, there are no changes in the variable after disturbance (Po = Co), so the community is resistant according to the Orwin and Wardle (Orwin and Wardle, 2004) resistance index. If the resistant option is rejected, it can be verified whether the disturbance implies a decrease (Co > Po) or an increase (Co < Po) in the response variable. Besides checking the direction of the modification of the variable, the workflow in Fig. 4 includes a second check-point in which the continuity of the control variable in time is analysed. If the control is maintained in time (Cx = Co) it possible to continue the classification of the resilience of the variable. So, if the null hypothesis of this check-point is rejected, the variable is changing over time (TV) due to causes other than disturbance and resilience cannot be analysed.

If the null hypothesis is accepted, the last check-point is the resilience index itself (or alternatively if the community has returned to the values prior to the disturbance, Px = Co). If the iRL bootstrap distribution includes the value of 1, the community can be considered to have fully recovered for the analysed variable, and is classified as fully resilient (RL). This decision is quite conservative since the iRL distributions of many variables will include the value of 1 if the biological replicates are not very homogeneous and produce large standard deviations in the bootstrap replicates.

If the resilience of the variables is rejected the iRL index values allow the response variable to be classified in the following scenarios: CR in recovery; CT the effect of the disturbance continues or RB rebound effect.

This workflow was applied to classify the normalized abundance of

3682 OTUs (operational taxonomic unit, they are groups of closely related individuals or sequences, OTUs are commonly used in the analysis of bacterial communities rather than species) found in the field study. The classification of all of them is shown in Table 2, using linear models and Bootstrap distributions in the three check points.

2098 OTUs (57.7%) were classified as resistant (RS) with linear models. It clearly seems to be an overestimation given the large differences in diversity found between communities from burned and control areas. Bootstrap analysis indicates that only 171 OTUs (4.7%) should be considered statistically resistant (Table 2). However, the number of OTUs considered TV (with temporal variations between controls) with the Bootstrap is higher (717, 19.7 % vs 240, 6.6 %) because many of them had passed the first check-point and had been considered RS with the linear model.

The number of OTUs classified as resilient with bootstrap (1829, 50.3 %) at the third check-point is three times higher than with linear models (605, 16.6 %). Many of the OTUs classified as RL with bootstrap and not with linear models come from those classified as RS with models. There are also variations between the RC, RB and CT both due to the result obtained in the check-points and because the mean value of the iRL index of the bootstrap distribution was used instead of the values obtained with the biological replicates. It is noteworthy that with bootstrap no OTU has been classified as RB, unlike what happens with linear models (62, 1.7 %)

The proposed methodology facilitates the calculation of the indices and the workflow is easy to apply automatically. As a result, numerous variables can be classified according to their response to a disturbance and thus the behaviour of the community in terms of stability can be understood. Although the application of linear models to the biological
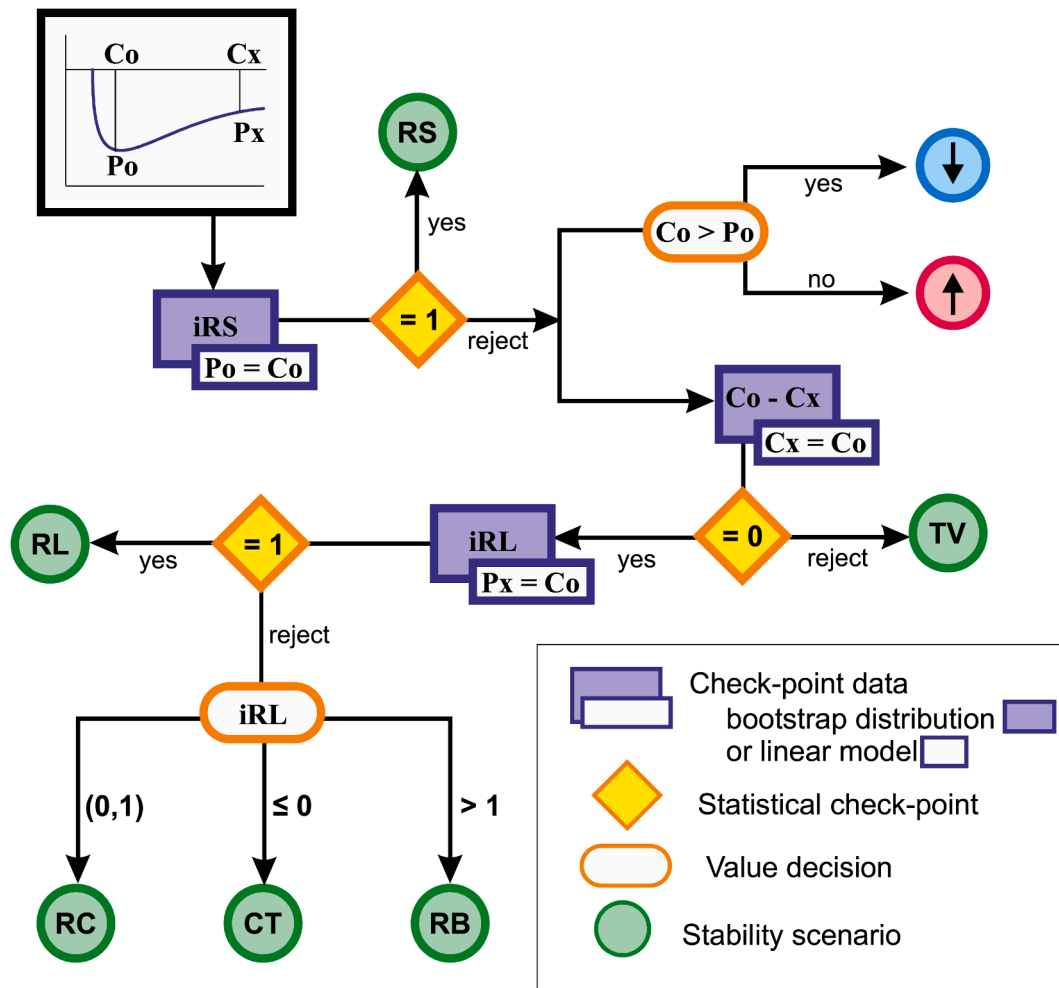
Fig. 4. Workflow to classify the response variables. The variables are classified into 4 resilience scenarios (RL, RC, CT and RB), resistant to disturbance (RS) or show variations over time in the controls (TV), so that resilience could not be correctly analysed. The direction of the changes of the variables is also indicated by arrows. The classification is carried out through statistical tests using bootstrap or linear models in three check-points or by the values of the variables or the resilience index.

Table 2

Classification of abundance of 3638 OTUs of a wildfire. It was performed according to their stability (resistance and resilience) using the Fig. 4 work-flow and the proposed new resilience index.

Classification	RS	TV	RL	RC	RB	CT
Linear model						
Co > Po ↓		226	546	293	60	258
Co < Po ↑		14	59	36	2	45
Total	**2098**	**240**	**605**	**329**	**62**	**303**
Bootstrap distribution						
Co > Po ↓		599	1496	542	0	344
Co < Po ↑		118	333	19	0	15
Total	**171**	**717**	**1829**	**561**	**0**	**359**

The response variable is the abundance of each OTU, the samples were taken just after burning (t_0) and after two years of recovery (t_x). They are classified as follows: resistant (RS), with time variation between controls (TV), resilient (RL), recovering (RC), rebounds (RB) and continuing the pulse effect (CT).

replicates in the three check-points of the workflow may be valid, the use of Bootstrap resampling provides an estimation of the error of the resistance and resilience indices that seems essential to analyse the stability of communities in the face of pulse-disturbances.

4. Conclusions

In this work, a new resilience index is proposed to carry out an automatic classification of a wide variety of ecological variables after pulse-disturbance. The advantage of the new proposed index is related to the ability of quantifying resilience and classifies the communities into different behaviour scenarios. This classification is simple and robust mainly when Bootstrap analytics are used, and provides the error of the indices.

The main advantages of the proposed index in relation to those already proposed by other authors are (i) its normalization for the impact value, (ii) the output classification is independent of the variation produced by the disturbance, (iii) the simplicity of interpreting the results, (iv) it has been developed to evaluate a large number of variables and a large number of samples automatically. Therefore, it is suitable for the informatics analysis, with a high number of samples or parameters, and other types of analysis.

CRediT authorship contribution statement

Sáenz de Miera, Pinto, Calvo, Ansola: Conceptualization, Data curation, Formal analysis, Writing original draft and Writing -review & editing. **Calvo, Ansola:** Funding acquisition, Resources. **Sáenz de Miera, Pinto:** Software, Validation, Visualization.

Declaration of Competing Interest

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