CO₂-driven experimental acidification effects on aquatic macroinvertebrates in a tropical stream

Crystal C. Klem and Pablo E. Gutiérrez-Fonseca

ABSTRACT
Experimental acidification of a tropical stream was conducted to measure the effects of declining pH on aquatic macroinvertebrates. The mechanisms by which anthropogenic acidification occur in a freshwater stream system are relatively well understood, while little is known about the natural phenomena of acidification or the corresponding effects on macroinvertebrate assemblages. Previous studies have attempted to model stream acidification using strong acids; however, this is one of the first studies which models stream acidification using the addition of gaseous CO₂. This method is a more natural means of modeling stream acidification conditions arising from increased levels of dissolved CO₂. We hypothesized that if experimental acidification was expressed most strongly at the injection site and produced a pH gradient downstream, macroinvertebrates should respond to the gradient and employ an escape mechanism to avoid the adverse conditions. Three macroinvertebrate sampling strategies were used: drift nets, leaf pack samples and benthic Surber samples. Samples were evaluated in the lab for macroinvertebrate abundance and taxonomic richness per m³ for drift net samples and benthic samples, and per g leaf material in the leaf pack samples. A maximum decline of 2 units in pH along a gradient was observed associated with the injection of CO₂. Results obtained from drift net and benthic sample analysis were inconclusive, possibly because of low stream flow, although analysis of the leaf pack samples indicates lower macroinvertebrate composition at areas of lower pH. The leaf pack samples also show significant macroinvertebrate sensitivity to the most severe pH decline at the injection site.

KEYWORDS
Macroinvertebrates; experimental acidification; tropical stream; Puerto Rico; CO₂; freshwater stream; climate change

1. Introduction
Stream acidification has been shown to have negative effects on freshwater ecosystems (Malmqvist & Rundle 2002; Dangles et al. 2004; Camargo & Alonso 2006). Freshwater may be acidified either through the impact of dilution of acid neutralizing capacity, atmospheric deposition (i.e. sulfur and nitrogen deposition), oxidation–reduction (redox) reactions, organic acid inputs, or elevated concentrations of dissolved carbon dioxide (CO₂). The mechanisms by which anthropogenic acidification occur are relatively well understood, while little is known about the natural phenomena of acidification. Small et al. (2012) observed that an increase in labile carbon storage in the forest soil associated with the most severe drought during ENSO-1998 produced a pH decline of ~3 units in...
some streams at La Selva (Costa Rica). Small et al. (2012) suggested that the pulse of CO₂-saturated runoff when the rains returned contributed to higher levels of dissolved CO₂, lowering the pH of shallow streams. Similar findings have been observed by Johnson et al. (2008) in groundwater springs, where high soil CO₂ concentrations contribute to a pH of 4.65, compared with first- and second-order streams with lower CO₂ concentrations and a neutral pH.

According to the hypothesis of acidification mediated by CO₂, global climate change could intensify natural acidification events due to a redistribution of precipitation across different regions, which would result in changes in rainfall patterns. Seasonal wet and dry patterns are expected to be intensified in a given area as the effects of global warming increase, and droughts are predicted to have both earlier onset and greater severity (Trenberth et al. 2014). These droughts could increase carbon concentration in the forest soil due to increased microbial respiration and tree root death. By these mechanisms, more severe and frequent droughts are expected to stimulate strong and unpredictable pH changes in streams. These unusual acidification events can cause significant stress on aquatic communities, with detrimental effects on both stream biota and ecological functions (e.g. Ramírez et al. 2006).

Macroinvertebrate responses to acidification events in tropical streams are poorly understood (Ramírez et al. 2006; Small et al. 2012), but have been well documented in temperate streams (e.g. Rosemond & Reice 1992; Lepori et al. 2003; Lepori & Ormerod 2005). Stream acidification simplifies macroinvertebrate assemblages and reduces taxonomic richness (Lepori & Ormerod 2005; Durance & Ormerod 2007), particularly among Ephemeroptera and grazers in general (e.g. Rosemond & Reice 1992). A common response of macroinvertebrates to decreases in pH is entering into drift and allowing the current to transport them to a new area. Ardón et al. (2013) observed increased macroinvertebrate drift in response to experimental acidification in tropical lowland streams; the drift was dominated by Ephemeroptera and Chironomidae. Some experimental studies have attempted to demonstrate the effects of acidification on stream macroinvertebrates using strong acids; some examples are the use of nitric acid by Dangles and Guérol (2000), and hydrochloric acid in a study on tropical streams (Ardón et al. 2013). Strong acids are good alternatives for the simulation of acid rain or the effects of mining, but not for the study of the effects of CO₂ pulses related to organic matter decomposition.

The goal of this study was to understand the effects of episodic acidification events on macroinvertebrates in a tropical stream. In order to model stream conditions resulting from increased dissolved CO₂, gaseous CO₂ was added directly to the stream to cause an experimental decline in pH. It is well known that a major stream buffering system is the carbonate-bicarbonate system, in which gaseous CO₂ exchange at the water surface reacts with water molecules to form H₂CO₃, a weak acid which protonates easily to produce H⁺ and HCO₃⁻ ions (Small et al. 2012; Ardón et al. 2013; Hasler et al. 2016). With the addition of injected gaseous CO₂, the equilibrium of this reaction may be driven to produce more H₂CO₃ and cause the stream to become acidified. We hypothesized that (1) the injected CO₂ would react with the water and cause a decline in stream pH, which could result in adverse conditions for aquatic organisms, and (2) macroinvertebrates exposed to the increased acidity of the environment would respond by entering into drift or employing an escape mechanism. Consequently, we predicted to find (1) the experimental acidification expressed most strongly at the CO₂ injection site and a pH gradient to be formed downstream, and (2) if the acidification effect was acute enough to induce changes in macroinvertebrate composition, the macroinvertebrate assemblages would respond to the pH gradient and have the most organisms in drift (or in fewer numbers in the benthic layer and leaf litter) near to the CO₂ injection site.

2. Site description

The study was performed in July 2014 at El Verde Field Station, which is within the Luquillo Experimental Forest (latitude 18°19′ N, longitude 65°45′ W). El Verde Field Station is located at an elevation of 350 m and is part of El Yunque National Forest. El Yunque is a subtropical wet forest under
the Holdridge Life Zone classification which encompasses 113.32 km² and has mountains that reach 1076.9 m asl (Ewel & Whitmore 1973). The annual rainfall at El Verde averages over 3460 mm and is evenly distributed throughout the year. The average pH of rainfall measured at El Verde was found to be 4.92 (Gioda et al. 2013). The driest period begins in January and ends in April with a relative humidity ranging from 60% to 100%. The mean temperature is 22.8 °C, while mean maximum temperature is 27 °C and mean minimum temperature is 21.1 °C.

The Buruquena stream, a first-order stream at El Verde, was chosen as the study site. The stream is a tributary in the Espiritu Santo watershed that drains a small, steep catchment and is characterized by a series of pools interspersed with boulder-lined riffles. The stream is bordered by dense riparian vegetation dominated by Dacryodes excelsa (tabonuco), Guarea guidonia, Cecropia sp. and Prestoea montana (sierra palm). Stream temperature ranges from 22.1 °C to 23.5 °C (unpublished data). Discharge is variable, and is strongly influenced by local storm conditions. Dominant substrates were bedrock, large boulders, and cobble, as well as some gravel and sand found mainly in pools.

3. Methods

3.1. Experimental acidification

The Buruquena stream was acidified by bubbling compressed gaseous CO₂ from a tank through a hose submerged in the stream. Acidification was for one hour, beginning at 1255 when the CO₂ tank was opened and ending at 1355 when the tank was closed. The whole experiment took place during the same day. The stream pH was measured at 1100, 1200, 1300, 1345, 1400 and 1500. Measurements were taken at the CO₂ injection site and at four stations located over a total distance of 35 m. One station was located 4 m upstream from the CO₂ injection site (Upstream) and the other stations were located at 4 m (First station), 10 m (Second station) and 29.5 m downstream from the injection site (Third station), respectively.

3.2. Macroinvertebrate assemblage characterization

3.2.1. Surber samples

To characterize the aquatic macroinvertebrate assemblages in the Buruquena stream before the experiment, three Surber samples (area: 0.036 m²; mesh size: 500 μm) were taken prior to the CO₂ injection at each sample site except the injection site. The same procedure was used the next day after the CO₂ injection to assess any change caused by the decline in pH. All material collected was preserved in the field with 95% ethanol and transported to the laboratory, where aquatic macroinvertebrates were separated using a stereoscopic microscope. All macroinvertebrates collected were identified to the family level for aquatic insects using Merritt et al. (2008), Springer et al. (2010), and Gutiérrez-Fonseca et al. (2013). Specimens were deposited in the UPRRP El Verde Field Station insect collection.

3.2.2. Leaf pack samples

Another measure of macroinvertebrate response to acidification was taken using leaf packs. Each pack was composed of approximately 7 g of G. guidonia (Meliaceae) leaves, a key riparian tree widely used in leaf-litter decomposition experiments in the Luquillo Experimental Forest (e.g. Rincón & Covich 2014). Six packs were placed at the Upstream station, directly at the injection site, and at the First and Second stations, with an additional seventh pack at each station in case a pack was lost due to some environmental disturbance. The leaf packs were left in the stream to develop for 5 days before the acidification experiment. Before the CO₂ injection, three packs were collected from each station and preserved in the field with 95% ethanol. The CO₂ tank was then opened for 1 hour from 1220 to 1320 of the same day. After the acidification, three more leaf packs were
collected from each of the stations and preserved in the same way. Macroinvertebrates were separated from each sample using a stereoscopic microscope and preserved according to the procedure given for the Surber samples. The leaf pack samples were evaluated in the laboratory for abundance and taxonomic richness per g.

3.3. Drift response

Macroinvertebrate samples were collected using three small drift nets (mouth: 0.06 m²; length: 0.5 m; mesh size: 250 µm) attached to a board 0.32 m apart. Samples were collected before the acidification at 1100 and 1200, and after the acidification at 1300 (beginning of CO₂ injection), 1400 (end of CO₂ injection), and 1500. For each sample taken, the drift net was completely submerged and the water column was filtered for 15 min. All samples were processed in the same manner as above.

Stream velocity was measured by a Marsh McBirney® current meter for each drift net. Macroinvertebrate drift density and taxonomic richness were calculated by dividing the number of individuals in a sample by the volume of water sampled. Water volume was calculated by multiplying net area, current velocity, and sampling time. Each sample was evaluated for abundance and taxonomic richness per m³.

4. Statistical analyses

For the macroinvertebrate drift experiment, a mixed model analysis of variance (ANOVA) was used to compare the macroinvertebrate assemblage structure at each station before and after the acidification. Separate analyses were run for macroinvertebrate abundance and richness. Time was specified as a random factor. The independent factors incorporated in the between effects design were station, treatment (before/after acidification), and the interaction between station, treatment, and time. The Euclidean distance was used as the distance measure.

To analyze changes in the Surber samples and the leaf pack samples, a mixed model ANOVA was also used to compare the macroinvertebrate structure at each station before and after the acidification. Separate analyses were run for macroinvertebrate abundance and richness and for each individual station. Station was specified as a random factor to account for the lack of independence in letting each station serve as its own control before and after the acidification. The independent factor incorporated in the between effects design was treatment (before/after acidification). All analyses were performed using Statistica v12 (Statsoft Inc., Tulsa, OK, USA).

5. Results

5.1. Experimental acidification

During the CO₂ injection experiment, we observed a decline in stream pH. The Upstream station maintained a constant pH over the experiment, remaining very near 7 with fluctuations between 6.88 and 7.22. The injection site experienced the largest drop in pH: the initial measurement was 7.13 before the injection, and after the CO₂ tank was opened at 1255 and the pH measured at 1300, it had dropped to 5.48. This low pH persisted and was measured as 5.43 at 1345 until the CO₂ tank was closed at 1355, at which point the pH began to rise to neutral levels of 7.24 at 1400 and 7.17 at 1500. The same trend may be seen with less severity at the other stations and follows a gradient that lessens in acidity farther away from the source of injection. The First station experienced a pH decrease from ~7 to 5.96 at 1300 and 5.83 at 1345 during the time the tank was open, the Second station dropped from ~7 before the injection to 6.68 at 1300 and 6.38 at 1345, and the Third station experienced little change during the injection and fluctuated between 6.94 and 6.6 (Figure 1).
5.2. Macroinvertebrate assemblage characterization

5.2.1. Surber samples
The most prevalent groups in the benthic samples were Leptophlebiidae (Ephemeroptera), Chironomidae (Diptera), and Hydropsychidae (Trichoptera). No significant changes in macroinvertebrate abundance or taxonomic richness were observed associated with acidification for the Upstream station ($F_{1,4} = 0.39, p > 0.05$, n.s.; $F_{1,4} < 0.01, p > 0.999$, n.s., respectively), First station ($F_{1,4} = 2.40, p > 0.05$, n.s.; $F_{1,4} = 4.00, p > 0.05$, n.s., respectively), Second station ($F_{1,4} = 0.06, p > 0.05$, n.s.; $F_{1,4} = 0.01, p > 0.05$, n.s., respectively), or Third station ($F_{1,4} = 1.93, p > 0.05$, n.s.; $F_{1,4} = 2.50, p > 0.05$, n.s., respectively).

5.2.2. Leaf pack samples
Leaf pack analysis showed the clearest macroinvertebrate response to the CO$_2$ acidification. At all stations the dominant group collected was Leptophlebiidae (Ephemeroptera), followed by Coenagrionidae (Odonata). No significant difference was found in macroinvertebrate abundance before and after acidification at the Upstream station ($F_{1,4} > 0.01, p > 0.05$, n.s.; Figure 2); however, a significant difference was found in the taxonomic richness at the Upstream station associated with acidification ($F_{1,4} = 8.00, p = 0.047$; Figure 3). At the injection site, there was a highly significant difference between the macroinvertebrate abundance ($F_{1,4} = 36.89, p = 0.004$; Figure 2) and taxonomic richness ($F_{1,4} = 21.13, p = 0.010$; Figure 3) before and after the CO$_2$ injection. At the First station, no significant difference was found in macroinvertebrate abundance ($F_{1,4} = 1.58, p > 0.05$, n.s.; Figure 2) or taxonomic richness ($F_{1,4} = 0.29, p > 0.05$, n.s.; Figure 3) associated with acidification. Similarly, no significant differences were found in macroinvertebrate abundance ($F_{1,4} = 0.11, p > 0.05$, n.s.; Figure 2) or taxonomic richness ($F_{1,4} = 0.20, p > 0.05$, n.s.; Figure 3) at the Second station. Although significant differences were not observed in the First and Second stations in either measure of macroinvertebrate composition, a trend showing a decline of macroinvertebrates in terms of abundance (Figure 2) and taxonomic richness (Figure 3) was observed after the addition of CO$_2$ at...
these stations. A trend was also observed at the Upstream station showing an increase in macroinvertebrate abundance after CO₂ was added (Figure 2).

5.3. Drift response

Macroinvertebrate drift density was consistently low and the effects of acidification were not clear. The drift samples were dominated by Baetidae (Ephemeroptera). No significant change in macroinvertebrate abundance ($F_{1,15} = 3.09, p > 0.05, \text{n.s.}$) or taxonomic richness ($F_{1,15} = 3.41, p > 0.05, \text{n.s.}$) was observed associated with acidification. A significant difference was found in the taxonomic richness measured between stations ($F_{1,15} = 3.69, p = 0.036$), although no significant differences were detected between stations using the Tukey HSD post hoc comparison. For abundance between stations, no significant differences were detected ($F_{1,15} = 2.81, p > 0.05, \text{n.s.}$).

6. Discussion

The hypothesis that the injection of CO₂ in the Buruquena stream should decrease the pH and strongly affect the composition and structure of aquatic macroinvertebrates was supported by a decline of up to 2 pH units at the site of injection and a change in richness and abundance of aquatic organisms. The pH decline observed in this study is consistent with the hypothesis put forward in Small et al. (2012) and Ardón et al. (2013) which place increased concentrations of CO₂ as one of the primary factors driving acidification in streams. This study further demonstrated that acidic conditions could be created in a stream with the addition of CO₂, and that the injection would create severe enough conditions to induce a macroinvertebrate response. We did not observe an increase in drift during the drift experiment, which could be because of low stream flow due to a drought that
affected the study site when it was conducted. Nevertheless, we observed a clear effect on the macroinvertebrate composition and structure in the leaf pack samples as a product of the acidification.

Leaf pack analysis showed the clearest and most compelling decrease in macroinvertebrate composition. At the site of the CO₂ injection, a vast difference was seen in insect abundance and a similarly large difference was seen in taxonomic composition after the stream was acidified. These provide good evidence that some kind of escape is being made at the site where the pH decrease is most severe. Possibly, increased CO₂ concentrations may have an effect on leaf litter decomposition in streams and the development of microorganism communities, which could be why the leaf pack samples showed the most change due to acidification. This would corroborate the results of Mulholland et al. (1987), who showed that both microorganism communities and leaf litter decomposition are negatively impacted by decreased pH. According to Sterner and Elser (2002), leaf litter exhibits a wider range of C:P:N ratios than living leaves, making it a strong factor in nutrient cycling. The increase in macroinvertebrate composition present at the Upstream station after the CO₂ injection in the leaf pack samples could also be the result of upstream taxis, another mechanism by which insects can escape disturbance (Berghahn et al. 2012). These data reinforce the view that a significant macroinvertebrate response was caused by the acidification.

The dominance of Leptophlebiidae (Ephemeroptera), Chironomidae (Diptera), and Hydropsychidae (Trichoptera) in the Surber samples is understandable since these insects most commonly make their habitat in the gravel and leaf litter at the stream bottom. The primary escape mechanism of these families, particularly Leptophlebiidae, is burrowing or hiding under rocks to avoid the disturbance (Ramírez & Hernández-Cruz 2004), so their presence in the benthic level of the stream is not surprising. Leptophlebiidae also predominated in the leaf pack samples, which was expected as they can be commonly found in larger masses of decomposing vegetation (Jandry et al. 2014). Studies have shown that mayflies prefer to feed on algae and cyanobacteria (Dudgeon 2008), and the microorganisms present on the surface of the leaf substrata would make the leaf packs a good

![Figure 3. Leaf pack sample taxonomic richness at each sampling station before and after acidification (*p < 0.05).](image-url)
environment for Leptophlebiidae. The overwhelming presence of Baetidae in the drift net samples concurs with a study by Berghahn et al. (2012) and can be explained because the primary escape mechanism of this family is entering into drift to avoid adverse conditions. The behavior of the shrimp at the First station below the injection site was very interesting, because once the CO₂ tank had been opened during the acidification experiment they began swimming downstream and leaping out of the water to avoid the pH change. The next day when we returned to conduct the post-acidification Surber samples, we found several dead shrimp at the First station, which we believed was due to the acidification experiment the previous day. The behavior of the shrimp in response to acidification would make a good subject for a future study.

It was expected that the number of insects collected in drift nets at each station throughout the acidification experiment would reflect the changing pH. A decrease in pH at a station ought to have corresponded to a proportional increase in specimens collected. However, the drift results for this experiment were inconclusive, especially as compared with the results of Ardón et al. (2013). For the drift experiment in this study, a significant difference in macroinvertebrate richness was detected after the injection of CO₂. However, this result was only barely statistically significant and post hoc comparisons could not detect significant differences between the stations. The macroinvertebrate drift measured in response to short-term acidification by HCl by Ardón et al. (2013) showed significant results below the HCl injection site, where the macroinvertebrate drift was found to be higher than above the injection site. The drift results were significant not only at the station 10 m below the injection but also 100 m below in both the well-buffered and poorly-buffered streams (Ardón et al. 2013). A possible reason for this is that unlike dissolved CO₂, HCl has no way of leaving the stream once it is introduced, while CO₂ is able to dissolve back out of solution by passive diffusion when the atmospheric concentration becomes lower than the concentration in the stream. According to Cole et al. (2007), excess CO₂ dissolved in the stream in the form of HCO₃⁻ is respired as CO₂ ‘meters to kilometers downstream of its origin when it evaporates across the stream surface’, which is consistent with the formation of a pH gradient we observed corresponding to the distance downstream from the CO₂ injection site. The macroinvertebrate response observed using HCl could be very different from the response caused by increased CO₂ (Ardón et al. 2013), possibly because no mechanism is present which would allow HCl to leave solution.

One assumption held from the beginning of the experiment was that a consistent population of insects existed along the extent of stream being studied. One explanation for our inconclusive drift and benthic data is the possibility that this assumption was not met and there were varying existing insect populations at each site. In addition, the summer during which this study was conducted was considered abnormally dry, so the low streamflow present could explain the low drift samples collected. According to Berghahn et al. (2012), under normal and low-flow stream conditions, macroinvertebrate drift must be considered a minor downstream shift within a range of only a few decimeters to meters. The macroinvertebrates that entered into drift could have been unable to drift far enough downstream to be caught in the sampling nets, or could have not entered into drift at all and used another mechanism to escape the acidic conditions. The low stream flow experienced over the course of this study, while impeding our understanding of macroinvertebrate drift, was beneficial however for facilitating a more pronounced drop in pH than would have been possible in a stream with greater volume.

In summary, the results obtained from this study are noteworthy because the correlation between CO₂ injection and decreased pH could indicate the possible effects that could result from an increase in dissolved CO₂ due to climate change. Decreased pH has been shown by this study to be linked to increased concentrations of gaseous CO₂ dissolving into the stream. There is also now empirical data associated with the tolerance of macroinvertebrate taxa, particularly insects, to deviations from environmental CO₂ levels, an area Hasler et al. (2016) identify as a knowledge gap. According to Small et al. (2012), seasonal pH patterns are amplified by drought years, with lower pH drops experienced during the ensuing wet season. Therefore, the pH in the Buruquena Stream may be expected to drop more significantly when the wet season returns to Puerto Rico because of the dry summer
experienced during this study. The *in situ* nature of our study allows inferences to be made within a natural ecological system with its many levels and factors in play, and stream acidification was mediated by a well-recognized buffering system rather than by the addition of strong acids in order to put episodic acidification in the context of CO₂ pulses. The Earth’s carbon cycle is projected to continue to respond to climate change and increasing atmospheric CO₂ (Ciais et al. 2013), which will increasingly impact overall ecosystems into the future. The acidic conditions created with the injection of CO₂ in this study were shown to have an effect on macroinvertebrate assemblages, and the role of increased dissolved CO₂ on these communities may now be better understood.

**Acknowledgements**

This research was made possible by the Research Experience for Undergraduates (REU) program funded by the National Science Foundation (NSF-DBI1062769, Ramírez). In particular, the authors wish to thank the REU Site: Tropical Ecology and Evolution at El Verde Field Station, and Alonso Ramírez, for providing the opportunity, facilities and support needed to complete this project. Thanks are also extended to Alonso Ramírez for editing the manuscript, Pedro J. Torres and Bethany Vázquez for helping with sampling, and all the 2014 REU students and mentors for their input.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the National Science Foundation [grant number NSF-DBI1062769, Alonso Ramírez].

**Notes on contributors**

Crystal C. Klem received her biology BS from University of Dallas in 2015, and is currently pursuing an entomology PhD from Purdue University.

Pablo E. Gutiérrez-Fonseca received his ecology and systematics PhD in June 2016. He is currently a post-doctoral associate with the Luquillo LTER program at University of Puerto Rico, Rio Piedras.

**ORCID**

Crystal C. Klem [http://orcid.org/0000-0003-3026-363X](http://orcid.org/0000-0003-3026-363X)

Pablo E. Gutiérrez-Fonseca [http://orcid.org/0000-0003-0777-8889](http://orcid.org/0000-0003-0777-8889)

**References**

Ardón M, Duff JH, Ramírez A, Small GE, Jackman AP, Triska FJ, Pringle CM. 2013. Experimental acidification of two biogeochemically-distinct neotropical streams: buffering mechanisms and macroinvertebrate drift. Sci Total Environ. 443:267–277.

Berghahn R, Mohr S, Hubner V, Schmiediche R, Schmiedling I, Svetich-Will E, Schmidt R. 2012. Effects of repeated insecticide pulses on macroinvertebrate drift in indoor stream mesocosms. Aquat Toxicol. 122–123:56–66.

Camargo JA, Alonso A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int. 32(6):831–849.

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, et al. 2013. Carbon and Other Biogeochemical Cycles. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, editors. Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge (UK): Cambridge University Press.
